Different Hereditary Contribution of the *CFH* Gene Between Polypoidal Choroidal Vasculopathy and Age-Related Macular Degeneration in Chinese Han People

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Purpose. To investigate whether 11 variants in complement factor H gene contributed differently in patients with neovascular age-related macular degeneration (nAMD) and polypoidal choroidal vasculopathy (PCV) of Chinese descent.

Methods. We performed a case-control study in a group of Chinese patients with nAMD (n = 344) or PCV (n = 368) and contrasted the results against an independent control group comprising 511 mild cataract patients without any evidence of age-related maculopathy. Association analysis of allele and genotype frequencies was performed for 11 haplotype-tagging single-nucleotide polymorphisms (SNPs) at the *CFH* locus (rs1061170, rs1329428, rs1410996, rs2284664, rs375396, rs529825, rs551397, rs7540032, rs800292, rs2274700, and rs1065489). Multinomial logistic regression analyses were performed to estimate and compare the effect of these 11 CFH polymorphisms on AMD and PCV, using the wild-type genotype as reference. Differences in the observed genotypic distributions between cases and controls were tested by using χ^2 tests, with age and sex adjusted for using logistic regression.

Results. CFH rs1065489 was not significantly associated with the nAMD phenotype in Chinese collections either on univariate or multivariate analysis ($P > 0.05$ for all comparisons). The other 10 SNPs of CFH were significantly associated with the nAMD phenotype. As for PCV, all 11 SNP markers were significantly associated with risk of PCV before or after correction for age and sex differences. Eight of the 11 SNP markers showed significant evidence of heterogeneity between AMD and PCV ($P < 0.05$ for all comparisons).

Conclusions. Our data suggest that the genetic architecture at the CFH locus is complex with some markers showing significant skewing of the genotypes toward nAMD or PCV in Asians. This further supports the clinical observation that nAMD and PCV could have distinct pathogenesis mechanisms, which will require larger studies to accurately dissect.

Keywords: neovascular age-related macular degeneration, polypoidal choroidal vasculopathy, complement factor H, single-nucleotide polymorphism

Age-related macular degeneration (AMD) is a major cause of severe visual loss in elderly populations in the developed countries.[1] Late-stage AMD can be divided into two forms: geographic atrophy (dry AMD) and neovascular AMD (wet AMD, or nAMD). Neovascular AMD is an advanced form of disease, characterized by the development of choroidal neovascular (CNV) membranes, which is the main cause of visual impairment in macular degeneration.[2]

Polypoidal choroidal vasculopathy (PCV) is characterized by aneurysmal dilations with interconnecting vessels, which are often best demonstrated by indocyanine green (ICG) angiography.[3] Sharing some similar clinical manifestations, it still remains controversial whether PCV represents a subtype of nAMD or is a specific entity on its own. However, the prevalence of PCV and nAMD varies depending on ethnicity.[4,5] Besides, emerging evidence suggests some other differences in basic pathomechanisms may exist between nAMD and PCV, including clinical morphologic features, histopathology features, clinical behavior, disease progression, and responses to photodynamic therapy or anti-VEGF therapy.[4,6–8]

Table 1. Demographic Distribution of the Study Subjects

	nAMD, $n = 344$	PCV, $n = 368$	Controls, $n = 511$
Females, *n*	125	143	285
Males, *n*	219	225	226
Age range, y*	50–90	42–87	45–96
Age, mean ± SD, y	69.2 ± 8.7	66.6 ± 9.6	67.2 ± 9.6

SD, standard deviation.
* Age at presentation.

Recently, genetic variants in the complement factor H gene (*CFH*) have been found to play an important role in the pathogenesis of both CNV and PCV. Although results vary with different ethnicities and different single-nucleotide polymorphisms (SNPs), most studies support the association between CFH variants and both nAMD and PCV. It remains uncertain whether the CFH SNPs play the same role in nAMD and PCV and there is a lack of sufficient evidence to interpret the difference between the two diseases.

To see whether the differences in clinical feature between these two phenotypes could be attributed to differences in genetic components, we attempted to investigate the relationships between these CFH SNPs and nAMD and PCV, and their effects between nAMD and PCV were compared as well.

Methods

Subjects

For eight of the 11 SNPs, 1223 unrelated Chinese subjects were studied in this case-control cohort. A total of 344 patients had nAMD, and 368 patients had PCV; 511 individuals without age-related maculopathy (ARM) were studied as controls. For rs800292, rs2274700, and rs1065489, 900 unrelated Chinese subjects were studied in this case-control cohort: 300 nAMD, 300 PCV, and 300 controls. The sex and age 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 Declaration of Helsinki, and consent forms were signed by 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, and ICG angiograms with HRA2 (Heidelberg Engineering, Heidelberg, Germany). The diagnosis of nAMD or ARM was defined by International Classification System for ARM.[9] The diagnosis of PCV was based on ICG angiography results that showed a branching vascular network terminating in aneurysmal enlargements. Exclusion criteria included any eye with any other macular abnormalities, such as

Table 2. Hardy-Weinberg Equilibrium Test of the Study Subjects

SNP	Test	A1	A2	GENO	O(HET)	E(HET)	*P*
rs1061170	AMD	C	T	8/52/252	0.1667	0.1942	0.01822
rs1061170	PCV	C	T	2/63/271	0.1875	0.1795	0.5549
rs1061170	Control	C	T	1/57/403	0.1236	0.1198	1
rs1065489	AMD	G	T	75/137/88	0.4567	0.4991	0.1646
rs1065489	PCV	G	T	52/142/104	0.4765	0.4848	0.8111
rs1065489	Control	G	T	79/156/66	0.5183	0.4991	0.5639
rs1329428	AMD	A	G	46/149/148	0.4344	0.4558	0.407
rs1329428	PCV	A	G	28/167/172	0.455	0.423	0.1748
rs1329428	Control	A	G	109/259/141	0.5088	0.498	0.6569
rs1410996	AMD	T	C	41/147/153	0.4311	0.4461	0.5445
rs1410996	PCV	T	C	25/160/180	0.4384	0.4098	0.2035
rs1410996	Control	T	C	103/252/150	0.499	0.4957	0.9285
rs2274700	AMD	A	G	35/125/131	0.4296	0.4456	0.5984
rs2274700	PCV	A	G	24/120/144	0.4167	0.4132	1
rs2274700	Control	A	G	60/137/95	0.4692	0.4928	0.4078
rs2284664	AMD	A	G	34/145/164	0.4227	0.4282	0.8017
rs2284664	PCV	A	G	19/147/198	0.4038	0.3791	0.2681
rs2284664	Control	A	G	81/241/188	0.4725	0.478	0.7818
rs3753396	AMD	A	G	80/163/101	0.4738	0.4981	0.3867
rs3753396	PCV	A	G	63/173/128	0.4753	0.4841	0.7456
rs3753396	Control	A	G	141/258/110	0.5069	0.4981	0.7225
rs529825	AMD	T	C	36/137/170	0.3994	0.4237	0.3078
rs529825	PCV	T	C	22/130/215	0.3542	0.3617	0.6667
rs529825	Control	T	C	81/251/178	0.4922	0.4819	0.7131
rs551397	AMD	A	G	35/122/155	0.391	0.426	0.1453
rs551397	PCV	A	G	22/129/191	0.3772	0.3779	1
rs551397	Control	A	G	81/211/166	0.4607	0.4828	0.3337
rs7540032	AMD	T	C	41/106/137	0.3732	0.4429	0.01054
rs7540032	PCV	T	C	23/122/153	0.4094	0.4048	1
rs7540032	Control	T	C	79/165/106	0.4714	0.497	0.3345
rs800292	AMD	A	G	34/123/141	0.4128	0.4355	0.3546
rs800292	PCV	A	G	19/115/164	0.3859	0.3816	1
rs800292	Control	A	G	48/147/104	0.4916	0.4825	0.8108

GENO, Genotype counts; O(HET), observed heterozygosity; E(HET), expected heterozygosity.

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Table 3. Genotype Frequency Distribution of the 11 CFH SNPs and the Results of Association Tests (*P* Trend)*

CHR	SNP	A1	A2	Test	Control	PCV	AMD	*P* (PCV vs. Control)	*P* (AMD vs. Control)	Phet†
1	rs529825	T	C	TREND	413/607	174/560	209/477	3.86E-13	0.0000292	0.005023
1	rs551397	A	G	TREND	373/543	173/511	192/432	3.61E-10	0.0001195	0.03104
1	rs800292	A	G	TREND	243/355	151/437	191/405	5.62E-08	0.0023	0.065
1	rs1061170	C	T	TREND	59/863	67/605	68/556	0.007927	0.002196	0.594
1	rs2274700	A	G	TREND	257/327	166/402	195/387	3.78E-07	0.00034	0.28
1	rs3753396	A	G	TREND	540/478	299/429	323/365	9.09E-07	0.01407	0.02873
1	rs1410996	T	C	TREND	458/552	210/520	229/453	2.20E-12	1.731E-06	0.04928
1	rs7540032	T	C	TREND	323/377	168/428	188/380	1.10E-10	7.956E-06	0.07994
1	rs2284664	A	G	TREND	403/617	185/543	213/473	8.65E-10	0.0004103	0.01726
1	rs1329428	A	G	TREND	477/541	223/511	241/445	2.68E-12	1.892E-06	0.0551
1	rs1065489	G	T	TREND	312/286	241/347	287/313	0.00012	0.14	0.099

CHR, chromosome.

* *P* trend: *P* value for association with nAMD and PCV.

† Phet: *P* value for heterogeneity between the associations observed for nAMD and PCV.

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 nAMD 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 extraction. Genomic DNA was extracted from venous blood leukocytes by using a genomic extraction kit (Beijing eBios Biotechnology Co., Ltd., Beijing, China), and genotyping was performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), as previously described.[10] Briefly, approximately 30 ng genomic DNA was used to genotype each sample. The primer sequences for 11 SNPs of *CFH* gene are shown in Supplementary Table S1. 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 SpectroCHIP array (Sequenom, San Diego, CA, USA). Allele detection was performed by using MALDI-TOF-MS. The mass spectrograms were analyzed by using MassARRAY Typer software version 4.0 (Sequenom, San Diego, CA, USA).

Statistical Analysis

Data were analyzed by using SAS9.1.3 software (SAS Institute Inc., Cary, NC, USA). Descriptive statistics were calculated for the demographic and clinical variables according to the presence or absence of AMD and PCV. Multinomial logistic regression analyses were performed to estimate and compare the effect of CFH polymorphisms on AMD and PCV, using the wide-type genotype as reference. The results are reported as odds ratios (ORs) with their accompanying 95% confidence intervals. $P = 0.05$ was used as the threshold for declaring statistical significance. For primary analysis, we measured the association between CFH SNP markers and disease status by using the trend test, modeling for a trend-per-copy of the minor allele.

Results

Demographics of this study are shown in Table 1. Overall, genotype and allele frequencies of the reported SNPs were analyzed in the 344 nAMD patients, 368 PCV patients, and contrasted against those of the 511 controls. For all study groups, the distributions of the genotypes are shown in Table 3. All 11 SNPs but rs1061170 and rs529825 showed no significant deviation from Hardy-Weinberg equilibrium in the AMD group ($P > 0.05$) (Table 2). The details of the allele, genotype frequencies, and summary statistics for these 11 SNPs are shown in Table 3 (Supplementary Tables S2–S4).

In keeping with findings from Western collections,[11] the CFH rs1065489 polymorphism was not significantly associated with the nAMD phenotype ($P > 0.05$), despite showing strong association with PCV ($P < 0.0005$, with or without adjustment for age and sex). All of the 11 SNPs tested at the *CFH* locus were significantly associated with PCV (Table 3), and 8 of 11 SNP markers tested showed significant evidence of heterogeneity between AMD and PCV ($P < 0.05$ for all comparisons) after adjusting for age and sex (Table 4). We noted no evidence of heterogeneity between PCV and nAMD for rs1161170, which encodes for the Y402H mutation, which was first reported to be very strongly associated with late-stage AMD in Europeans.[12] Haplotype analysis revealed strong evidence of linkage disequilibrium across most of the 11 common polymorphisms in AMD (Fig. A) and in PCV (Fig. B).

Discussion

Our study comprehensively evaluated 11 SNPs of CFH in both nAMD and PCV, and contrasted the differences in genotype distribution between controls, nAMD, and PCV. In keeping with earlier Western reports on nAMD, most SNPs of CFH appeared to be shared genetic risk factors for both nAMD and PCV, whereas SNP rs1065489 appeared to be more confined to PCV. Of note, no association has been observed between *CFH* rs1065489 and severe nAMD in patients of European descent,[11] consistent with our observations of no association with nAMD in Chinese persons.

With the increased knowledge about the genetic determinants for AMD, researchers have tried to find genetic evidence to determine the association between nAMD and PCV. Single-nucleotide polymorphisms of CFH, replicated in multiple cohorts, have been proved to be associated with both nAMD and PCV, which plays as major genetic evidence to support the similarity between nAMD and PCV. In our study, significant heterogeneity between nAMD and PCV were observed in 8 of 11 SNPs tested of CFH. The result may indicate that although they share some common genetic determinants and even further functional mechanisms, PCV seems not to be just a simple variant of AMD, but could be a distinct disease entity.

Table 4. Logistic Regression Adjustment for Sex and Age

		PCV*		PCV†		AMD‡		AMD§		Phet	
SNP	A1	OR	*P*	OR	*P*	OR	*P*	OR	*P*	Phet‖	Phet¶
rs529825	T	0.4544	1.298E-12	0.4471	1.10E-12	0.6462	3.357E-05	0.6506	6.445E-05	0.00523	0.003022
rs551397	A	0.5028	7.56E-10	0.4903	3.74E-10	0.6629	0.0001333	0.6583	0.0001447	0.03155	0.02065
rs800292	A	0.4991	8.292E-8	0.4933	9.15E-08	0.6927	0.002417	0.6839	0.002555	0.01699	0.01091
rs1061170	C	1.651	0.008483	1.789	0.002657	1.733	0.002561	1.743	0.002824	0.5942	0.6279
rs2274700	A	0.5314	4.517E-07	0.5333	8.36E-07	0.6528	0.0003731	0.6511	0.0006022	0.1152	0.08588
rs3753396	A	0.6161	1.156E-06	0.6273	0.000003999	0.7581	0.01431	0.7817	0.01496	0.02909	0.02114
rs1410996	T	0.476	6.187E-12	0.4758	1.26E-11	0.6118	2.178E-06	0.6089	3.317E-06	0.04976	0.0275
rs7540032	T	0.4672	2.879E-10	0.465	3.39E-10	0.6059	9.888E-06	0.5987	1.078E-05	0.08055	0.05915
rs2284664	A	0.5161	1.617E-09	0.5221	4.74E-09	0.6924	0.0004413	0.7029	0.0009987	0.01763	0.01066
rs1329428	A	0.4799	7.357E-12	0.4772	1.16E-11	0.6156	2.365E-06	0.612	3.357E-06	0.05558	0.03596
rs1065489	G	0.6414	0.0001818	0.6517	0.0003729	0.8443	0.1389	0.8571	0.192	0.02679	0.01701

Phet, *P* value for heterogeneity between the associations observed for nAMD and PCV; OR, odds ratio for association with nAMD and PCV.
* PCV before adjustment for sex and age.
† PCV after adjustment for sex and age.
‡ AMD before adjustment for sex and age.
§ AMD after adjustment for sex and age.
‖ Phet before adjustment for sex and age.
¶ Phet after adjustment for sex and age.

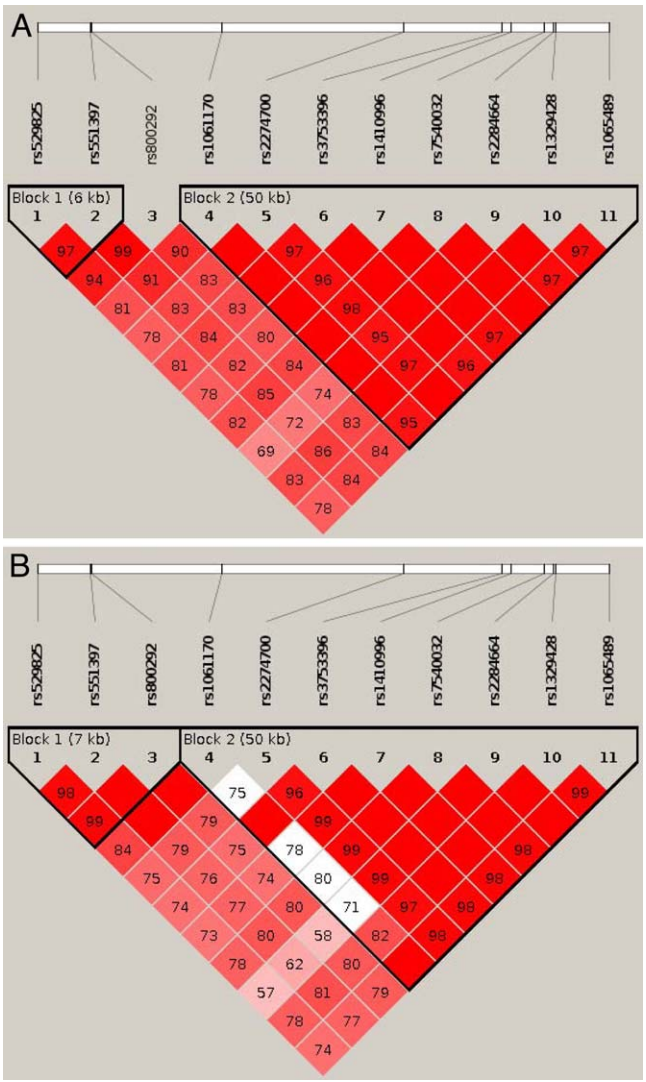

Figure. **(A)** Analysis of pairwise LD across CFH SNPs in nAMD cohort. **(B)** Analysis of pairwise LD across CFH SNPs in PCV cohort. LD, linkage disequilibrium.

We also noted significant heterogeneity between 8 of the 11 CFH SNP markers tested between nAMD and PCV (Table 4), a difference which is unlikely to occur by chance alone. The reason why these different effects could not be detected previously may lie in the clinical classification, which may lead to misclassification between PCV patient samples and nAMD patients. Ethnic differences may also affect the associative significance. Besides, different effects of these SNPs of CFH on PCV and nAMD may indicate different SNP functions.

The G allele of CFH rs1065489 has been found to confer resistance against meningococcal sepsis in Europeans,[13] as well as exert an opposite effect: certain alleles are associated with disease in atypical hemolytic syndrome but are protective against immunoglobulin A nephropathy, central serous chorioretinopathy, and AMD.[14,15] It can be speculated that environmental factors and interactions with other multiple genetic loci may affect phenotypic expression of variant alleles.

Our result may imply a different pathogenesis, although further studies are necessary to clarify the link. Since it is still unclear how genetic differences play a role in the pathogenesis of diseases, studies with larger sample sizes and different cohorts may be necessary to reveal the effect of these alleles. Besides, functional studies of each independent SNP may be carried out for further information.

In conclusion, this study provided evidence that *CFH* acts somehow as a susceptibility gene for both nAMD and PCV, and that the complement pathway has an important role in the pathogenesis. The different effects of the nine SNPs on PCV and AMD suggest there may be different pathogenesis mechanisms and different genetic factors that may play a role. This finding provides further insight into the underlying genetic character and pathophysiology of the development of nAMD and PCV.

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