Differentiation of Exudative Age-Related Macular Degeneration and Polypoidal Choroidal Vasculopathy in the *ARMS2/HTRA1* Locus

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Purpose. To differentiate the associations of exudative age-related macular degeneration (AMD) and polypoidal choroidal vasculopathy (PCV) with the *ARMS2/HTRA1* locus.

Methods. The entire *ARMS2* sequence was sequenced and *HTRA1* rs11200638 genotyped in 568 unrelated Chinese individuals: 156 exudative AMD patients, 164 PCV patients, and 248 controls. A meta-analysis was performed to examine the effects of rs10490924 and rs11200638 at the *ARMS2/HTRA1* locus in PCV.

Results. In total, 31 polymorphisms in *ARMS2* were identified. Significant associations with both exudative AMD and PCV were observed in 11 of them and *HTRA1* rs11200638, with different genotypic distributions between exudative AMD and PCV ($P < 0.001$). After adjusting for rs11200638, *ARMS2* rs10490924 remained significantly associated with exudative AMD ($P = 0.011$), but not with PCV ($P = 0.077$). Meta-analysis showed consistent allelic associations of rs10490924 and rs11200638 with PCV in different study populations.

Conclusions. There is a strong and consistent association of the *ARMS2/HTRA1* locus with both exudative AMD and PCV, suggesting the two disorders share, at least partially, similar molecular mechanisms. Different effect sizes indicate the existence of additional genetic and environmental factors affecting them to different extents. (*Invest Ophthalmol Vis Sci.* 2012;53:3175–3182) DOI:10.1167/iovs.11-8135

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness among elderly people in developed countries, affecting approximately 50 million individuals worldwide.[1,2] Early AMD is characterized by the presence of drusen and/or retinal pigmentary abnormalities, while late AMD can be classified into non-neovascular (dry or nonexudative) and neovascular (wet or exudative) forms.[3] In Asians aged 40 to 79 years, prevalence of early and late AMD are 6.8% and 0.56% respectively,[4] similar to Caucasians (5.7% and 0.8%, respectively).[5] Overall prevalence of late AMD is projected to increase by more than 50% by year 2020.[6]

Familial [7,8] and twin studies [9,10] implicate the role of genetic predisposition in AMD.[11] Genome-wide association studies identified the complement factor H (*CFH*) gene on chromosome 1q32 as a susceptibility gene for AMD.[12–14] Later, a single nucleotide polymorphism (SNP; rs10490924, c.205G > T, A69S) in the age-related maculopathy susceptibility 2 (*ARMS2*) gene on chromosome 10q26 was reported to be strongly associated with AMD.[15] Moreover, we previously discovered a SNP (rs11200638, −625G > A) in the promoter region of the high temperature requirement factor A1 (*HTRA1*) gene, which is in strong linkage disequilibrium (LD) with rs10490924, was associated with exudative AMD in our Hong Kong Chinese cohort.[16] The association and interactions of these genes with AMD have been widely replicated in different populations.[17–23]

Recently, polypoidal choroidal vasculopathy (PCV), a disease sharing similar phenotypes with exudative AMD, was reported to be associated with *CFH* and *ARMS2/HTRA1* in Caucasian,[24] Japanese,[25–32] and Singapore's Chinese[33] populations. PCV is more prevalent in Asians.[34] PCV is an inner choroidal vascular abnormality in the macular region. Indocyanine green angiography (ICGA) could be used to characterize typical lesions of PCV by the branching choroidal vasculature basal to the retinal pigment epithelium (RPE) with various sized polypoidal structures connected to the branching vascular network.[34,35] Important clinical features of PCV, such as hemorrhagic RPE detachment and vitreous hemorrhage, share similar hallmarks with choroidal neovascularization (CNV) in exudative AMD.[35] On the contrary, patients with PCV tend to be younger and lacking drusen.[34] Responses to treatment vary between PCV and exudative AMD patients. The former respond better to photodynamic therapy but poorer to anti-vascular endothelial growth factor therapy.[36,37] It remains unclear whether exudative AMD and PCV are distinct disease entities.[34]

In this study, we investigated the genetic determinants of exudative AMD and PCV to highlight their genetic differentiation. We screened the entire *ARMS2* gene and genotyped the *HTRA1* rs11200638 in a Chinese cohort. A meta-analysis was also performed to examine the association of *ARMS2/HTRA1* with PCV among different populations.

Materials and Methods

Study Subjects

A total of 568 unrelated Han Chinese subjects were recruited from the Prince of Wales Hospital in Hong Kong and the Hong Kong Eye

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Table 1. Demographic Distribution of the Study Subjects of *ARMS2* and *HTRA1* Screening

	Exudative AMD ($n = 156$)	PCV ($n = 164$)	Controls ($n = 248$)	*P*
Males, *n* (%)	82 (52.6%)	111 (69.4%)	111 (44.9%)	
Females, *n* (%)	74 (47.4%)	49 (30.6%)	136 (55.1%)	
Age* range (Years)	60–94	43–87	60–94	
Mean age ± SD (Years)	75.9 ± 7.4	67.5 ± 9.0	73.6 ± 7.4	
AMD vs. control				0.003
PCV vs. control				5.51×10^{-13}
AMD vs. PCV				1.43×10^{-17}

SD, standard deviation.
* Age of presentation.

Hospital, including 156 exudative AMD patients, 164 PCV patients and 248 normal controls (Table 1). All study subjects, including patients and controls, were given complete ophthalmic examinations. AMD was graded according to an international classification and grading system.[3] Two vitreoretinal specialists were involved in the grading. Since they were also involved in treatment and follow-up of the patients, they were not masked. Patients with exudative AMD had nondrusenoid RPE detachment, choroidal neovascularization, serous or hemorrhagic retinal detachments, subretinal or sub-RPE hemorrhage or fibrosis. The diagnosis of PCV was distinguished from AMD by fluorescein angiography (FA) and ICGA.[38] PCV patients had subretinal red or orange nodules and hemorrhagic pigment epithelial detachment and characteristic sacculated vascular abnormalities in the inner choroid as visualized on ICGA. All of the included patients were examined by FA and ICGA (Model TRC-50IX; TOPCON, Tokyo, Japan). Patients with geographic atrophy or early signs of AMD were excluded. Some patients at late stage of exudative AMD may possess fibrosis and disciform scar, which made it difficult to be clearly differentiated from PCV by FA and ICGA, were also excluded in this study. The control subjects were recruited from elderly people (i.e., >60 years of age) who did not have any identifiable signs of AMD, PCV or other major eye diseases except for mild senile cataract and slight refractive errors. The mean refractive error of all our exudative AMD, PCV patients and control subjects was −0.8 diopter (D), ranging from −1.5D to +1.5D. Subjects with severe cataracts were also excluded. From the fundus examination of all study subjects, we found no pseudophakic patients with fundus findings consistent with high myopia. The study protocol, approved by the Ethics Committee for Human Research at the Chinese University of Hong Kong, is in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from each study subject.

Sequence Analysis

Genomic DNA from whole blood was extracted (Qiagen QIAamp DNA Blood Mini kit; Qiagen, Hiden, Germany) according to the supplier's instructions. The entire *ARMS2* gene (ENSG00000228258) was screened and the rs11200638 in *HTRA1* (ENSG00000166033) genotyped by polymerase chain reaction (PCR) with specific primers,[21,22] followed by direct DNA sequencing (BigDye Terminator Cycle Sequencing Reaction Kit, v3.1; Applied Biosystems, Foster City, CA) on a DNA sequencer (ABI 3130xL; Applied Biosystems).

Statistical Analysis

All the identified polymorphisms were assessed for Hardy-Weinberg equilibrium using χ^2 analysis. Allelic and genotypic distributions among different groups were compared using the χ^2 test or Fisher's exact test, and logistic regression analysis was performed to identify the strongest associated SNPs in the *ARMS2/HTRA1* locus (SPSS, version 16.0; SPSS Science, Chicago, IL). LD and haplotype-based association analyses were performed (Haploview, version 4.2, http://www.broadinstitute.org/).[39] Bonferroni method was used for multiple testing corrections.

For meta-analysis, an internet-based PubMed literature search was first conducted, using the term "polypoidal choroidal vasculopathy" or "PCV" and "genetics" or "gene" or "association" or "HTRA1" or "ARMS2" or "LOC387715." Only case-control studies and reports on the association of the two SNPs, *ARMS2* rs10490924 and *HTRA1* rs11200638, were included. Allelic associations of the two SNPs in different studies were then analyzed (Review Manager, version 5.0.25; The Cochrane Collaboration, Copenhagen, Denmark).

Results

A total of 31 polymorphisms were identified in *ARMS2* (Table 2). Five novel polymorphisms (c.324+373T > G, c.324+418C > T, c.324+570T > C, c.324+682C > T, and c.324+780G > A) were located within the deleted segment of the c.372_815del443ins54 variant, in which their genotypes of some subjects could not be identified. Therefore, they were excluded from further analysis. Two polymorphisms, IVS1-484G > A and rs11412729 (IVS1-4delT, were also excluded since they did not follow Hardy-Weinberg equilibrium. The remaining 24 SNPs were taken for further analysis. Among them, nine uncommon SNPs (frequency <5%) and one common SNP (c.324+965T > C) did not show significant association with exudative AMD or PCV. Three polymorphisms (rs2736911 [c.112C > T, R38X], rs2736912 [IVS1-829C > T, and c.324+838C > T) showed a significant association with PCV ($P = 4.4\times10^{-4}$, odds ratio [OR] = 0.42, 95% confident interval [CI]: 0.26–0.60; $P = 0.0011$, OR = 0.45, 95% CI: 0.27–0.70, and $P = 3.0\times10^{-4}$, OR = 0.39, 95% CI: 0.23–0.60, respectively) in a dominant model. Their associations with exudative AMD were mild and statistically insignificant ($P = 0.037$, $P = 0.10$, and $P = 0.038$, respectively). The remaining 11 polymorphisms in *ARMS2*, including rs10490924 (c.205G > T, A69S), rs61544945 (IVS1+63_IVS1+64insTG), rs36212731 (IVS1+436G > T), rs36212732 (IVS1+658A > G), rs36212733 (IVS1+671T > C), rs3750848 (IVS1+775T > G), rs3750847 (IVS1+881C > T), rs3750846 (IVS1-858T > C), rs10664316 (IVS1-37_IVS1-38insAT), the indel (c.372_815del443ins54), and rs2014307 (c.324+1183T > G), together with *HTRA1* rs11200638 (−625G > A), showed a significant association with both exudative AMD and PCV. Homozygous carriers of rs10490924-T allele had increased risk of 7.91 and 3.15-fold to exudative AMD and PCV respectively, and rs11200638-A of 6.95 and 2.82-fold respectively. Furthermore, the genotypic distributions of these 12 associated polymorphisms also showed significant differences between exudative AMD and PCV ($P < 0.001$, under recessive model). The ORs of these SNPs for exudative AMD were ≥2.47-fold higher as compared with PCV. Significant associations of these 12 polymorphisms with both AMD and PCV were also found

Table 2. The Polymorphisms in *ARMS2* and *HTRA1* Identified in the Screening Study of Exudative AMD, PCV, and Controls

				Genotypic Frequency†			AMD-control		PCV-control		AMD - PCV	
Location	db SNP ID	Nucleotide Change	Residual Change	AMD $n = 156$	PCV $n = 164$	Control $n = 248$	P^*	OR (95% CI)	P^*	OR (95% CI)	P^*	OR (95% CI)
ARMS2												
Promoter	Novel	−9G > A	–	0/2/151	0/0/164	0/1/247	0.561	–	1.000	–	0.234	–
Exon1	rs10490923	c.8G > A	R3H	0/0/151	0/1/161	0/6/242	0.090	–	0.253	–	1.000	–
Exon1	rs2736911	c.112C > T	R38X	1/33/117	3/24/135	5/75/168	0.037#	0.61 (0.38–0.97)	0.00044#	0.42 (0.26–0.69)	0.19#	1.45 (0.83–2.55)
Exon1	rs10490924	c.205G > T	A69S	90/46/15	60/79/23	39/119/90	1.01×10^{-19}	7.91 (4.93–12.67)	8.25×10^{-7}	3.15 (1.98–5.03)	6.52×10^{-5}	2.51 (1.59–3.96)
Intron1	rs61544945	IVS1+63_IVS1+64insTG	–	92/46/15	60/79/23	40/118/90	8.35×10^{-20}	7.84 (4.91–12.53)	1.44×10^{-6}	3.06 (1.92–4.87)	4.14×10^{-5}	2.56 (1.63–4.04)
Intron1	rs36212731	IVS1+436G > T	–	92/44/15	60/79/23	40/117/90	3.66×10^{-20}	8.07 (5.04–12.92)	1.61×10^{-6}	3.04 (1.91–4.85)	2.38×10^{-5}	2.65 (1.68–4.19)
Intron1	rs36212732	IVS1+658A > G	–	89/44/15	60/79/23	40/117/90	1.99×10^{-19}	7.81 (4.87–12.52)	1.61×10^{-6}	3.04 (1.91–4.85)	4.79×10^{-5}	2.56 (1.62–4.06)
Intron1	Novel	IVS1+664A > G	–	0/1/152	0/2/160	0/2/246	1.000	–	0.652	–	1.000	–
Intron1	rs36212733	IVS1+671T > C	–	89/44/15	60/79/23	40/117/90	1.99×10^{-19}	7.81 (4.87–12.52)	1.61×10^{-6}	3.04 (1.91–4.85)	4.79×10^{-5}	2.56 (1.62–4.06)
Intron1	Novel	IVS1+773T > C	–	0/0/153	0/0/162	0/1/247	1.000	–	1.000	–	1.000	–
Intron1	rs3750848	IVS1+775T > G	–	89/44/15	60/79/23	40/117/90	1.99×10^{-19}	7.81 (4.87–12.52)	1.61×10^{-6}	3.04 (1.91–4.85)	4.79×10^{-5}	2.56 (1.62–4.06)
Intron1	rs3750847	IVS1+881C > T	–	89/45/15	60/78/24	40/117/90	3.38×10^{-19}	7.68 (4.79–12.29)	1.61×10^{-6}	3.04 (1.91–4.85)	6.28×10^{-5}	2.52 (1.60–3.98)
Intron1	rs3750846	IVS1-858T > C	–	89/45/15	60/78/24	42/116/89	2.05×10^{-18}	7.24 (4.54–11.54)	4.65×10^{-6}	2.87 (1.81–4.55)	6.28×10^{-5}	2.52 (1.60–3.98)
Intron1	rs2736912	IVS1-829C > T	–	1/35/120	3/24/137	4/72/172	0.10#	0.68 (0.43–1.08)	0.0011#	0.45 (0.27–0.73)	0.14#	1.52 (0.87–2.65)
Intron1	Novel	IVS1-746A > G	–	0/1/153	0/1/163	0/1/246	1.000	–	1.000	–	1.000	–
Intron1	Novel	IVS1-484G > A	–	0/2/151	0/0/161	0/0/246	0.146	–	1.000	–	0.237	–
Intron1	rs10664316	IVS1-37_IVS1-38insAT	–	121/29/2	96/59/8	101/111/33	7.04×10^{-14}	5.57 (3.48–8.90)	4.68×10^{-4}	2.04 (1.37–3.06)	7.27×10^{-5}	2.72 (1.65–4.51)
Intron1	rs36213074	IVS1-26T > C	–	0/0/149	0/1/161	0/6/239	0.090	–	0.251	–	1.000	–
Intron1	rs7088128	IVS1-13A > G	–	0/0/149	0/2/161	0/6/239	0.090	–	0.485	–	0.499	–
Intron1	rs11412729	IVS1-4delT	–	0/1/149	0/0/163	0/0/245	0.380	–	1.000	–	0.479	–
3′-UTR	Novel	c.324+163insC	–	0/0/150	0/0/164	0/1/245	1.000	–	1.000	–	1.000	–
3′-UTR	Reported	c.372_815del443ins54	–	92/46/15	61/77/26	44/117/87	3.05×10^{-18}	6.99 (4.42–11.07)	9.19×10^{-6}	2.75 (1.74–4.33)	4.43×10^{-5}	2.55 (1.62–4.01)
3′-UTR	Novel	c.324+373T > G	–	0/0/63	1/1/100	4/2/198	–	–	–	–	–	–
3′-UTR	Novel	c.324+418C > T	–	0/0/64	0/0/102	1/2/204	–	–	–	–	–	–
3′-UTR	Novel	c.324+570T > C	–	0/0/63	0/1/101	1/0/203	–	–	–	–	–	–
3′-UTR	Novel	c.324+682C > T	–	0/1/14	0/0/26	0/0/86	–	–	–	–	–	–
3′-UTR	Novel	c.324+780G > A	–	0/0/15	0/0/26	0/1/85	–	–	–	–	–	–
Intergenic	Reported	c.324+838C > T	–	1/29/119	3/20/140	5/68/174	0.038#	0.60 (0.37–0.98)	0.00030#	0.39 (0.23–0.66)	0.16#	1.53 (0.85–2.78)
Intergenic	Reported	c.324+965T > C	–	0/9/147	1/18/144	0/29/219	0.054	–	0.991	–	0.075	–
Intergenic	Novel	c.324+1096G > A	–	0/0/156	0/0/164	0/1/247	1.000	–	1.000	–	1.000	–
Intergenic	rs2014307	c.324+1183T > G	–	125/29/2	94/60/8	103/112/33	2.59×10^{-14}	5.68 (5.57–9.06)	0.001	1.95 (1.30–2.91)	2.09×10^{-5}	2.92 (1.77–4.82)
HTRA1												
Promoter	rs11200638	−625G > A	–	93/47/16	61/76/26	41/105/88	9.88×10^{-18}	6.95 (4.37–11.06)	8.02×10^{-6}	2.82 (1.77–4.47)	7.34×10^{-5}	2.47 (1.57–0.87)

* The association was calculated under recessive model ($P < 0.05/32 = 0.0016$ is considered to be significant).

† The genotype presented as homozygous/heterozygous/wide-type.

The association was assessed under dominant model.

The missing values were the genotypes of five novel polymorphisms (c.324+373T > G, c.324+418C > T, c.324+570T > C, c.324+682C > T, and c.324+780G > A). They are located within the deleted segment of the c.372_815del443ins54 variant, in which the genotypes of some subjects could not be identified.

Table 3. The Associations and Odds Ratios of *ARMS2/HTRA1* Polymorphisms with Age and Sex Stratification

db SNP ID	Nucleotide Change	AMD vs. Control			PCV vs. Control		
		Adjusted *P*	OR	95% CI	Adjusted *P*	OR	95% CI
rs2736911	c.112C > T	0.048	0.64	(0.41–1.00)	0.001	0.44	(0.27–0.72)
rs10490924	c.205G > T	6.79×10^{-16}	4.03	(2.87–5.65)	1.22×10^{-7}	2.48	(1.77–3.46)
rs61544945	IVS1+63_IVS1+64insTG	5.19×10^{-16}	4.01	(2.87–5.61)	1.42×10^{-7}	2.46	(1.76–3.43)
rs36212731	IVS1+436G > T	4.79×10^{-16}	4.04	(2.88–5.65)	1.45×10^{-7}	2.46	(1.76–3.43)
rs36212732	IVS1+658A > G	1.21×10^{-15}	3.96	(2.83–5.55)	1.45×10^{-7}	2.46	(1.76–3.43)
rs36212733	IVS1+671T > C	1.21×10^{-15}	3.96	(2.83–5.55)	1.45×10^{-7}	2.46	(1.76–3.43)
rs3750848	IVS1+775T > G	1.21×10^{-15}	3.96	(2.83–5.55)	1.45×10^{-7}	2.46	(1.76–3.43)
rs3750847	IVS1+881C > T	1.30×10^{-15}	3.95	(2.82–5.53)	2.29×10^{-7}	2.41	(1.73–3.35)
rs3750846	IVS1-858T > C	3.64×10^{-15}	3.81	(2.73–5.32)	7.47×10^{-7}	2.30	(1.65–3.19)
rs2736912	IVS1-829C > T	0.119	0.71	(0.45–1.09)	0.003	0.47	(0.29–0.77)
rs10664316	IVS1-37_IVS1-38insAT	1.33×10^{-12}	5.03	(3.22–7.87)	0.001	1.81	(1.27–2.58)
Reported	c.372_815del443ins54	7.29×10^{-15}	3.70	(2.66–5.15)	3.10×10^{-6}	2.15	(1.56–2.96)
Reported	c.324+838C > T	0.059	0.64	(0.41–1.02)	0.001	0.41	(0.25–0.69)
rs2014307	c.324+1183T > G	4.52×10^{-12}	4.46	(2.92–6.80)	0.002	1.76	(1.24–2.51)
rs11200638	−625G > A	4.36×10^{-15}	3.69	(2.66–5.11)	1.31×10^{-6}	2.21	(1.60–3.05)

The adjusted *P* value, odds ratio (OR), and 95% confident intervals (CI) were calculated by logistic regression with age and sex stratification.

under dominant model (data not shown). Similar results were observed when allelic frequencies were analyzed (data not shown). Three SNPs, c.112C > T, IVS1-829C > T, and c.324+838C > T, showed significant associations with PCV in the dominant model ($P < 0.0011$ and OR < 0.45), but not with AMD. After stratification by age and sex, the associations among AMD, PCV, and control subjects remained significant (Table 3).

The statistical power of the SNP *ARMS2* rs10490924 in this study was > 99% (with $p_{corr} = 0.017$) in different comparison (AMD versus controls, PCV versus controls, and AMD versus PCV), which is sufficient for detecting the significant associations. The formula for minimum sample size was $n = (Z_\alpha + Z_\beta)^2 \times [\pi_1 (1 - \pi_1) + \pi_2 (1 - \pi_2)]/\delta$ (n was referred to the minimum sample size required, Z_α to the Z value when power reached 0.05, Z_β to the Z value corresponding to p_{corr} in this study). The estimated number of minimum sample size for detecting significant difference between AMD and PCV through *ARMS2* rs10490924 was <27 for AMD patient and <55 for PCV patients ($n_{AMD} = 26.8$ and $n_{PCV} = 54.3$ [with $p_{corr} = 0.0001$], respectively).

Haplotype analysis revealed an extensive LD across all the 16 common polymorphisms, except c.324+965T > C, in AMD (Fig. 1A) and 2 conjoint LD blocks in PCV (Fig. 1B). Since c.324+965T > C was not associated with exudative AMD or PCV, it was excluded from further haplotype-based association analysis. Furthermore, 8 polymorphisms, rs10490924 (c.205G > T), rs61544945 (IVS1+63_IVS1+64insTG), rs36212731 (IVS1+436G > T), rs36212732 (IVS1+658A > G), rs36212733 (IVS1+671T > C), rs3750848 (IVS1+775T > G), rs3750847 (IVS1+881C > T), rs3750846 (IVS1-858T > C), and the indel, were in complete LD (D' = 1). Therefore, only rs10490924 and the indel were used as proxies, leaving a total of eight polymorphisms for haplotype analysis. Among the haplotypes defined by a non-risk SNP rs2736911 and 5 risk SNPs (rs10490924, the indel, rs10664316, rs2014307, and rs11200638), a risk haplotype CT22GA and a non-risk haplotype CG11TG were significantly associated with both exudative AMD ($P = 1.40\times10^{-22}$; OR = 4.47, 95% CI: 3.27–6.11 and $P = 7.96\times10^{-16}$; OR = 0.20, 95% CI: 0.13–0.30, respectively) and PCV ($P = 3.36\times10^{-9}$, OR = 2.38, 95% CI: 1.79–3.17 and $P = 9.00\times10^{-4}$, OR = 0.52, 95% CI: 0.38–0.71, respectively; Table 3). Moreover, haplotype analysis of

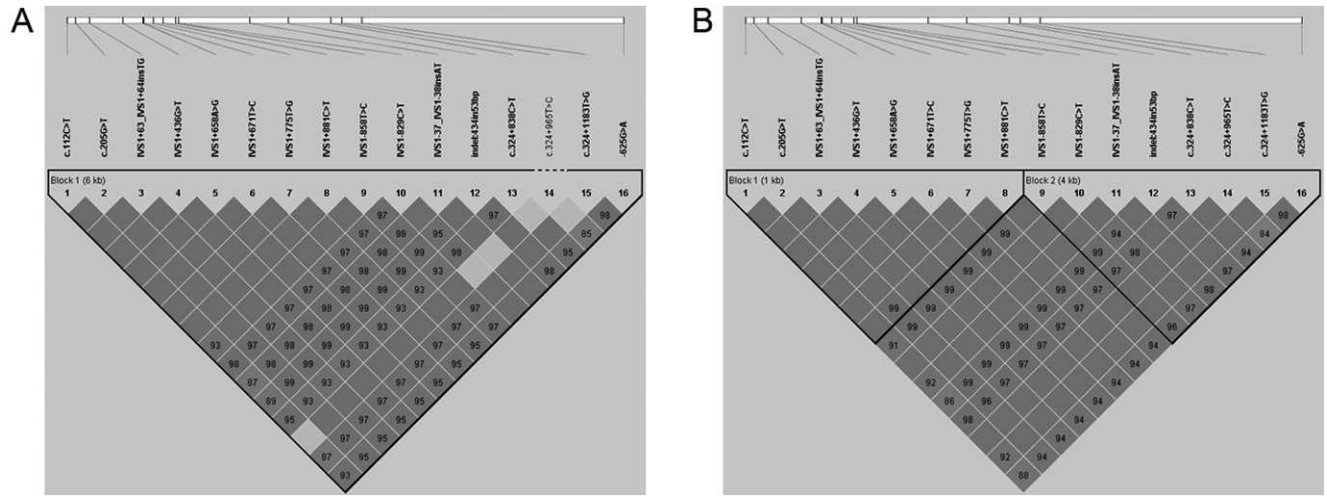

Figure 1. Haplotype block structure for the 16 common polymorphisms. The haplotype analysis revealed (**A**) a linkage disequilibrium (LD) block lying across all of the 16 common polymorphisms except for the c.324+965T > C in AMD and (**B**) 2 conjoint LD blocks in PCV group.

TABLE 4. Haplotype Analysis of *ARMS2* and *HTRA1* Polymorphisms in Exudative AMD and PCV

No.	SNPs Included	Haplotype†	Frequency			AMD - Control		PCV - Control		AMD - PCV	
			AMD	PCV	Control	*P**	OR (95% CI)	*P**	OR (95% CI)	*P**	OR (95% CI)
1	rs2736911.rs10490924, rs10664316, the indel, rs2014307, rs11200638	CT22GA	0.735	0.594	0.384	1.40×10^{-22}	4.47 (3.27–6.11)	3.36×10^{-9}	2.38 (1.79–3.17)	3.00×10^{-4}	1.86 (1.33–2.61)
		CG11TG	0.100	0.228	0.354	7.96×10^{-16}	0.20 (0.13–0.30)	9.00×10^{-4}	0.52 (0.38–0.71)	2.42×10^{-5}	0.38 (0.24–0.60)
		TG21GG	0.113	0.088	0.157	0.439	–	0.031	0.53 (0.34–0.83)	0.91	–
2	rs2736911, rs2736912, c.324+838C > T	CCC	0.867	0.907	0.827	0.14	–	0.0017	2.06 (1.32–3.20)	0.44	–
		TTT	0.114	0.083	0.155	0.26	–	0.0036	0.50 (0.31–0.79)	0.45	–
3	rs10490924, rs11200638	TA	0.740	0.594	0.384	7.47×10^{-23}	4.51 (3.30–6.17)	3.41×10^{-9}	2.38 (1.79–3.17)	2.00×10^{-4}	1.89 (1.35–2.65)
		GG	0.240	0.378	0.588	4.48×10^{-22}	0.22 (0.16–0.31)	3.56×10^{-9}	0.42 (0.31–0.56)	4.00×10^{-4}	0.54 (0.38–0.76)
4	rs2736911, rs10490924	CT	0.748	0.614	0.397	5.42×10^{-22}	4.51 (3.29–6.19)	1.20×10^{-9}	2.42 (1.81–3.22)	3.00×10^{-4}	1.87 (1.33–2.63)
		CG	0.136	0.293	0.431	3.69×10^{-18}	0.21 (0.14–0.30)	6.51×10^{-5}	0.55 (0.41–0.74)	1.81×10^{-6}	0.38 (0.25–0.57)
		TG	0.116	0.093	0.171	0.09	–	0.006	0.49 (0.32–0.77)	0.79	–

† For the polymorphisms that were not a single nucleotide change, the wide-type was denoted as 1 and the variant denoted as 2.
* The *P* value of haplotypes was corrected by permutation tests (number of permutations = 10,000).

rs10490924 and rs11200638 revealed that two haplotypes TA and GG were significantly associated with both exudative AMD ($P = 7.47\times10^{-23}$, OR = 4.51, 95% CI: 3.30–6.17 and $P = 4.48\times10^{-22}$, OR = 0.22, 95% CI: 0.16–0.31, respectively) and PCV ($P = 3.41\times10^{-9}$, OR = 2.38, 95% CI: 1.79–3.17 and $P = 3.56\times10^{-9}$, OR = 0.42, 95% CI: 0.31–0.56, respectively). Significant differences in haplotype frequencies between exudative AMD and PCV were also observed ($P = 2.00\times10^{-4}$, OR = 1.89, 95% CI: 1.35–2.65 and $P = 4.00\times10^{-4}$, OR = 0.54, 95% CI: 0.38–0.76, respectively).

The haplotypes CCC and TTT defined by 3 non-risk polymorphisms (rs2736911, rs2736912, and c.324+838C > T) were significantly associated with PCV ($P = 0.0017$, OR = 2.06, 95% CI: 1.32–3.20 and $P = 0.0036$, OR = 0.50, 95% CI: 0.31–0.79, respectively), but not with exudative AMD (Table 4). Meanwhile, different association was also found in a haplotype defined by rs2736911 and rs10490924, which the non-risk haplotype TG was significantly associated only with PCV ($P = 0.006$; OR = 0.49, 95% CI: 0.32–0.77), but not with exudative AMD ($P = 0.09$).

We included the eight polymorphisms used for haplotype analysis in logistic regression analysis. SNPs *ARMS2* rs10490924 and *HTRA1* rs11200638 were chosen for comparison since they showed the strongest associations. In exudative AMD, rs10490924 remained statistically significant ($P = 0.011$) after adjusting for other SNPs, including rs11200638 (Table 5). However, rs11200638 became statistically insignificant ($P = 0.077$) when adjusting for rs10490924. In PCV, neither rs10490924 nor rs11200638 remained significant when adjusting for each other ($P > 0.07$). In addition, rs10490924, but not rs11200638, remained significant in both exudative AMD ($P = 0.01$) and PCV ($P = 0.009$) when adjusting for the indel variant.

To further examine the effect of *ARMS2/HTRA1* locus in PCV, meta-analysis was performed. A total of 10 publications from 3 different populations were included in the analysis.[23–32] Allelic associations of *ARMS2* rs10490924 and *HTRA1* rs11200638 with PCV were consistent in different populations ($P > 0.05$ in the test of heterogeneity; Fig. 2). The ORs of rs10490924 were similar in Chinese (OR = 2.28, 95% CI: 1.79–2.90), Japanese (OR = 2.14, 95% CI: 1.95–2.34), and Caucasian (OR = 1.85, 95% CI: 1.19–2.88) participants. The ORs of rs11200638 were also similar in Chinese (OR = 2.30, 95% CI: 1.80–2.93) and Japanese (OR = 2.37, 95% CI: 1.89–2.99) subjects. At present, the association of rs11200638 with PCV has not been reported in Caucasian populations.

DISCUSSION

Exudative AMD and PCV are important macular disorders sharing similar phenotypes and serious clinical complications, including hemorrhagic RPE detachment and vitreous hemorrhage,[35] both of which have been used to classify PCV as a subtype of exudative AMD.[41] However, there are discernible differences in their natural courses,[34] responses to treatments and overall visual prognosis,[37] indicating that PCV could be a type of macular disease that is different from AMD. Based on the reported AMD-associated genes,[12–17] genetic studies have been initiated to investigate the molecular mechanisms underlying the two diseases. Results of genotype analysis have indicated that exudative AMD and PCV may share common genetic backgrounds.[26–28] Recently, associations of the *ARMS2* rs10490924 and *HTRA1* rs11200638 with both exudative AMD and PCV have been reported in a Japanese study.[28] However, neither allelic nor genotypic frequencies showed significant differences.[28] Similar associations of rs10490924 with advanced AMD and PCV have also been reported in Caucasians.[24]

Table 5. Logistic Regression Analysis of SNPs in *ARMS2* and *HTRA1* between AMD and PCV

			P* Value of rs10490924 and rs11200638 after Adjusting for the following SNPs			
			AMD		**PCV**	
db SNP ID	**Sequence Change**	**Codon Change**	***ARMS2* rs10490924**	***HTRA1* rs11200638**	***ARMS2* rs10490924**	***HTRA1* rs11200638**
rs11200638	−625G > A	–	0.011	–	0.077	–
rs10490924	c.205G > T	A69S	–	0.74	–	0.74
rs2736911	c.112C > T	R38X	6.39×10^{-16}	1.21×10^{-14}	5.47×10^{-7}	2.47×10^{-6}
rs2736912	IVS1-829C > T	–	9.94×10^{-17}	4.99×10^{-16}	2.68×10^{-8}	2.49×10^{-6}
rs10664316	IVS1-37_IVS1-38insAT	–	1.25×10^{-6}	7.14×10^{-6}	2.03×10^{-5}	8.27×10^{-5}
Reported	c.372_815del443ins54	–	0.010	0.140	0.009	0.084
Reported	c.324+838C > T	–	3.02×10^{-16}	1.48×10^{-15}	3.76×10^{-7}	3.92×10^{-6}
rs2014307	c.324+1183T > G	–	7.11×10^{-7}	3.37×10^{-6}	9.45×10^{-6}	4.12×10^{-5}

* $P < 0.05$ was considered to be significant.

In a recent Caucasian study, rs10490924 posed a higher OR in its association with AMD (OR = 2.66) comparing with PCV (OR = 1.63).[23] In a Japanese cohort, a stronger association of rs10490924 with PCV ($P < 0.0001$) was found.[27] In comparison, a much stronger significant associations was detected in our Chinese cohort ($P = 8.25\times10^{-7}$, OR = 3.15, 95% CI: 1.98–5.03), while the effect on AMD ($P = 1.01\times10^{-19}$, OR = 7.91, 95% CI: 4.93–12.67) was also stronger than PCV (Table 2). Similar trends were obtained in rs11200638 when our results in Chinese were compared with the Japanese.[27]

In this study, despite lack of masked graders, we investigated the genetic profiles of exudative AMD and PCV through analysis of the *ARMS2/HTRA1* locus. A total of 12 polymorphisms in *ARMS2* and *HTRA1* were found to be associated with both diseases (Table 2). Their genotype frequencies were all significantly different between exudative AMD and PCV ($P < 0.001$). These results indicate resembling genetic effects in the *ARMS2/HTRA1* locus between the two diseases, but the size of the effects were different. Therefore, other genetic variations might also determine the development of exudative AMD and PCV. It is noted that, while the *P* values and ORs between the individual SNPs with AMD and with PCV may differ, the trend of associations remained the same (Tables 2, 4, 5). Therefore, the results showed that AMD and PCV are subject to the same genetic influence as far as *ARMS2* and *HTRA1* SNPs are concerned.

ARMS2 and *HTRA1* are the AMD candidate genes on 10q26.[40] In this study, strong associations of these two genes with exudative AMD and PCV were found, and this was further confirmed by the haplotype analysis. Strong LD across all these associated SNPs was observed in both exudative AMD and PCV. Logistic regression analysis identified the strongest associated SNP within the LD block. The *ARMS2* rs10490924 ($P = 0.011$), but not *HTRA1* rs11200638 ($P = 0.077$), showed a significant association with exudative AMD when adjusting for each other. In contrast, *HTRA1* rs11200638 was not significantly associated with either AMD ($P = 0.74$) or PCV ($P = 0.74$) after adjusting for *ARMS2* rs10490924. Thus, rs10490924 represented the strongest associated maker for AMD but not for PCV.

A non-synonymous variant in *ARMS2* (rs2736911) located in a non-risk haplotype (defined by rs2736911, rs10490924, the indel and rs11200638) was associated with AMD in Han Chinese and Caucasian cohorts.[42] Although the association could not be replicated in our exudative AMD cohort ($P = 0.20$), significant association with PCV was found ($P = 0.011$, OR = 0.51, and 95% CI: 0.33–0.80; data not shown). Meanwhile, rs2736911 together with other two SNPs, rs2736912 and c.324+838C > T, showed differential associa-

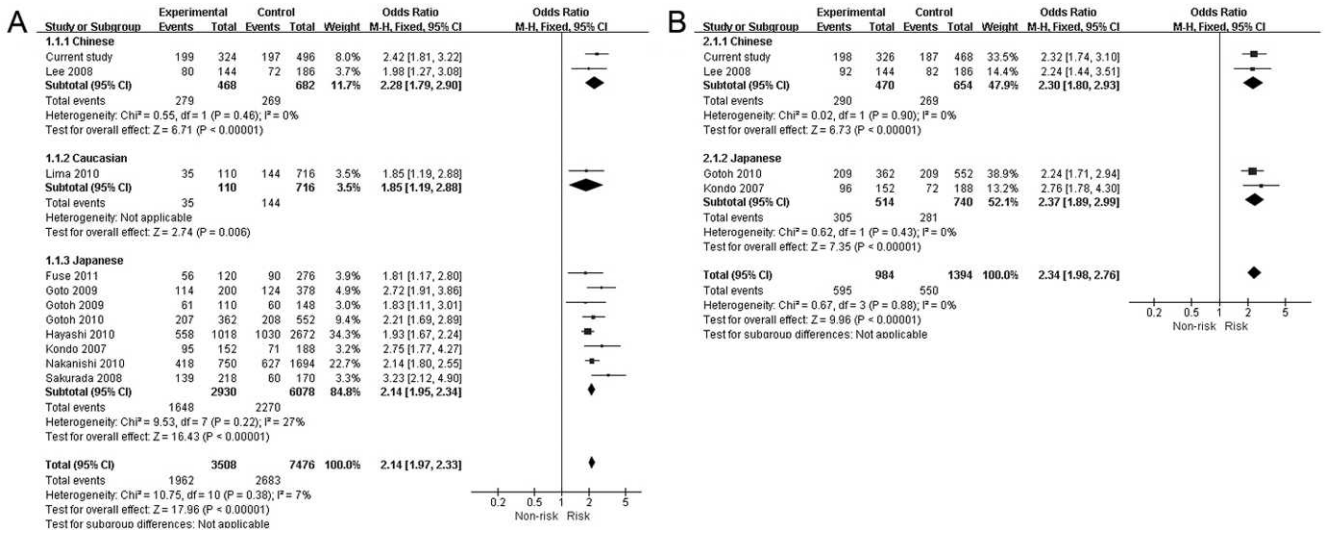

Figure 2. Meta-analysis of rs104090924 and rs11200638 in PCV association studies. The allelic association of (**A**) rs10490924 and (**B**) rs11200638 with PCV was consistent in different populations. The odds ratios (OR) for risk allele carriers were approximately 2-fold higher than non-risk allele carriers. *Squares* indicate study-specific OR; the size of box is proportional to the weight of the study; *horizontal lines* indicate 95% confidence interval (CI); *diamond* indicates summary OR with its corresponding 95% CI.

tions in exudative AMD and PCV. These results indicate different genetic influences between the two diseases and variations in different study cohorts or ethnic groups.

The sex distribution of PCV patients in our study was approximately 2:1 (male/female, 111:49), whereas for exudative AMD patients and control subjects the distributions were approximately 1:1 (Table 1). This was compatible to one of the previous studies.[23] Other reported studies have AMD patients matching with PCV patients in sex (approximately 3:1), but not with the control subjects (approximately 1:1 or 2:1).[24,27] In this study, the difference in sex distribution among groups did not affect the associations. Even after stratification by age and sex, the associations among AMD, PCV and control subjects remained significant (Table 3).

In summary, results of this *ARMS2* sequence analysis in our Chinese study subjects indicated a strong and persistent association of the *ARMS2/HTRA1* locus with both exudative AMD and PCV, suggesting similar genetic effects of the *ARMS2/HTRA1* locus on these two disorders. However, different effect sizes may implicate the existence of additional genetic and environmental factors affecting them to different extents.

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