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LABORATORY STUDY

Gene–gene interaction of *CFH*, *ARMS2*, and *ARMS2/HTRA1* on the risk of neovascular age-related macular degeneration and polypoidal choroidal vasculopathy in Chinese population

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Abstract

Purpose **To evaluate the association and interaction of five single-nucleotide polymorphisms (SNPs) in three genes (*CFH, ARMS2,* and *ARMS2/HTRA1*) with neovascular age-related macular degeneration (nAMD) and polypoidal choroidal vasculopathy (PCV) in Chinese population.**

Methods **A total of 300 nAMD and 300 PCV patients and 301 normal subjects participated in the present study. The allelic variants of rs800292, rs2274700, rs3750847, rs3793917, and rs1065489 were determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Gene–gene interactions were evaluated by the data mining approach multifactor-dimensionality reduction (MDR) method.**

Results **The risk alleles of *CFH* rs800292, rs2274700, *ARMS2* rs3057847, and *ARMS2/HTRA1* rs3793917 showed significant difference between nAMD or PCV patients and controls (all $P<0.01$). The homozygosity of risk alleles for rs800292, rs2274700, rs3750847, and rs3793917 were significantly different between nAMD patients and controls (all $P<0.01$), and predisposed to PCV patients (all $P<0.01$). After cross-validation consistency (CVC) and permutation tests, the two-locus model rs2274700_rs3750847 has a balanced accuracy of 64.37% in predicting nAMD disease risk. The one-marker model, rs3750847, and two-locus model rs2274700_rs3750847 has a balanced accuracy of 66.07% and 65.89% in predicting PCV disease risk, respectively. Furthermore, *CFH* rs1065489 did not show significant association with nAMD ($P>0.01$), but was strongly associated with PCV in Chinese patients ($P<0.001$).**

Conclusions **In this study, we found that the interaction of *ARMS2* and *ARMS2/HTRA1* is significantly associated with nAMD, and the interaction of *CFH* and *ARMS2* is pronounced in PCV development in Chinese population.**

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Introduction

Age-related macular degeneration (AMD) is the most common cause of legal blindness in the elderly in developed countries. Advanced AMD, including neovascular AMD (wet AMD, or nAMD) and geographic atrophy (dry AMD), is associated with substantial, progressive visual impairment.[1] AMD is characterized as a chronic and progressive degeneration of photoreceptors, the underlying retinal pigment epithelium, Bruch's membrane, and potentially, the choriocapillaris in the macula.[2] Polypoidal choroidal vasculopathy (PCV) is associated with a decrease in vision in the elderly Asian population, and is characterized by a network of vessels with two distinct components: a complex of branching vessels and multiple, terminal, reddish-orange polypoidal lesions.[3–5] PCV has been described as a distinct clinical entity from AMD and the other diseases associated with subretinal neovascularization.[6] Nevertheless, whether PCV represents a subtype of AMD remains controversial.[7] Evidence suggests that

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nAMD and PCV, despite their different phenotypic manifestations, may share common genetic risk factors.[8–12] Susceptibility to nAMD is complex, involving genetic, lifestyle, and environmental factors, although the specifics of the etiology remain unresolved.

Complement factor H (*CFH*) is a major inhibitor of the alternative complement pathway and is found within drusen,[13] which is an important sign in early AMD. *CFH* has been identified by various studies as a major AMD susceptible gene in the Chinese, Japanese, and Caucasian populations.[14–17] Previous studies have proved that multiple variants of *CFH* gene, such as I62V (rs800292), are associated with the risk of nAMD in different ethnic groups.[18–21]

Beyond the complement pathway, the Age-Related Maculopathy Susceptibility 2 *(ARMS2)* locus at chromosome 10q26 has been implicated as another major genetic contributor to the nAMD disease process. *ARMS2* rs3750847 has been demonstrated to have a strong association with both nAMD and PCV in Chinese population.[22] *HTRA1* (High-Temperature Requirement factor A 1), another major locus at chromosome 10q26, was recently shown to have an important role in the pathogenesis of nAMD or PCV.[23] Previous studies proved that gene variants like rs3793917 localized to the intergenic region between *ARMS2* and *HTRA1* were high-risk factors for nAMD.[24,25]

To identify multilocus interactions in the pathogenesis of nAMD in Chinese population, we applied the multifactor-dimensionality reduction (MDR) analysis, which has been used to characterize gene–gene interactions in nAMD.[26–28]

In this study, we aimed to evaluate the association of three genes (*CFH, ARMS2,* and *ARMS2/HTRA1*) with nAMD and PCV, and to determine their possibility as biomarkers for genetic factors predisposing to nAMD and PCV in Chinese population. We analyzed the genetic association of the reported major risk SNPs (rs800292, rs2274700, rs3750847, rs3793917, and rs1065489) in *CFH, ARMS2*, and *ARMS2/HTRA1* genes, and examined gene–gene interactions using the MDR method in unrelated Chinese patients with nAMD and PCV.

Methods

Subjects

A total of 901 unrelated Chinese subjects were studied in this case–control cohort. Three hundred patients had nAMD, and three hundred patients had PCV. Three hundred and one individuals without age-related maculopathy (ARM) were studied as controls. The genders and ages of the controls and cases are given in Supplementary 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 Helsinki Declaration, 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 (OCT), and indocyanine green angiograms with HRA2 (Heidelberg Engineering, Heidelberg, Germany). The diagnosis of nAMD or ARM was defined by the International Classification System for ARM.[29] The diagnosis of PCV was based on indocyanine green angiography (ICGA) results that showed a branching vascular network terminating in aneurysmal enlargements, which typify polypoidal lesions. Exclusion criteria included any eye with any other macular abnormalities, such as 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 was extracted. Genomic DNA was extracted from venous blood leukocytes using a genomic extraction kit (Beijing eBios Biotechnology, Beijing, China), and genotyping was performed with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, as previously described.[30] Briefly, ~30 ng of genomic DNA was used to genotype each sample. 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 using 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, USA).

Statistical analysis

The data were analyzed using SPSS (version 16.0; SPSS Science, Chicago, IL, USA). All identified polymorphisms were assessed for Hardy–Weinberg equilibrium using

χ^2-tests. We also used χ^2-test to determine significant difference in risk allele of SNPs, and single-marker association analysis was performed using logistic regression incorporating an additive genetic model as previously described.[31] Logistic regression model was used to calculate the odds ratio (OR) and 95% confidence interval (CI) of nAMD or PCV, comparing case groups to the control group, and adjusted for age, gender, different genotypes, and various genetic models. A *P*-value of <0.01 was considered statistically significant. Potential locus–locus interaction was evaluated using the nonparametric MDR software (version 2.0 alpha, www.multifactordimensionalityreduction.org) with three SNPs for *CFH*, one SNP for *ARMS2* and one SNP for *ARMS2/HTRA1* each with risk alleles. Briefly, the multilocus genotypes were pooled into high-risk and low-risk groups, effectively reducing the genotype predictors to one dimension. The new, one-dimensional multilocus-genotype variable was then evaluated for its ability to classify and predict disease status through cross-validation and permutation testing. A detailed explanation on the MDR method has been described elsewhere.[26]

Results

A total of 901 subjects participated in the study, including 301 control subjects (mean age ± SD, 65.1 ± 9.5 years; 159 females and 142 males), 300 cases with nAMD (mean age ± SD, 69.4 ± 8.9 years; 111 females and 189 males) in one or both eyes, and 300 cases with PCV (mean age ± SD, 66.8 ± 9.7 years; 112 females and 188 males) in at least one eye. The general characteristics of the study subjects are shown in Supplementary 1.

SNP analysis

The allele frequencies and genotype frequencies for patients and controls are shown in Tables 1 and 2. All SNPs showed no significant deviation from Hardy–Weinberg equilibrium in the control group ($P>0.05$), except rs3750847 and rs3793917 in the nAMD group ($P=0.03$, $P=0.01$,respectively; shown in Supplementary 2).

The risk allele frequencies of *CFH* rs800292, rs2274700, *ARMS2* rs3057847, and *ARMS2/HTRA1* rs3793917 showed significant difference between nAMD or PCV patients and controls (all $P<0.01$), and *CFH* rs1065489 was significantly different between PCV patients and controls, but showed no difference between nAMD patients and controls ($P=0.149$; shown in Table 1).

The adjusted ORs, 95% CIs, and *P*-values of the positive SNPs are shown in Table 2. The homozygosity of risk alleles for two SNPs in *CFH* (rs800292, rs2274700), one SNP in *ARMS2* (rs3750847), and one SNP in *ARMS2/HTRA1* (rs3793917) conferred a 1.994-fold (95% CI, 1.458,2.568), 1.503-fold (95% CI, 1.189,1.900), 2.438-fold (95% CI, 1.913,3.107), and 2.420-fold (95% CI, 1.901,3.081) increased likelihood of nAMD, and conferred 2.021-fold (95% CI, 1.561,2.617), 1.718-fold (95% CI, 1.360,2.171), 3.221-fold (95% CI, 2.494,4.159), and 3.060-fold (95% CI, 2.376,3.940) increased likelihood of PCV. Besides, *CFH* rs1065489 was associated with increased risk of PCV with OR 1.528 (95%CI, 1.208,1.933), but showed no significant association with nAMD ($P=0.166$).

Gene–gene interaction evaluation using the nonparametric MDR method

Interaction between *CFH*, *ARMS2*, and *ARMS2/HTRA1* polymorphisms in relation to the risk of nAMD and PCV was evaluated by the nonparametric MDR method. Table 3 shows the results of cross-validation (CV) consistency, training accuracy, testing accuracy, and testing OR (95% CI) obtained from MDR analysis.

In relation to the risk of nAMD, after cross-validation and permutation tests, we identified the one-marker model (rs3793917), which had a maximum cross-validation consistency of 90% and a maximum prediction

Table 1 Allele frequency distribution and the result of χ^2-tests

	Risk allele	*nAMD*	*PCV*	*Total*	*Control*	*nAMD vs control*		*PCV vs control*	
						P-*value*	*OR (95% CI)*	P-*value*	*OR (95% CI)*
CFH									
rs800292	G	405	445	850	355	0.002	1.451 (1.145,1.840)	0.000	1.991 (1.557,2.545)
rs2274700	G	387	409	796	327	0.000	1.560 (1.230,1.987)	0.000	1.972 (1.545,2.518)
rs1065489	T	401	424	825	250	0.149	1.189 (0.948,1.492)	0.000	1.550 (1.233,1.947)
AMRS2									
rs3750847	G	408	424	832	258	0.000	3.353 (2.640,4.260)	0.000	3.353 (2.640,4.260)
AMRS2/HTRA1									
rs3793917	T	313	351	664	287	0.000	3.156 (2.485,4.008)	0.000	3.156 (2.485,4.008)

$P<0.01$ was considered significant.

Table 2 Genotype frequency and the results of association tests

Gene	*Variant*	*Genotype*	*nAMD*	*PCV*	*Control*	*Adjusted association analysis*			
						nAMD vs control		*PCV vs control*	
						P-*value*	*OR (95% CI)*	P-*value*	*OR (95% CI)*
CFH	rs800292	AA	34	19	48	0.000	1.994 (1.458,2.568)	0.000	2.021 (1.561,2.617)
		GA	123	115	147				
		GG	141	165	104				
	rs2274700	AA	35	21	60	0.001	1.503 (1.189,1.900)	0.000	1.718 (1.360,2.171)
		AG	125	121	137				
		GG	131	144	95				
	rs1065489	CC	41	29	105	0.166	1.178 (0.934,1.487)	0.000	1.528 (1.208,1.933)
		CT	117	118	138				
		TT	142	153	56				
AMRS2	rs3750847	CC	40	30	100	0.000	2.438 (1.91,3.107)	0.000	3.221 (2.494,4.159)
		CG	112	116	138				
		GG	148	154	60				
AMRS2/HTRA1	rs3793917	GG	75	52	79	0.000	2.420 (1.901,3.081)	0.000	3.060 (2.376,3.940)
		GT	137	143	155				
		TT	88	104	66				

$P < 0.01$ was considered significant.

Table 3 CFH, ARMS2, and ARMS2/HTRA1 interaction models for nAMD and PCV patients from MDR analysis

Model	*Training accuracy*	*Testing accuracy*	*Cross-validation consistency*	P-*value*[a]	*Testing OR (95% CI)*
nAMD					
rs3793917	64.71	64.34	9/10	0.0209	3.832 (1.189, 12.344)
rs2274700_rs3750847	65.98	64.37	8/10	0.0284	3.266 (1.116, 9.565)
rs800292_rs3793917_rs1065489	66.83	61.95	6/10	0.0673	2.674 (0.922, 7.754)
PCV					
rs3750847	66.07	66.07	10/10	0.0098	4.596 (1.388, 15.216)
rs2274700_rs3750847	67.90	65.98	7/10	0.0150	3.767 (1.269, 11.183)
rs800292_rs2274700_rs3750847	68.91	65.12	7/10	0.0213	3.491 (1.183, 10.297)

[a]1000-fold permutation test.

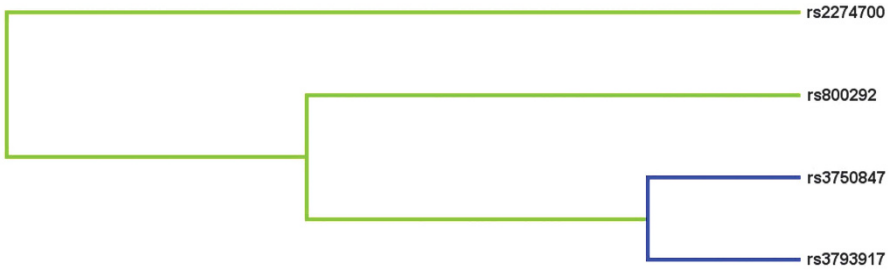

Figure 1 nAMD: interaction dendrogram for the best four-locus SNP model selected by the MDR. The blue line indicates a strong interaction whereas the green line indicates weak interaction.

accuracy of 64.34% ($P = 0.0209$ on the basis of 1000-fold permutation testing), and the two-locus models, rs2274700_rs3750847, with 64.37% balanced accuracy and a cross-validation consistency of 80% in predicting nAMD disease risk ($P = 0.0284$ on the basis of 1000-fold permutation testing). An interaction dendrogram highlighting the amount of information gained about nAMD-control using the MDR indicated that the set including rs3793917_rs3750847 variations had a synergistic effect (Figure 1).

In relation to the risk of PCV, after cross-validation and permutation tests, we identified the one-marker model

Figure 2 PCV: interaction dendrogram for the best four-locus SNP model selected by the MDR. The blue line indicates a strong interaction whereas the green line indicates weak interaction.

(rs3750847), which had a maximum cross-validation consistency of 100% and a maximum prediction accuracy of 66.07% ($P=0.0098$ on the basis of 1000-fold permutation testing), and the two-locus models, rs2274700_rs3750847, with 65.89% balanced accuracy and a cross-validation consistency of 70% in predicting PCV disease risk ($P=0.015$ on the basis of 1000-fold permutation testing). An interaction dendrogram highlighting the amount of information gained about PCV-control using the MDR indicated that the set including rs800292_rs2274700_rs3750847 variations had a synergistic effect (Figure 2).

Discussion

AMD is a complex disease caused by multiple environmental and genetic risk factors. Several genes have been proved to be associated with nAMD and PCV.[32,33] Genetic variants in the *Complement Factor H (CFH)* gene on chromosome 1q32 and two tightly linked genes—*ARMS2* and *HTRA1* on 10q26 have been identified as major contributors to nAMD development.[14–16,34] Our study identified that *CFH* rs800292 and rs2274700 showed significant difference between nAMD or PCV patients and controls, which was consistent with previous study in our laboratory.[35] We also identified that *CFH* rs1065489 was a risk factor in PCV, which had been proved in Japanese population.[36] Besides, we also found that *ARMS2* rs3750847 and *ARMS2/HTRA1* rs3793917 showed the strongest association with nAMD and PCV by SNP analysis, and might have important function in the pathogenesis of these diseases. Our findings are in keeping with genome-wide association study, which identified rs3750847 was a susceptibility locus of nAMD in Japanese population.[20] *ARMS2/HTRA1* rs3793917 has also been identified as a risk factor of nAMD in different ethnic groups.[35,37,38]

Several studies have been performed to explore the interactions between *CFH*, *ARMS2*, or *HTRA1* as risk factors in nAMD, but the results have been conflicting: some studies provided the evidence that the three genes appeared to be independent contributors to nAMD,[16,39,40] but others suggested otherwise.[41] Moreover, few studies explore the interactions of *CFH, ARMS2*, and *HTRA1* in the development of PCV in Chinese population. In our study, we use MDR method to examine the role of gene–gene interactions in the pathogenesis of nAMD and PCV. This method defines a single variable that incorporates information from several loci and/or environmental factors that can be divided into high-risk and low-risk combinations, and the new variable can be evaluated using cross-validation (CV) and permutation testing or other conventional analytic methods.[26,27,42]

According to the MDR analysis, we found that *ARMS2/HTRA1* rs3793917 had the most significant association with nAMD ($P=0.0209$), and that the two-locus model, rs2274700_rs3750847, conferred OR of 3.266. The interaction dendrogram indicated that the set including rs3793917_rs3750847 variations had a synergistic effect, suggesting the interaction between *ARMS2/HTRA1* and *ARMS2* is more important in nAMD development. The coinheritance of rs3750847 and rs3793917 in patients suggests the important role of interaction of *ARMS2* and *ARMS2/HTRA1* in the pathogenesis of nAMD in Chinese population. Our results indicated that a combined phenotype of rs3750847 and rs3793917 might contribute to risk assessment when early AMD signs are present. Further studies with larger sample sizes and different cohorts and functional studies are needed to understand these effects

In relation to the risk of PCV, *ARMS2* rs3750847 showed the most significant association with PCV, as it was most significant both in one-marker model and two-locus model by MDR analysis. In the dendogram, the gene–gene interaction between *CFH* rs2274700 and *ARMS2* rs3750847 was more significant. From the analysis, one-marker model, rs3750847 and the two-locus model including rs3750847 and rs2274700 variations were associated with a significantly increased risk in PCV patients. To our knowledge, it is our first time to identify the association between *ARMS2* rs3750847 and PCV, and to find the important role of the coinheritance of

rs3750847 and rs2274700 in PCV development. These findings provide further insight into pathogenesis of PCV.

In our study, we found no significant association between *CFH* rs1065489 and nAMD. Neither of the results of SNP analysis nor MDR analysis showed significant association between *CFH* rs1065489 and nAMD (all $P > 0.01$), and our result is consistent with the observation of no association in a European population.[43] However, this SNP showed a strong association with PCV ($P < 0.001$). It can be speculated that PCV may have some distinct genetic characteristics from nAMD, though they share some common genetic background.

In our study, we found rs3750847 and rs3793917 are departures from Hardy–Weinberg equilibrium in nAMD group. There are several explanations for this departure. First, in SNP analysis, we found rs3750847 and rs3793917 showed the most significant association with nAMD. These results suggest that rs3750847 and rs3793917 may be candidate loci for nAMD. The candidate genes could lead to the excess of homozygotes in patient group, which could result in departure from Hardy–Weinberg equilibrium. Second, systematic errors in genotyping or nonrandom patterns of missing data may generate departure from Hardy–Weinberg equilibrium. Our study recruited 300 nAMD patients, and the number of patients is not large enough to ignore systemic errors. So studies with larger sample size are needed.

In conclusion, we found that the risk alleles of *CFH* rs800292, rs2274700, *ARMS2* rs3057847, and *ARMS2/HTRA1* rs3793917 had significant association with nAMD and PCV. We also noted that gene–gene interaction of rs3793917_rs3750847 was significant associated with nAMD, and interaction of rs3750847_rs2274700 has association with PCV. However, more functional studies are necessary to make clear the nature of the gene–gene interaction in the development of these diseases, which will help us understand the complex genetic basis of nAMD and PCV.

Summary

What was known before

- The variants of CFH, ARMS2, and ARMS2/HTRA1 had an important role in the development of nAMD and PCV in China.

What this study adds

- We explored the gene–gene interaction of CFH, ARMS2, and ARMS2/HTRA1 in the pathogenesis of nAMD or PCV in Chinese population.
- We found that interaction of ARMS2 variants and ARMS2/HTRA1 variants was more significant in development of nAMD and interaction of CFH variants and ARMS2 variants was more important in PCV development, which will help us understand the complex genetic basis of nAMD and PCV better.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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