Association of *ABCG1* With Neovascular Age-Related Macular Degeneration and Polypoidal Choroidal Vasculopathy in Chinese and Japanese

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Purpose. We investigated the association of the *ATP-binding cassette, subfamily G, member 1* (*ABCG1*) gene with polypoidal choroidal vasculopathy (PCV) and neovascular age-related macular degeneration (nAMD) in independent Chinese and Japanese cohorts.

Methods. A total of 12 haplotype-tagging single-nucleotide polymorphisms (SNPs) and the SNP rs57137919 in the *ABCG1* gene were first analyzed in a Hong Kong Chinese cohort of 235 nAMD, 236 PCV, and 365 controls, using TaqMan genotyping assays. Two SNPs (rs57137919 and rs225396) that showed a disease-association were genotyped in a Shantou Chinese cohort of 189 nAMD, 187 PCV, and 670 controls, and an Osaka Japanese cohort of 192 nAMD, 204 PCV, and 157 controls, totaling 2435 subjects. Association analysis was performed in individual cohorts, followed by a pooled analysis of the data from all three cohorts.

Results. In the Hong Kong cohort, SNP rs57137919 was associated with PCV (odds ratio [OR] = 1.35). A tagging SNP rs225396 was associated with nAMD (OR = 1.28) and PCV (OR = 1.32). In the Osaka cohort, SNP rs225396 was associated with nAMD (OR = 1.42) and PCV (OR = 1.74). In the pooled analysis involving the 3 study cohorts, rs225396 showed an enhanced association with nAMD ($P = 0.01$, OR = 1.21, $I^2 = 14\%$) and PCV ($P = 0.0001$, OR = 1.35, $I^2 = 46\%$).

Conclusions. In this study, we have newly identified a haplotype-tagging SNP, rs225396, in *ABCG1* to be associated with PCV and nAMD in Chinese and Japanese cohorts. This provides new evidence to support *ABCG1* as a susceptibility gene for PCV and nAMD. Further replication in other populations should be warranted.

Keywords: *ABCG1*, gene, association, age-related macular degeneration, polypoidal choroidal vasculopathy

Age-related macular degeneration (AMD) is a leading cause of irreversible blindness in the elderly.[1] Neovascular AMD (nAMD), characterized by choroidal neovascularization (CNV), can cause severe vision loss. Polypoidal choroidal vasculopathy (PCV) is a maculopathy with inner choroidal vascular network terminating in polypoidal lesions, which are the best visualized in indocyanine green angiography (ICGA).[2] Polypoidal choroidal vasculopathy was considered a subtype of nAMD due to the similarities in clinical features. However, PCV is more prevalent in Asians than in Caucasians. It has been estimated that 24.5% to 54.7% of Asian patients with nAMD are suffering from PCV, compared to less than 10% in Caucasians.[3] In contrast, the prevalence of nAMD is similar in Caucasians and Asians.[4]

Both AMD and PCV are complex diseases with multiple environmental and genetic risk factors. Genome-wide association studies (GWAS) have identified more than 30 susceptibility genes for AMD, including the *complement factor H* (*CFH*) gene and the *ARMS2-HTRA1* locus.[5–8] Most of the AMD genes also were associated with PCV, though the effect sizes of some (e.g., *ARMS2*) were different between PCV and AMD.[9] A recent whole-exome sequencing study identified a missense variant in *FGD6* to confer increased risk towards PCV but not AMD.[10] These evidences suggest that there are similarities and differences in the genetic components between AMD and PCV.

In a recent study of genes in the high density lipoprotein (HDL) cholesterol metabolism pathway, a single-nucleotide polymorphism (SNP), rs57137919, in the *ATP-binding cassette, sub-family G, member 1* (*ABCG1*) gene showed a borderline association with PCV ($P = 0.03$), but not with nAMD.[11] However, rs57137919 was the only SNP that was selected from *ABCG1*, and there was no replication in other study cohorts.[11] In the present study, we performed a haplotype-tagging SNP association analysis to confirm the association of *ABCG1* with nAMD and PCV in Chinese and Japanese.

Materials and Methods

Study Participants

This study involved a total of 2435 participants from three independent cohorts: a Hong Kong Chinese cohort of 235 nAMD patients, 236 PCV patients, and 365 controls, a Shantou Chinese cohort of 189 nAMD, 187 PCV, and 670 controls, and an Osaka Japanese cohort of 192 nAMD, 204 PCV, and 157 controls. The Hong Kong sample included 128 new participants, while the remainder had been included in the previous study, which involved 200 nAMD patients, 233 PCV patients, and 275 control subjects.[11] All Chinese participants are unrelated Han Chinese, enrolled from the Hong Kong Eye Hospital, the eye clinics of the Prince of Wales Hospital, Hong Kong, and the Joint Shantou International Eye Center, Shantou, China.[12] The Osaka cohort included unrelated Japanese participants recruited from the Department of Ophthalmology, Osaka University Graduate School of Medicine, Osaka, Japan. The study protocol had been approved by the Ethics Committee at respective collaborating institution. Written informed consent was obtained from each participant and the study procedures were performed in accordance with the tenets of the Declaration of Helsinki.

All patients underwent complete ophthalmic investigations, including best-corrected visual acuity (BCVA), ocular tonometry, slit-lamp biomicroscopy, fundus photographs, fluorescein angiography (FFA) and ICGA. Clinical diagnosis and classification of AMD followed the standardized Age-Related Eye Disease Study criteria.[13] All AMD patients recruited in this study had nAMD in at least one eye. Polypoidal choroidal vasculopathy was diagnosed based on choroidal polypoidal lesions as shown by ICGA.[11,14,15] Patients with secondary CNV, such as myopic CNV, or with CNV and PCV in the same or fellow eye, were excluded. Unrelated control subjects were recruited from attendants to the clinics for ophthalmic examinations. Some Osaka control participants were recruited from the staff and volunteers in the hospital. All control subjects underwent complete ophthalmic investigations and were: aged $\geq$60 years, except for some Osaka staff and volunteer individuals with younger age (40 to 60 year-old); without macular degeneration and changes of any cause or pigment abnormalities; and without other major ophthalmic diseases, except for mild senile cataract or mild refractive errors.

SNP Selection and Genotyping

Haplotype-tagging SNPs in the *ABCG1* gene were selected from HapMap Beijing Han Chinese (CHB) population (International HapMap Project, available in the public domain at http://hapmap.ncbi.nlm.nih.gov/, HapMap Genome Browser release #27, accessed April 29, 2014). We selected 12 SNPs using the tagger-pairwise method, with an r^2 cutoff of 0.8. The minor allele frequency (MAF) of all SNPs was >10%. The previously reported SNP rs57137919 in *ABCG1* also was included in this study. Genomic DNA was extracted from peripheral blood using a QIAamp Blood Kit (Qiagen, Hilden, Germany) according to the protocol from the manufacturer. The 13 *ABCG1* SNPs were genotyped in all of the Hong Kong participants by using TaqMan genotyping assays (Applied Biosystems, Foster City, CA, USA) in a Roche LightCycle 480 Real-Time PCR System (Roche Diagnostics, Basel, Switzerland), according to the manufacturer's instructions. Two SNPs (rs57137919 and rs225396) that showed a disease-association in the Hong Kong cohort were genotyped in all participants from the Shantou and Osaka cohorts, using the same genotyping method.

Statistical Analysis

Hardy-Weinberg equilibrium (HWE) of each individual SNP was assessed in the control group in each cohort using the χ^2 test. Hardy-Weinberg equilibrium is the static relationship between genotypic frequency and allelic frequency of a SNP.[16] An SNP deviated from HWE in controls may indicate population substructure or technical issues, such as nonspecificity and genotyping errors.[17] Therefore, the HWE test is important in association studies for examining whether the observed genotypes conform to HWE distribution. Allelic distributions were compared by the χ^2 test between cases and controls to determine disease association, and between nAMD and PCV to assess for interdisease difference. The odds ratio (OR) and 95% confidence intervals (CI) for each SNP were calculated with the major allele as reference. Logistic regression was conducted to evaluate the genetic effects of the *ABCG1* SNPs in the context of age and sex. These analyses were performed in PLINK (v1.07, available in the public domain at http://pngu.mgh.harvard.edu/~purcell/plink/).

We performed a pooled analysis on data from the 3 cohorts to obtain the combined ORs and 95% CIs for the 2 SNPs (rs57137919 and rs225396), using the Mantel-Haenszel χ^2 test under a fixed-effect ($I^2 \leq 50\%$) or random-effect ($I^2 > 50\%$) model based on heterogeneity test results.[18] The pooled analyses were performed using Review Manager (RevMan, version 5.2, The Cochrane Collaboration, Copenhagen, Denmark). In the association analysis among the Hong Kong exploratory cohort, an SNP with *P* value less than 0.05 was considered statistically significant and further analyzed in the replication cohorts. In the pooled-analysis, we adopted a study-wide Bonferroni correction to adjust the *P* values for multiple testing. Thus, a pooled *P* value of less than 0.0042 (~0.05/12, where 12 was the number of SNPs included in exploratory analysis) defined a significant disease association.

Results

Association of *ABCG1* With nAMD and PCV in the Hong Kong Cohort

Table 1 showed the demographics of the study subjects in the 3 independent cohorts. We analyzed the genetic associations with or without age and sex adjustment, and found no significant controversies between the results. In the Hong Kong Chinese cohort, the genotype call rates of all SNPs were 100%. All candidate SNPs but rs1044317 conformed to HWE in the control group. Thus, rs1044317 was removed from further analysis. In single-marker analysis, the *ABCG1* SNP rs57137919 was associated with PCV ($P = 0.022$; OR = 1.35; 95% CI, 1.04–1.74) but not with nAMD ($P = 0.80$; OR = 1.03; 95% CI, 0.79–1.34; Table 2). In contrast, a tagging SNP rs225396 showed an association with nAMD ($P = 0.048$; OR = 1.28; 95% CI, 1.00–1.64) and PCV ($P = 0.026$; OR = 1.32; 95% CI, 1.03–1.69; Table 2). In logistic regression, SNP rs57137919 remained associated with PCV after adjustment for rs225396 ($P = 0.027$; OR = 1.33; 95% CI, 1.03–1.71), while rs225396 remained associated with nAMD ($P = 0.041$; OR = 1.31; 95% CI, 1.01–1.69) and PCV ($P = 0.037$; OR = 1.29; 95% CI, 1.02–1.63) after adjustment for rs57137919, indicating independent effects of the two SNPs. However, the *P* values did not withstand Bonferroni correction ($P > 0.0042$). None of the *ABCG1* SNPs showed a significant difference between nAMD and PCV (Table 2).

Table 1. Characteristics of the Study Subjects

Study Cohort	nAMD	PCV	Control	nAMD vs. Control, *P* Value*	PCV vs. Control, *P* Value*
Hong Kong cohort	$n = 235$	$n = 236$	$n = 365$		
Sex, male/female	129/106	163/73	154/211	<0.05	<0.05
Mean age ± SD, y	75.3 ± 7.6	68.5 ± 9.0	74.4 ± 7.7	0.18	<0.05
Age range, y	50–94	46–90	60–94	NA	NA
Shantou cohort	$n = 189$	$n = 187$	$n = 670$		
Sex, male/female	131/58	134/53	286/384	<0.05	<0.05
Mean age ± SD, y	67.3 ± 10.1	63.1 ± 10.5	73.8 ± 6.8	<0.05	<0.05
Age range, y	50–90	44-81	60–97	NA	NA
Japan cohort	$n = 192$	$n = 204$	$n = 157$		
Sex, male/female	129/63	157/47	52/105	<0.05	<0.05
Mean age ± SD, y	74.3 ± 7.3	72.2 ± 8.0	47.9 ± 15.1	<0.05	<0.05
Age range, y	60–92	60–89	40–83	NA	NA

NA, not applicable.
* Sex proportions were compared by χ^2 test; mean ages were compared by independent *t*-test.

Association of *ABCG1* SNPs With nAMD and PCV in the Shantou and Osaka Cohorts

In replication studies, we genotyped SNPs rs57137919 and rs225396 in the Shantou and Osaka cohorts. These 2 SNPs followed HWE in the controls of these two cohorts. In the Shantou cohort, the ORs of rs225396-T for nAMD (OR = 1.05) and PCV (OR = 1.18) were toward the same trend as the Hong Kong cohort, although the associations were not statistically significant (Table 2).

In the Osaka cohort, SNP rs225396 was significantly associated with PCV ($P = 6.1 \times 10^{-4}$; OR = 1.74; 95% CI, 1.27–2.38) and marginally with nAMD ($P = 0.036$; OR = 1.42; 95% CI, 1.02–1.96; Table 2). In view of the inclusion of young control subjects in the Osaka cohort, we performed a sensitivity analysis to exclude the younger controls aged 40 to 60 years ($n = 65$) and reanalyzed the association using only the older control subjects (aged >60 years, $n = 92$). The association remained significant (PCV: $P = 7.0 \times 10^{-4}$; OR = 1.94; 95% CI, 1.32–2.85; nAMD: $P = 0.022$; OR = 1.58; 95% CI, 1.07–2.34) and notably, the ORs became larger. In addition, there was no significant difference in the allelic ($P = 0.31$) and genotypic ($P = 0.38$) distributions between younger (40–60 years) and older (>60 years) controls.

Association of *ABCG1* SNPs With nAMD and PCV in Pooled Chinese and Japanese

We pooled the association results of the Hong Kong, Shantou, and Osaka cohorts. Single nucleotide polymorphism rs225396 showed an enhanced association with nAMD ($P = 0.01$; OR = 1.21; 95% CI, 1.04–1.41; $I^2 = 14\%$; Fig. 1A) and PCV ($P = 0.0001$; OR = 1.35; 95% CI, 1.16–1.56; $I^2 = 46\%$; Fig. 1B). Also, rs225396 remained associated with PCV ($P = 0.006$; OR = 1.28; 95% CI, 1.07–1.53) and nAMD ($P = 0.02$; OR = 1.22; 95% CI, 1.04–1.44) after adjusted for age and sex (Supplementary Fig. S1). In contrast, SNP rs57137919 was not significantly associated with nAMD or PCV in the pooled subjects (Fig. 2).

Discussion

In this study, we newly identified a haplotype-tagging SNP rs225396 in the *ABCG1* gene to be associated significantly with PCV ($P = 0.0001$) and moderately with nAMD ($P = 0.01$) in pooled Hong Kong, Shantou, and Osaka study subjects. The risk allele T conferred a 1.35-fold of increased risk for PCV and 1.21-fold for nAMD. These findings confirm *ABCG1* as a susceptibility gene for PCV and nAMD in the Chinese and Japanese populations.

The *ABCG1* gene, located on chromosome 21, encodes a transmembrane cholesterol efflux transporter protein ABCG1, which is expressed in many cell types and tissues, including the RPE cells and choroid.[19–22] It has an important role of regulating cellular free cholesterol efflux to HDL in lipid homoeostasis.[23] In the retina, oxidized lipids were transferred to HDL-like lipoprotein particles, which then were internalized and excreted back into the circulation through ABCG1 on the base of the RPE.[24] Defects of ABCG1 may accumulate oxidized lipids in the retina, and the excessive products could initiate inflammation and abnormal angiogenesis, which contribute to the pathogenesis of nAMD and PCV.[25,26] In this study, a tagging SNP rs225396, located in intron 3 of the *ABCG1* gene, conferred a risk effect for PCV and nAMD. To our knowledge, this is the first time that *ABCG1* rs225396 is associated with a human disease. Lipid metabolism pathway has been hypothesized to be involved in the pathogenesis of AMD.[27] The protein ABCG1 is highly expressed in the retina and choroid. Loss of the protein leads to accumulation of oxysterols in the retina in transgenic animal models.[20,21,28] Thus, ABCG1 could have a role in the pathogenesis of AMD and PCV through lipid metabolism. Although located in the intronic region, rs225396 may influence the gene expression pattern by disrupting regulatory elements that control gene splicing, transcription, and/or translation.

Previously, an *ABCG1* SNP, rs57137919, was found to reduce the risk of coronary artery disease (CAD; AG + AA versus GG, OR = 0.73) and associate with the angiographic severity of CAD (AG + AA versus GG, OR = 0.40), probably through interfering with cholesterol homeostasis.[29] Recently, this SNP was associated with an increased macrophage apoptosis, which may be due to the accumulation of oxysterol in macrophages caused by decreased ABCG1-mediated cholesterol efflux.[30] In our recent study, we found that rs57137919 was associated with PCV.[11] The A allele conferred a 1.36-fold of increased risk of PCV, a distinct effect from that of CAD.[29] However, in the present study, the association of rs57137919 with PCV was not significant in the Shantou (OR = 0.86) or Osaka (OR = 1.10) cohort. Association of another *ABCG1* SNP, rs225374, with CAD (OR = 1.19) has been reported in a Chinese cohort.[31] In our present study, rs225374 was not associated with AMD or PCV. A recent GWAS on Alzheimer's disease (AD) showed a significant association between an *ABCG1* marker Chr21:43678066 and neuritic plaques ($P = 8.0 \times 10^{-9}$, OR = 8.27).[32] In another study, an *ABCG1* SNP

Table 2. Allelic Association of SNPs in the *ABCG1* Gene With nAMD and PCV

SNP	Nucleotide Change	Minor Allele	Minor Allele Frequency			Allelic Association					
						nAMD-Control		PCV-Control		nAMD-PCV	
			nAMD	PCV	Control	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)
Hong Kong cohort			$n = 235$	$n = 236$	$n = 365$						
rs57137919*	c.49-6763G>A	A	0.264	0.320	0.259	0.80	1.03 (0.79–1.34)	0.022	1.35 (1.04–1.74)	0.093	0.78 (0.59–1.04)
rs9976024	c.49-4124G>A	A	0.268	0.307	0.256	0.64	1.06 (0.82–1.38)	0.053	1.29 (1.00–1.66)	0.30	0.86 (0.64–1.15)
rs881395	c.292+6386C>T	T	0.296	0.333	0.316	0.45	0.91 (0.71–1.17)	0.56	1.08 (0.84–1.38)	0.22	0.84 (0.63–1.11)
rs225374	c.293-19547G>C	C	0.332	0.371	0.351	0.50	0.92 (0.72–1.18)	0.48	1.09 (0.86–1.39)	0.32	0.87 (0.66–1.14)
rs225378	c.293-15486A>G	G	0.387	0.394	0.353	0.23	1.16 (0.91–1.47)	0.15	1.19 (0.94–1.51)	0.85	0.97 (0.75–1.27)
rs183436	c.293-13349C>A	A	0.434	0.458	0.418	0.58	1.07 (0.85–1.35)	0.17	1.18 (0.93–1.49)	0.63	0.94 (0.72–1.22)
rs225396	c.293-3826C>T	T	0.353	0.360	0.299	0.048	1.28 (1.00–1.64)	0.026	1.32 (1.03–1.69)	0.71	0.95 (0.72–1.25)
rs225398	c.293-2586G>C	C	0.487	0.485	0.464	0.44	1.10 (0.87–1.38)	0.48	1.09 (0.86–1.37)	0.96	0.99 (0.77–1.29)
rs3787997	c.594+2041G>A	A	0.294	0.273	0.266	0.29	1.15 (0.89–1.49)	0.77	1.04 (0.80–1.35)	0.75	1.05 (0.78–1.40)
rs225410	c.595-1477C>T	T	0.366	0.352	0.369	0.93	0.99 (0.78–1.26)	0.55	0.93 (0.73–1.18)	0.74	1.05 (0.80–1.38)
rs2234721	c.1231-64G>T	T	0.268	0.288	0.282	0.59	0.93 (0.72–1.21)	0.82	1.03 (0.80–1.33)	0.74	0.95 (0.71–1.27)
rs9975333	c.1660-135C>A	A	0.466	0.462	0.440	0.37	1.11 (0.88–1.40)	0.45	1.09 (0.87–1.38)	0.74	0.96 (0.74–1.24)
Shantou cohort			$n = 189$	$n = 187$	$n = 670$						
rs57137919*	c.49-6763G>A	A	0.238	0.233	0.260	0.39	0.89 (0.68–1.16)	0.29	0.86 (0.66–1.13)	0.86	1.03 (0.74–1.44)
rs225396	c.293-3826C>T	T	0.347	0.372	0.335	0.67	1.05 (0.83–1.34)	0.18	1.18 (0.93–1.49)	0.47	0.90 (0.67–1.21)
Osaka cohort			$n = 192$	$n = 204$	$n = 157$						
rs57137919*	c.49-6763G>A	A	0.206	0.230	0.213	0.80	0.95 (0.66–1.38)	0.59	1.10 (0.77–1.57)	0.40	0.87 (0.62–1.21)
rs225396	c.293-3826C>T	T	0.352	0.400	0.277	0.036	1.42 (1.02–1.96)	6.1×10^{-4}	1.74 (1.27–2.38)	0.16	0.81 (0.61–1.09)

* Single nucleotide polymorphism that was reported to be associated with PCV in a previous study.[11]

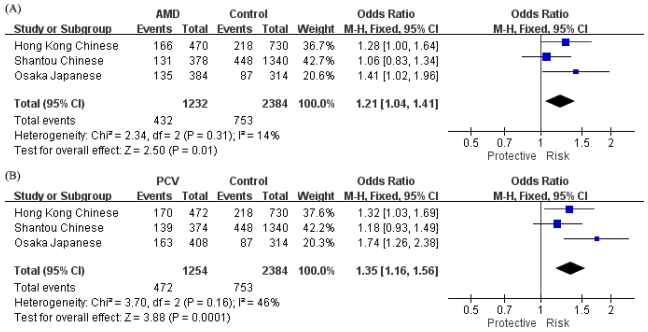

Figure 1. The forest plots of meta-analysis for rs225396. (**A**) nAMD. (**B**) PCV. Individual and pooled ORs were estimated for the A allele frequencies. *Squares* indicate the study-specific OR. The size of the box is proportional to the percent weight that each cohort contributed in the pooled OR. *Horizontal lines* indicate 95% CI. A *diamond* indicates the pooled OR with its corresponding 95% CI.

rs692383, located in intron 2, was associated with AD (OR = 1.82).[33] In our present study, a new tagging SNP rs225396 was associated with nAMD and PCV. Thus, the *ABCG1* gene could be a common genetic factor for AD, CAD, and AMD/PCV, but with allelic heterogeneities.

Results of this study provided new evidence supporting *ABCG1* as a susceptibility gene for nAMD and PCV. In this study, we selected haplotype-tagging SNPs with an r^2 cutoff of 0.8. A r^2 threshold of >0.8 is adequately stringent in selecting tag-SNPs for a candidate gene association study.[34] Thus, analysis of the tag-SNP set can comprehensively interrogate for the main effects of common SNPs that are either directly assayed or exceed the threshold level of linkage disequilibrium ($r^2 > 0.8$) with the assayed SNPs in *ABCG1*. However, whether the SNP rs225396 identified in our study is the causal SNP or is in linkage disequilibrium with another functional SNP remains to be elucidated by gene sequence analysis or biological assays.

There are several limitations in this study. First, the *P* values detected for the "associated SNPs" were moderate in the Hong Kong cohort, which could not withstand correction for multiple testing. Therefore, we conducted replication in 2 independent cohorts and found that the ORs of SNP rs225396 were toward the same direction in nAMD and PCV among the three study cohorts. However, while the *P* values in the Osaka cohort were statistically significant ($P < 0.05$), the *P* values in the Shantou cohort did not achieve statistical significance. Thus, there might be intercohort heterogeneity in the SNP effect sizes. In the pooled analyses of SNP rs225396, enhanced associations were observed for nAMD and PCV, and there were mild to moderate intercohort heterogeneities ($I^2 < 50\%$), indicating that the effects of the *ABCG1* SNP on nAMD and PCV are consistent among the three cohorts. Second, in the Osaka cohort, we included younger control subjects aged 40 to 60 years. Since nAMD is a late-onset disease, the inclusion of young subjects may compromise the power for detecting a SNP with risk effect. Notably, however, when these young control subjects were removed from analysis, the ORs of rs225396 became greater for nAMD and PCV. Moreover, the allelic and genotypic distributions of rs225396 were not significantly different between the younger and older control subjects. Furthermore, the association of rs225396 remained a similar level of significance with nAMD and PCV after adjusting for age and sex (Supplementary Fig. S1). These data altogether suggested that the inclusion of younger controls did not compromise the association results. In addition, the ORs of rs225396 were consistently higher, though not reaching statistical significance, for PCV (OR = 1.32, 1.18, and 1.74 in the Hong Kong, Shantou, and Osaka cohorts, respectively) than

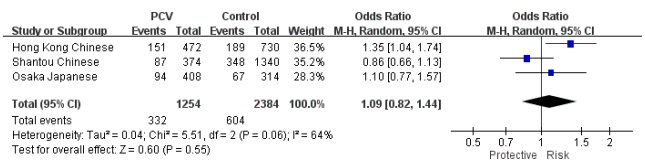

Figure 2. The forest plots of meta-analysis for rs57137919(A) with PCV. *Squares* indicate the study-specific OR. The size of the box is proportional to the percent weight that each cohort contributed in the pooled OR. *Horizontal lines* indicate 95% CI. A *diamond* indicates the pooled OR with its corresponding 95% CI.

for nAMD (OR = 1.28, 1.05, and 1.42 in respective study cohorts), suggesting that *ABCG1* may confer a stronger effect for PCV than for nAMD, which awaits confirmation in larger cohorts.

In summary, we have newly identified a haplotype-tagging SNP rs225396 in the *ABCG1* gene to be associated with PCV and nAMD in Chinese and Japanese subjects. Our results supported *ABCG1* as a new susceptibility gene for PCV and nAMD. Further replication studies in other ethnic populations are warranted to confirm the role of *ABCG1* in nAMD and PCV.

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