Association Analysis of *CFH*, *C2*, *BF*, and *HTRA1* Gene Polymorphisms in Chinese Patients with Polypoidal Choroidal Vasculopathy

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PURPOSE. Polypoidal choroidal vasculopathy (PCV) is a major cause of serosanguinous maculopathy in Chinese patients with age-related macular degeneration (AMD). Variants in the *CFH* and *HTRA1/LOC387715* genes are strongly associated with AMD in Caucasians and Chinese. Variants in the *C2* and *BF* genes have been found to confer a significantly reduced risk of AMD. This study was undertaken to determine whether these associations occur in Chinese patients with PCV.

METHODS. Patients of Chinese ethnicity with clinically and angiographically diagnosed PCV and normal control subjects were recruited from the Singapore National Eye Centre. Five single-nucleotide polymorphisms (SNPs) in the *CFH* gene, two each within the *C2* and *BF* genes and two variants located in the *LOC387715* and *HTRA1* genes, were screened in all patients and control subjects.

RESULTS. Seventy-two patients with PCV and 93 normal control subjects were studied. A significant association was noted with *CFH* variants rs3753394 and rs800292 among the PCV cases ($P = 0.0015$ and $P = 0.0045$, respectively). Individuals homozygous for the TT genotype of rs3753394 had a significantly higher risk ($P = 0.0076$) of PCV (OR = 4.29; 95% CI: 1.47–12.50) than those carrying a single copy of the T allele ($P = 0.3210$; OR = 1.69; 95% CI: 0.60–4.78), after adjustment for such risk factors as age and sex. The genotype frequencies of rs11200638 and rs10490924 in *HTRA1* and *LOC387715*, respectively, were also found to be significantly different between patients with PCV and normal control subjects ($P = 0.00032$ and $P = 0.003$, respectively). The AA genotype of rs11200638 and TT genotype of rs10490924 conferred a 4.9-fold (95% CI: 1.85–12.95) and 4.89-fold (95% CI: 1.85–12.90) increased risk of PCV, respectively, after adjustment for age and sex. The Y402H variant of *CFH* (rs1061170) and the *BF* and *C2* variants were not significantly different in patients and normal control subjects.

CONCLUSIONS. The SNPs rs3753394 and rs800292 of *CFH* and rs11200638 of *HTRA1* are significantly associated with the risk
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Polypoidal choroidal vasculopathy (PCV) is a major cause of serosanguinous maculopathy in elderly Chinese and Japanese patients with choroidal neovascular anomaly[1,2] as a distinct form of age-related macular degeneration (AMD).[1–6] The incidence of PCV in the Chinese and Japanese populations with neovascular AMD has been reported to be as high as 24.5% and 54.7% respectively, compared with a much lower incidence in Caucasians.[4,6,7–9]

PCV is characterized on indocyanine green (ICG) angiography by a branching vascular network with polypoidal structures at the border of the choroidal vascular network.[10] These polypoidal structures lead to recurrent serosanguinous retinal pigment epithelial detachments and neurosensory retinal detachments with associated lipid exudates.[7] Soft drusen associated with AMD are often not seen in patients with PCV, and since disciform scarring does not always occur despite recurrent leakage and bleeding from the polypoidal structures, patients with PCV can often retain useful vision.[7] However, severe cases of PCV maculopathy can result in irreversible loss of vision.

The *CFH* gene has been shown to be strongly associated with AMD through the Y402H variant (single-nucleotide polymorphism [SNP] rs1061170) and significantly increases the risk for AMD in Caucasian and Taiwanese populations.[11–14] This gene, however, has not been found to be associated with AMD in Japanese patients.[15] Other variants in the *CFH* gene including the *CFH* promoter (rs3753394), I62V (rs800292), and IVS15 (rs1329428) have also been reported to be associated with AMD in Caucasians and Chinese.[16,17] Variants E318D (rs9332739) in the complement component 2 gene (*C2*) and L9H (rs4151667) and R32Q (rs641153) in the factor B gene (*BF*) have been found to confer a significantly reduced risk of AMD in Caucasians.[18] All these findings suggest a role of the complement system in the molecular pathogenesis of AMD. Recently, SNPs rs10490924 (*LOC387715* locus) and rs11200638 in the promoter region of *HTRA1*, a serine protease gene on chromosome 10, area q26, were found to be associated with wet AMD in Chinese, Caucasians, and Japanese.[19–21]

Our intention was to investigate whether these associations occur in Chinese patients with PCV from Singapore.

METHODS

Subjects

Patients of Chinese ethnicity, with clinically and angiographically diagnosed PCV and normal control subjects were recruited from the Singapore National Eye Centre. Written informed consent was obtained from all patients, and the study had the approval of the Ethics Committee of the Singapore Eye Research Institute and was performed according to the tenets of the Declaration of Helsinki.

All patients were examined by retina-fellowship–trained ophthalmologists, and detailed characterization of each patient's phenotype was performed with fundus photography, fundus fluorescein angiography (FFA), and ICG angiography (Fig. 1). Clinical features of patients with clearly defined PCV phenotypes revealed the presence of (1) subretinal red or orange nodules and hemorrhagic pigment epithelial detachment (PED) and (2) characteristic saccular vascular abnormalities in the inner choroid, as visualized on ICG angiography (Figs. 1C, 1D).

Subjects were excluded if they did not meet the criteria for diagnosis of PCV or if there was presence of choroidal neovascularization (CNV) on FFA and ICG angiography or drusen. Thus, all subjects included only had PCV, which we consider to be a neovascular form of AMD distinct from CNV in our population. Chinese subjects with normal results in a macular examination and without clinical signs of retinal disease, PCV, or AMD were recruited as the control.

Genotyping

Genomic DNA was extracted from peripheral white blood cells. The SNPs in the *CFH* gene (rs3753394, rs800292, rs1061170, rs2274700, and rs1329428), *C2* gene (rs9332739 and rs547154), *BF* gene (rs4151667, rs12614 and rs641153), *LOC387715* locus (rs10490924), and *HTRA1* gene (rs11200638) were amplified by polymerase chain reaction (PCR; Thermocycler 9700; Applied Biosystems, Inc. [ABI], Foster City, CA). PCR reactions were performed in 50-μL reaction volumes containing 10 mM Tris HCl (pH 8.9), 50 mM KCl, 1.5 mM MgCl$_2$, 25 picomoles of each primer, 200 μM of each dNTP, 50 to 100 ng of patient genomic DNA, and 0.7 units of *Taq* thermostable DNA polymerase (Promega, Madison, WI). Cycling parameters were 3 minutes at 95°C, followed by 35 cycles of 30 seconds at 95°C, 30 seconds at the melting temperature (T$_m$) of the primers (52°C–62°C), and 30 seconds to 1 minute at 72°C, with a final 5-minute extension at 72°C. PCR products were purified using PCR clean-up columns (GFX; GE Healthc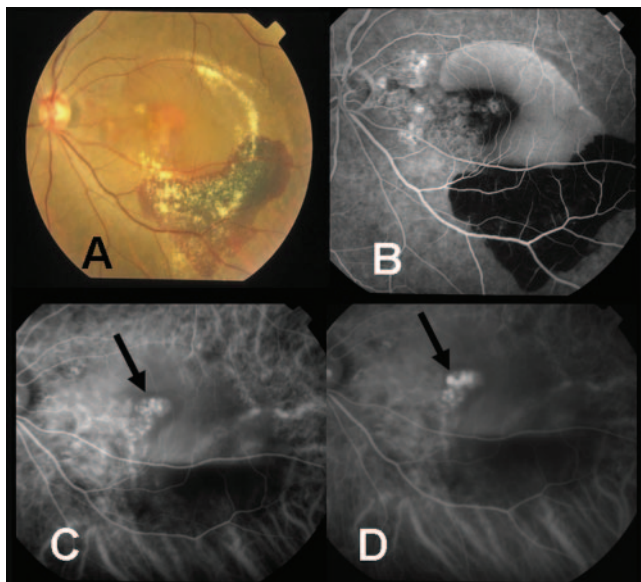are, Piscataway, NJ). Sequence variations were identified by automated bidirectional sequencing (BigDye terminator chemistry, ver. 3.1; ABI). An automated DNA sequencer (Prism 3100; ABI) was used. Primers for sequence reactions were the same as those for the PCR reaction.

Statistical Analysis

Fisher exact tests were used to test the allelic and genotypic associations of all the SNPs with PCV. Logistic regression was used to test genotypic associations adjusted for age and sex. Conditional analysis using logistic regression was also performed to examine the independent effect of an SNP conditional on another SNP. The Hardy-Weinberg equilibrium of the genotypic frequencies among cases and separately among the control subjects was also examined. All the analyses were performed with the software R.[22] Haploview was used to compute the LD statistics and the LD plot.[23] Haplotype association analysis was performed with the software package WHAP.[24] Joint associations of all the haplotypes and haplotype-specific and sole variant associations were determined with this program. A haplotype-specific test of association is used to examine the independent effect of any specific haplotype. For this test, under the null model none of the haplotypes is used, and under the alternative model the specific haplotype effect is entered. A likelihood-ratio test is then constructed to assess the significance of the haplotype-specific effect. Additional details regarding the haplotype associations implemented in the program can be found in the reference article cited.[24]

RESULTS

Demographics of Subjects

Seventy-two patients with PCV and 93 normal control subjects were recruited for the study. There were 26 female and 46 male patients with PCV, and all were Chinese. The mean age of the patients was 63.8 ± 7.6 years (range, 42–84), and the mean age of normal control subjects (53 females and 40 males) was 67.2 ± 4.60 years (range, 60–85). For the age- and sex-adjusted analysis, age was categorized into three groups: <65 (40%), 65–70 (36%), and >75 (24%) years.

Clinical Phenotype

There were 10 patients who had a diagnosis of bilateral disease. All patients had angiographically diagnosed PCV, with characteristic polypoidal choroidal lesions on ICG angiography. Symptoms at presentation included blurring of vision, decreased central vision, metamorphopsia, a black patch in the field of vision, and floaters. The duration of presenting symptoms ranged from 1 week to 3 years. Best corrected Snellen's visual acuity at presentation ranged from 6/6 to hand motions (HM). Patients with foveal involvement from the exudative lesion had poorer presenting visual acuity. Focal laser was the treatment of choice in patients with extrafoveal lesions, whereas patients with subfoveal lesions received photodynamic therapy.

SNP Analysis

The allelic and genotype frequency of the SNPs investigated are shown in Tables 1, 2, and 3. The genotype frequencies of the controls and cases followed Hardy-Weinberg equilibrium. In the analysis of *CFH* gene variants between PCV and control subjects, significant allelic associations were detected only with the *CFH* variants rs3753394 and rs800292 but not with rs1061170, rs2274700, or rs1329428. The frequency of the C risk allele at Y402H was 6.9% in PCV cases and 5.4% in controls, with no significant difference ($P = 0.64$). In contrast the T allele of rs3753394 and G allele of rs800292 conferred a 2.1-fold (95% CI: 1.30–3.43) and 2.0-fold (95% CI: 1.21–3.36) increased likelihood of PCV, respectively. The association sig-

FIGURE 1. (**A**) Color fundus photograph of a patient with serosanguinous maculopathy. There was a hemorrhagic pigment epithelial detachment with hard exudates, and an orange lesion was visible in the macula. (**B**) FFA showing the notched pigment epithelial detachment (PED) with a large area of blocked fluorescence from the hemorrhage. (**C, D**) The diagnosis of PCV was confirmed with ICG angiography. The polypoidal lesions (*arrow*) were best visualized with ICG angiography. Saccular outpouchings of the choroidal vessel in the same location as the orange lesion in (**A**) were seen. The typical polypoidal lesions were more distinct in the later ICG film (**D**), as the dye was slowly washed from the choroidal system.

TABLE 1. SNPs of the *CFH* Gene Investigated in Patients with PCV

SNP ID	Designation		Allele Distribution (%)		Allele Association (*P*)	Odds Ratio (95% CI)		Genotype Distribution (%)		Genotype Association *P*-value	*P* Odds Ratio (95% CI)
			Cases	Controls				Cases	Controls		
rs3753394	*CFH* Promoter (−257)	T	101 (70.1)	98 (52.7)	0.0015	2.10 (1.30–3.43)	TT*	36 (50.0)	25 (26.9)	0.0054	0.0055
		C	43 (29.9)	88 (47.3)			CT	29 (40.3)	48 (51.6)		**4.05 (1.38–13.13)**+ 0.35
							CC	7 (9.7)	20 (21.5)		1.72 (0.60–5.42)++ 0.016
											2.37 (1.13–5.02)+++
rs800292	*CFH* Exon 2 162V	G	109 (75.7)	113 (60.8)	0.0045	2.01 (1.21–3.36)	GG*	41 (56.9)	36 (38.7)	0.0175	0.011
		A	35 (24.3)	73 (39.2)			GA	27 (37.5)	41 (44.1)		**4.49 (1.29–20.15)**+ 0.1196
							AA	4 (5.6)	16 (17.2)		2.61 (0.73–11.88)++ 0.133
											1.72 (0.85–3.53)+++
rs1061170	*CFH* Exon 9 Y402H 1q31	C	10 (6.9)	10 (5.4)	0.6438	1.31 (0.47–3.62)	CC*	0 (0.0)	1 (1.1)	NA	NA
		T	134 (93.1)	176 (94.6)			CT	10 (13.9)	8 (8.6)		
							TT	62 (86.1)	84 (90.3)		
rs2274700	*CFH* Exon 10	C	102 (70.8)	113 (60.8)	0.0628	1.57 (0.96–2.57)	CC*	33 (45.8)	31 (33.3)	0.1084	NS
		T	42 (29.2)	73 (39.2)			CT	36 (50.0)	51 (54.8)		
							TT	3 (3.2)	11 (11.8)		
rs1329428	*CFH* IVS15	C	95 (66.0)	106 (57.0)	0.1115	1.46 (0.91–2.36)	CC*	29 (40.3)	27 (29.0)	0.2041	NS
		T	49 (34.0)	80 (43.0)			CT	37 (51.4)	52 (55.9)		
							TT	6 (8.3)	14 (15.1)		

Cases *n* = 72; controls *n* = 93. +, Comparing the likelihood of AMD in individuals with two copies of the risk allele versus individuals with no copies of the risk allele; ++, comparing the likelihood of PCV in individuals with one copy of risk allele versus no copies; +++, comparing the likelihood of PCV in individuals with two copies of risk allele versus one copy. Bold odds ratios represent significance at $P < 0.05/3 = 0.0167$ (Bonferroni correction).

* Homozygous for the risk allele.
† Homozygous for the protective allele.

nal with rs800292 was weaker than that for the *CFH* promoter variant rs3753394. Individuals homozygous for the TT genotype of the *CFH* variant rs3753394 had a significantly higher risk ($P = 0.0055$) of PCV (OR = 4.05; 95% CI: 1.39–13.13) than those carrying a single copy of the T allele ($P = 0.3494$; OR = 1.72, 95% CI: 0.60–5.42). The significant association of the TT genotype was consistent ($P = 0.0076$; OR = 4.29, 95% CI: 1.47–12.50) even after adjustment for the risk factors age and sex (Table 4). Similarly, the GG homozygote of rs800292 was significantly associated ($P = 0.0095$), conferring a 5.2-fold increased risk (95% CI: 1.5–18.2), compared with the homozy-

gous nonrisk genotype after adjustment for age and sex. We also used logistic regression to determine whether the effect at rs3753394 might explain the association at rs800292 and vice versa (Table 5). After conditioning on rs3753394, rs800292 was found not to be significantly associated with disease and vice versa, indicating that both SNPs are highly correlated and represent the same association at this locus.

The SNPs rs11200638 and rs10490924 in *HTRA1* and *LOC387715*, respectively, showed significant association to PCV with OR = 2.24, $P = 0.0004$ for allele A of rs11200638, and OR = 1.98, $P = 0.0027$ for allele T of rs10490924. The

TABLE 2. SNPs within *LOC387715* and *HTRA1* Genes Investigated in Patients with PCV

SNP ID	Designation		Allele Distribution (%)		Allele Association (*P*)	Odds Ratio (95% CI)		Genotype Distribution (%)		Genotype Association (*P*)	*P* Odds Ratio (95% CI)
			Cases	Control				Cases	Control		
rs11200638	*HTRA1* Promoter (−512) 10q26	A	92 (63.9)	82 (44.1)	0.0004	2.24 (1.40–3.60)	AA*	32 (44.4)	15 (16.1)	0.0003	0.0011
		G	52 (36.1)	104 (55.9)			AG	28 (38.9)	52 (55.9)		**4.53 (1.69–12.87)**+ 0.8358
							GG	12 (16.7)	26 (28.0)		1.16 (0.48–2.94)++ 4×10^{-4}
											3.92 (1.73–9.21)+++
rs10490924	*LOC387715* 10q26	T	80 (55.6)	72 (38.7)	0.0027	1.98 (1.24–3.15)	TT*	25 (34.7)	12 (12.9)	0.0034	0.0025
		G	64 (44.4)	114 (61.3)			TG	30 (41.7)	48 (51.6)		**3.97 (1.50–11.06)**+ 0.7078
							GG	17 (23.6)	33 (35.5)		1.21 (0.54–2.74)++ 0.005
											3.30 (1.36–8.37)+++

For an explanation of the data, see the footnote to Table 1.

TABLE 3. SNPs of the *C2* and *BF* Gene Investigated in Patients with PCV

SNP ID	Designation		Allele Distribution (%)		Allele Association (*P*)	Odds Ratio (95% CI)		Genotype Distribution (%)		Genotype Association (*P*)	Odds Ratio (95% CI)
			Cases	Controls				Cases	Controls		
rs9332739	*C2* E318D 6p21	C	2 (1.4)	4 (2.2)	0.7000	1.56 (0.22-17.45)	CC†	0 (0.0)	0 (0.0)	NA	NA
		G	142 (98.6)	182 (97.8)			CG	2 (2.8)	4 (4.3)		
							GG	70 (97.2)	89 (95.7)		
rs547154	*C2* IVS10	T	5 (3.5)	9 (4.8)	0.5940	1.41 (0.41-5.49)	TT†	0 (0.0)	0 (0.0)	NA	NA
		G	139 (96.5)	177 (95.2)			TG	5 (6.9)	9 (9.7)		
							GG	67 (93.1)	84 (90.3)		
rs4151667	*BF* Exon 1 L9H	A	3 (2.1)	4 (2.2)	1.0000	1.03 (0.17-7.16)	AA†	0 (0.0)	0 (0.0)	NA	NA
		T	141 (97.9)	182 (97.8)			AT	3 (4.2)	4 (4.3)		
							TT	69 (95.8)	89 (95.7)		
rs12614	*BF* Exon 2 R32W	T	4 (2.8)	6 (3.2)	1.0000	1.17 (0.27-5.73)	TT†	0 (0.0)	0 (0.0)	NA	NA
		C	140 (97.2)	180 (96.8)			TC	4 (5.6)	6 (6.5)		
							CC	68 (94.4)	87 (93.5)		
rs641153	*BF* Exon 2 R32Q	A	8 (5.6)	13 (7.0)	0.6552	1.28 (0.47-3.66)	AA†	0 (0.0)	0 (0.0)	NA	NA
		G	136 (94.4)	173 (93.0)			AG	8 (11.1)	13 (14.0)		
							GG	64 (88.9)	80 (86.0)		

For an explanation of the data, see the footnote to Table 1.

genotype frequencies of rs11200638 and rs10490924 were also found to be significantly different between patients with PCV and normal control subjects (*P* = 0.0003 and *P* = 0.0034 respectively). The AA genotype of rs11200638 and TT genotype of rs10490924 conferred a 4.9-fold (95% CI: 1.85–12.95), and 4.89-fold (95% CI: 1.85–12.90) of increased risk of PCV, respectively, even after adjustment for age and sex (Table 4). Conditional analysis showed that after conditioning on rs10490924, rs11200638 was only marginally associated with disease risk (*P* = 0.0449). Furthermore, after conditioning on rs11200638, rs10490924 also did not remain significantly associated with disease risk indicating that the effect of rs11200638 is mainly responsible for the association signal for PCV at the *HTRA1* locus and that these two SNPs are also correlated.

We also evaluated the role of epistasis between the *CFH* SNPs rs3753394 and rs800292 and the two *HTRA1* and *LOC387715* SNPs rs11200638 and rs10490924, which showed associations in our study. Using logistic regression, we did not

observe statistically significant interaction terms between any pair of these SNPs (data not shown). In support of this, the association of rs11200638 with PCV was found to be significant (*P* = 0.0028) when analyzed conditional on *CFH* SNP rs3753394, and the association of rs3753394 when conditional on rs11200638 was also found to be significant (*P* = 0.0077).

The *BF* and *C2* variants investigated were not significantly different in patients and normal control subjects. No patients or normal subjects were homozygous for the protective alleles in *C2* and *BF* genes. As the frequencies of the protective alleles were very low in the Chinese population, a larger sample size may be needed to reveal the protective effect of these alleles.

Linkage Disequilibrium (LD) and Haplotype Association Analysis

Within the *CFH* gene pair-wise LD analysis showed rs2274700 in high LD with rs1329428 (D' = 0.97, 95% CI: 0.89–1.0; Fig.

TABLE 4. Distribution of Unadjusted and Adjusted Odds Ratio for Risk Genotypes in *CFH* and *HTRA1*

Locus (SNP)	Genotype	OR (95% CI Unadjusted)	*P*	OR (95% CI Adjusted)*	*P*
CFH (rs3753394)	TT	4.05 (1.39-13.13)	0.0055	4.29 (1.47-12.50)	0.0076
	CT	1.72 (0.60-5.42)	0.3494	1.69 (0.60-4.78)	0.3210
CFH (rs800292)	GG	4.49 (1.29-20.15)	0.011	5.22 (1.50-18.20)	0.0095
	GA	2.61 (0.73-11.88)	0.1196	3.24 (0.91-11.50)	0.0687
HTRA1 (rs11200638)	AA	4.53 (1.69-12.87)	0.0011	4.90 (1.85-12.95)	0.0014
	AG	1.17 (0.48-2.94)	0.8358	1.01 (0.43-2.39)	0.9775
LOC387715 (rs10490924)	TT	3.97 (1.50-11.06)	0.0025	4.89 (1.85-12.90)	0.0013
	TG	1.21 (0.54-2.74)	0.7078	1.27 (0.57-2.80)	0.5591

* Adjusted for age and sex.

TABLE 5. Association between *CFH* and *HTRA1* Variants and PCV

| | | | | | | Conditional on: | | | |
| | | | | | | rs3753394 | | rs800292 | |
SNP	Risk Allele	Control Frequency (%)	Affected Frequency (%)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)
rs3753394	T	52.7	70.1	0.0015	2.10 (1.30–3.43)	—	—	0.0819	1.76 (0.93–3.32)
rs800292	G	60.8	75.7	0.0045	2.01 (1.21–3.36)	0.3976	1.33 (0.69–2.55)	—	—

| | | | | | | Conditional on: | | | |
| | | | | | | rs11200638 | | rs10490924 | |
SNP	Risk Allele	Control Frequency (%)	Affected Frequency (%)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)
rs11200638	A	44.1	63.9	0.0004	2.24 (1.4–3.6)	—	—	0.0449	1.99 (1.01–3.89)
rs10490924	T	38.7	55.6	0.0027	1.98 (1.24–3.15)	0.597	1.19 (0.62–2.29)	—	—

2). The rs3753394 was also in moderately high LD with rs800292 (D′ = 0.82, 95% CI: 0.68–0.91). Haplotype analysis using the five SNPs of *CFH* revealed nine different haplotypes among the patient and control individuals, after the rare haplotypes with frequencies less than 1% were trimmed. The estimated haplotype frequencies are presented in Table 6. A significant association was noted for the C-A-T-T-T (P = 0.0062) haplotype that was present approximately two times higher (29.8% vs. 17.1%) in controls than in cases, indicating that it could be protective. The association of PCV with at-risk haplotype T-G-T-C-C and protective haplotype C-A-T-T-T were only marginally significant after adjustment for age and sex. When only the two locus haplotype comprising the *CFH* SNPs (rs3753394 and rs800292) were considered, the risk haplotype (T-G) was found to be more strongly associated with disease (P = 0.0007; OR = 2.27 (95% CI: 1.40–3.68) even after adjusting for age and sex (P = 0.0011; OR = 2.26; 95% CI: 1.37–3.72).

The two SNPs rs11200638 and rs10490924 in *HTRA1* and *LOC387715* were in strong LD with each other (D′ = 0.83,

FIGURE 2. Analysis of pair-wise LD across the five CFH SNPs in the Chinese cohort.

95% CI: 0.71–0.91). Haplotype analysis of the two SNPs rs11200638 and rs10490924 identified four haplotypes (Table 7). Consistent with individual SNP analysis, the haplotype T-A conferred a 2.39-fold (95% CI: 1.48–3.85) increased likelihood of PCV (P = 0.0003) after adjustment for age and sex. This association was further supported by a nonsignificant adjusted probability (P = 0.069) in the sole-variant-haplotype test,[24]—a test of association of all other haplotypes excluding this haplotype, which indicated that the T-A haplotype contributes to the global test of association.

DISCUSSION

Patients with PCV can develop recurrent serous and hemorrhagic detachments of the retinal pigment epithelium and the neurosensory retina. The fundus typically lacks drusen, and most cases are unilateral and occur more frequently in males and at an earlier age than CNV occurs.[2,5,25] The pathogenesis of the disorder is unknown; however, unlike CNV, PCV involves primarily the choroidal vasculature with characteristic lesions seen as vascular outpouchings of the normal choroidal vessel, which may be seen as orange-reddish nodules or polyp-like structures at the posterior pole.[25] In Singapore, PCV is seen in more than 40% of cases of exudative AMD (unpublished hospital data, July 2007) and is a major cause of visual loss from serosanguinous maculopathy.

Our study shows that there is an association between *CFH* and *HTRA1* gene variants and PCV. The SNPs rs3753394 and rs800292 of *CFH*, rs10490924 of *LOC387715*, and rs11200638 of *HTRA1* were significantly associated with the risk of PCV in our Chinese patients. After adjustment for age and sex, all these SNPs showed a partially recessive effect on the association with PCV. Our study is the first to detect association between variants within the 10q locus (rs10490924 and rs11200638) and PCV in the Chinese. These two SNPs also showed a highly significant allelic association with PCV (rs10490924, P = 5.7 × 10^{-6}; rs11200638, P = 5.2 × 10^{-6}) in a recent Japanese study involving 76 PCV cases and 94 control subjects.[26] The risk allele A of rs11200638 was present at a lower frequency (38.3%) among the Japanese control samples than in our Chinese control samples (44.1%), whereas the frequency of this allele in PCV cases was similar between the Japanese (63.2%) and the Chinese (63.9%). The frequency of the risk allele of rs10490924 was also similar between Japanese and Chinese control subjects but differed between the Japanese (62.5%) and Chinese PCV cases (55.6%) of our study. These differences in allele frequencies may explain the differences in association
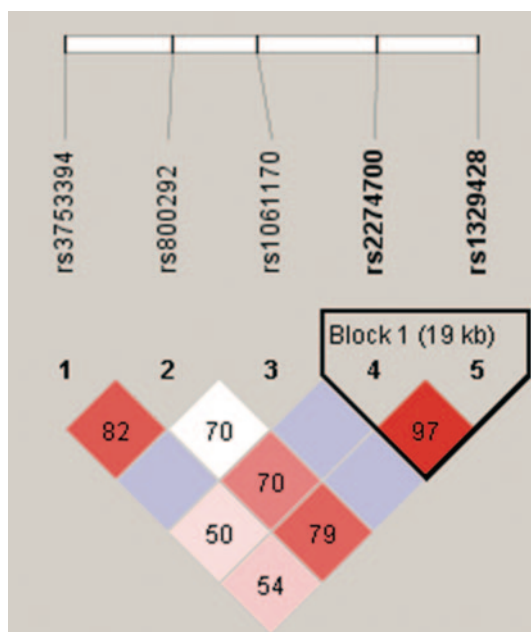
TABLE 6. Haplotype Analysis of SNPs in *CFH*

rs3753394	rs800292	rs1061170	rs2274700	rs1329428	Frequency* Controls	Frequency* Cases	OR (95% CI Unadjusted)	P‡	OR (95% CI Adjusted)†	P‡
		CFH Haplotypes								
T	G	T	C	C	0.428	0.554	1.764 (1.09-2.86)	0.0210	1.789 (1.07-2.97)	0.0272
C	A	T	T	T	0.298	0.171	0.467 (0.26-1.21)	0.0062	0.51 (0.28-0.92)	0.0214
T	G	T	T	T	0.060	0.081	1.439 (0.57-3.63)	0.4473	1.32 (0.50-3.48)	0.5775
C	G	C	C	C	0.045	0.065	1.45 (0.56-3.75)	0.4199	1.54 (0.57-4.16)	0.3964
C	G	T	C	C	0.058	0.023	0.444 (0.13-1.49)	0.1562	0.37 (0.11-1.26)	0.0915
T	A	T	T	T	0.035	0.030	0.865 (0.26-2.84)	0.8510	0.778 (0.22-2.69)	0.6532
C	A	T	C	T	0.018	0.043	2.58 (0.60-11.04)	0.2166	2.53 (0.56-11.34)	0.2635
C	A	T	C	C	0.042	0.010	0.168 (0.02-1.39)	0.0402	0.19 (0.02-1.57)	0.1119
T	G	T	C	T	0.015	0.022	1.53 (0.27-8.64)	0.5873	1.84 (0.31-10.79)	0.4867

* All haplotypes with frequency 1% in the combined case and control sample are shown.
† Adjusted for age and sex. Global unadjusted $P = 0.0743$; adjusted $= 0.0712$.
‡ P reported is the haplotype-specific test P obtained for 10,000 permutations.

signal strengths observed between our study and that of the Japanese, even though both involved a similar number of cases and controls.

In a previous study of Chinese exudative AMD cases, the *CFH* variants rs3753394, rs800292, and rs1329428 were found to be significantly associated with exudative AMD.[17] In our Chinese PCV cohort, we found a significant association with only two of the above SNPs (i.e., rs3753394 and rs800292). However, after correcting for multiple comparisons, we found that the genotype association signal with rs800292 was much weaker. Pair-wise LD analysis also indicated a strong LD between rs3753394 and rs800292 and the risk haplotype (T-G) was found to be strongly associated ($P = 0.0011$), conferring a 2.26-fold increased risk (95% CI: 1.37–3.72) of PCV after adjustment for age and sex. Our data therefore indicate rs3753394 in the *CFH* promoter to be one of the major AMD susceptibility polymorphisms in the Chinese population, although it was found not be associated with AMD in two previous studies conducted in Caucasians.[12,16] It is possible that a larger sample cohort would give more power to the association shown with the SNP rs800292, but it may not be so for the rs1329428 variant. Hence, such genetic differences may partly underlie the phenotypic variations/diversity observed between the two AMD phenotypes in the Chinese. The Y402H

variant of CFH (rs1061170) was not associated with the risk of PCV in our Chinese patients and variants in *C2* and *BF* did not confer a reduced risk of PCV. The frequency of the Y402H variant in the Chinese and Japanese populations have been reported to be low, and our study provides further evidence that other variants in the *CFH* gene, such as rs3753394, may play a more important role in AMD in Chinese populations.[14,15,17] Studies have shown that there are multiple AMD-predisposing variants in the *CFH* gene.[27] Our study was limited to only five *CFH* variants and therefore does not preclude the possibility of other *CFH* variants that may play a role in the pathogenesis of PCV in combination with environmental variables.

Our findings suggest a role of the complement system in the molecular pathogenesis of PCV. CFH specifically inhibits the alternative complement cascade and also regulates the common pathway.[28] It is hypothesized that variants in CFH, like Y402H, result in an aberrant inflammatory process with inappropriate complement activation and damage to the Bruch's membrane, with resultant neovascularization.[11,16,29] The *HTRA1* gene encodes a heat shock serine protein and is activated by cellular stress.[30] It is hypothesized that overexpression of HTRA1 alters the integrity of Bruch's membrane, facilitating invasion of choroidal capillaries, as HTRA1 appears to

TABLE 7. Haplotype Analysis of SNPs in *LOC387715* and *HTRA1*

LOC387715 rs10490924	HTRA1 rs11200638	Frequency* Controls	Frequency* Cases	OR (95% CI Unadjusted)	P‡	OR (95% CI Adjusted)†	P‡
G	G	0.525	0.360	0.49 (0.31-0.79)	0.0029	0.46 (0.28-0.75)	0.0015
T	A	0.353	0.554	2.22 (1.41-3.51)	0.0005	2.39 (1.48-3.85)	0.0003
G	A	0.088	0.085	0.97 (0.49-1.89)	0.8860	0.96 (0.48-1.92)	0.9018
T	G	0.034	0.002	NA	NA	NA	NA

* All haplotypes with frequency >1% in the combined case and control sample are shown.
† Adjusted for age and sex. Global test 0.0005; adjusted $P = 0.0008$.
‡ P is the haplotype-specific test P obtained for 10,000 permutations.

regulate the degradation of extracellular matrix proteoglycans.[31] We hypothesize that both of these distinct pathways are involved in the pathogenesis of PCV. That we did not observe any statistically significant interaction between rs3753394 of *CFH* and rs11200638 of *HTRA1* also suggests that these variants confer risk for PCV in an independent additive fashion. This result reflects what has been observed previously in other studies for AMD where *CFH* and *HTRA1* variants were found to confer independent risks.[32,33]

In conclusion, we have found significant association between *CFH* variants (rs3753394 and rs800292) and variants within the 10q locus (rs11200638 and rs10490924) and PCV in Chinese subjects, even after adjustment for risk factors such as age and sex. We note, however, that further studies in a larger population should be performed to ascertain the effects of rare variants and risk factors such as smoking, which has been found to modify the association of *CFH* polymorphisms and AMD.[34,35] Our findings suggest a role of two biological pathways, each contributing to the pathogenesis of PCV. The genetic risk factors (rs3753394, rs800292, rs11200638, and rs10490924) common to both exudative AMD and PCV also suggest that these two diseases have common pathogenic mechanisms.

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