Identification of *PGF* as a New Gene for Neovascular Age-Related Macular Degeneration in a Chinese Population

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Purpose. To determine the associations of the *VEGFA*, *VEGFB*, and placental growth factor (*PGF*) genes with neovascular age-related macular degeneration (nAMD) and polypoidal choroidal vasculopathy (PCV).

Methods. Seven single-nucleotide polymorphisms (SNPs) in *VEGFA*, three SNPs in *VEGFB*, and five SNPs in *PGF* were genotyped in 1722 unrelated Chinese participants, including a Hong Kong cohort of 214 nAMD patients, 236 PCV patients, and 365 controls, and an independent Shantou cohort of 189 nAMD patients, 187 PCV patients, and 531 controls, using TaqMan genotyping assays.

Results. Placental growth factor SNPs rs2268615 (G allele, $P = 0.0047$; odds ratio [OR] = 1.54, 95% confidence interval [CI], 1.14–2.08) and rs2268614 (G allele, $P = 0.015$; OR = 1.46, 95% CI, 1.07–1.97) were associated with nAMD. A significant omnibus haplotype association with nAMD was detected for a two-SNP window containing rs2268615 and rs2268614, with a haplotype G-G conferring a 1.54-fold increased risk ($P = 0.0042$) in the Hong Kong cohort and a 1.42-fold risk ($P = 0.012$) in the Shantou cohort. Pooling of the Hong Kong and Shantou data enhanced the association of nAMD with rs2268615 ($P = 0.0022$; OR = 1.38, 95% CI, 1.12–1.69; $I^2 = 0\%$), rs2268614 ($P = 0.0067$; OR = 1.33, 95% CI, 1.08–1.63; $I^2 = 0\%$), and the G-G haplotype ($P = 0.0013$; OR = 1.46, 95% CI, 1.16–1.84; $I^2 = 0\%$). In contrast, the *PGF* SNPs and haplotype were not associated with PCV. Our results also revealed no association of SNPs in *VEGFA* and *VEGFB* with nAMD or PCV.

Conclusion. Placental growth factor is a susceptibility gene for nAMD in a Chinese population, providing new evidence to support a biological role of PGF in choroidal neovascularization.

Keywords: PGF, AMD, genetic association

Age-related macular degeneration (AMD) is a leading cause of irreversible central visual impairment among elderly individuals in developed countries. Age-related macular degeneration is characterized by degenerative features at the macula affecting the photoreceptors and retinal pigment epithelium (RPE), often presenting with macular drusen in the early stage. Advanced AMD can be classified into geographic atrophy (dry AMD) and neovascular AMD (nAMD; or wet AMD). Prevalence of advanced AMD has been estimated to be 0.59% in Caucasian populations and 0.56% in Asian populations aged 40 to 79 years.[1] Neovascular AMD accounts for the majority of severe visual loss in Asian AMD patients. Retinal pigment epithelium detachment, subretinal fluid, hemorrhage, and fibrotic scar are common features in nAMD.[2]

Polypoidal choroidal vasculopathy (PCV) is another serious macular disease usually presented with subretinal polyp-like lesions, RPE detachment, retinal edema, and subretinal hemorrhage. In PCV, the inner choroidal vascular networks terminated in polypoidal lesions, which are different from the generalized choroidal neovascularization (CNV) in nAMD and best detected by indocyanine green angiography (ICGA).[3] Polypoidal choroidal vasculopathy is reportedly more prevalent among Asians and African-Americans compared with Caucasians.[4] It might account for approximately 20% to 50% of nAMD in Asian populations while less than 10% in Caucasians.[5] In Chinese, the prevalence of PCV was approximately 0.5% in people aged 40 years and older and over 1.3% in those aged 65 years and older.[6] It remains debatable whether PCV is a subtype of nAMD or a distinct clinical entity. Generally, PCV was considered a variant of nAMD due to the similarities in phenotypic features; however, they are dramatically different in natural history, prevalence, and treatment response. These discrepancies lead to believe that nAMD and PCV are two separate entities.[7]

Both AMD and PCV are multifactorial in etiology, involving multiple environmental and genetic risk factors. A recent genome-wide association study (GWAS) involving more than 77,000 study subjects showed that polymorphisms in 19 genetic loci, which included complement factor H (*CFH*), age-related maculopathy susceptibility 2 (*ARMS2*), and *VEGFA*, accounted for 15% to 65% of the total genetic contribution to AMD.[8] No GWAS of large scale has been conducted in PCV, but there are reported associations of PCV with AMD-associated genes such as *CFH*, *ARMS2-HTRA1*, complement component 2

(*C2*), and cholesteryl ester transfer protein (*CETP*).[9,10] In a recent systematic review and meta-analysis, the majority of genes that were associated with PCV were also associated with AMD. One clear exception was the *ARMS2-HTRA1* polymorphisms, which were significantly different between nAMD and PCV,[7] suggesting that the genetic components of AMD and PCV are partially different. Therefore, genetic studies involving both AMD and PCV will help to identify gene variants of similar or differential effects between the two.

Differences between nAMD and PCV also present in their treatment responses to anti-VEGF therapies. Most patients with nAMD respond well to anti-VEGF monotherapy, while PCV patients usually require combined anti-VEGF and photodynamic therapy.[5] In 2012, aflibercept, a fusion protein that blocks VEGFA, VEGFB, and placental growth factor (PGF), was approved for treating macular degeneration.[11] Intravitreal injection of aflibercept achieved equivalent effect in improving the best-corrected visual acuity (BCVA) and preventing BCVA loss in nAMD while required fewer injections when compared with ranibizumab.[12] Aflibercept is also effective for treatment-resistant nAMD.[13]

The PGF, a target of anti-VEGF therapy, plays an important role in angiogenesis.[14] We therefore hypothesize that *PGF* could be a susceptibility gene for nAMD and/or PCV. In this study, we determine the associations of the *PGF*, *VEGFA*, and *VEGFB* genes with nAMD and PCV separately, using haplotype-tagging single-nucleotide polymorphisms (SNPs) and reported functional SNPs. Our results revealed *PGF* as a novel putative gene for nAMD.

Materials and Methods

Study Participants in the Hong Kong Cohort

This study involved a total of 1722 unrelated Chinese participants recruited separately from Hong Kong and Shantou, two different cities in southern China. The Hong Kong cohort included 815 participants: 214 patients with nAMD, 236 with PCV, and 365 healthy controls (Supplementary Table S1), recruited from the eye clinics of the Prince of Wales Hospital and the Hong Kong Eye Hospital, Hong Kong. All patients received complete ophthalmic investigations, including BCVA measurement, applanation tonometry, slit-lamp biomicroscopy, fundus photographs, fluorescein fundus angiography (FFA), and ICGA. All AMD patients recruited in this study had nAMD in at least one eye. Polypoidal choroidal vasculopathy was diagnosed upon a choroidal origin of polypoidal lesions as shown by ICGA.[10,15–17] Diagnosis of nAMD and PCV were distinguished by FFA and ICGA. Subjects with any eye having nAMD and PCV lesions concurrently or other causes of CNV were excluded. Control subjects were recruited from people who attended the clinics for an unrelated eye condition and recruited according to the following criteria: (1) age 60 years and older, (2) no identifiable signs of AMD or macular degeneration of any cause, and (3) no any other major eye diseases, except for mild senile cataracts and/or mild refractive errors.

The study protocols were approved by institutional Ethics Committees in the Chinese University of Hong Kong and the Joint Shantou International Eye Centre. Written informed consents were obtained from all subjects after explaining the nature of the study. All study procedures were performed in accordance with the tenets of the Declaration of Helsinki.

Selection of SNPs and Genotyping

Haplotype-tagging SNPs were selected from the *PGF* gene in the HapMap CHB population, using the HapMap Genome Browser release #27 dataset (in the public domain, http://hapmap.ncbi.nlm.nih.gov/). The tagger-pairwise method was used, with an r^2 cut-off of 0.8 and a minor allele frequency (MAF) cut-off of 0.05. Three SNPs were generated, namely rs2359192, rs2268615, and rs2268616. These three SNPs captured all alleles across the *PGF* locus with a MAF greater than 0.05 and a mean r^2 of 0.974. In addition, two *PGF* SNPs, rs11850328 and rs2268614, which had been correlated with the serum level of PGF,[18] were also selected. Thus, a total of five *PGF* SNPs were included. The haplotype-tagging SNPs in the *VEGFB* gene were selected using the same criteria and two SNPs were picked with a mean r^2 of 1.0: rs4930152 and rs11603042. However, rs11603042 failed the assay design so that it was replaced by SNP rs594942 in high linkage disequilibrium (LD; $r^2 = 0.925$). In addition, a common coding variant (rs12366035, p.Asp136Asp) in *VEGFB* was selected.

In this study, we also assessed the association of the *VEGFA* gene with nAMD and PCV. We selected seven candidate SNPs based on previous studies. SNP rs943080 had a significant association with AMD in European populations[8] and a marginal association with AMD in Asians.[19] In a recent meta-analysis two *VEGFA* SNPs, rs1413711 and rs833061, were linked to AMD susceptibility.[20] SNP rs699947 was a strong determinant of the anatomical outcome after photodynamic therapy in AMD.[21] SNP rs833070 was associated with AMD risk and retinal thickness in patients receiving anti-VEGF therapy.[22] SNP rs833069 was associated with the development and progression of AMD.[23] Another SNP rs3025039 was associated with a decreased serum level of VEGF.[24]

Genomic DNA from the whole blood was extracted using a commercial kit (Qiagen QIAamp DNA Blood Mini kit; Qiagen, Hiden, Germany) according to the manufacturer's protocol. All of the 15 candidate SNPs, including five in *PGF*, three in *VEGFB*, and seven in *VEGFA*, were genotyped in all of the Hong Kong participants, using *TaqMan* SNP assays (Applied Biosystems, Foster City, CA, USA) with the Roche LightCycler 480 Real-Time PCR System (Roche, Basel, Switzerland) according to the manufacturer's instructions.

Replication Study

The two *PGF* SNPs (rs2268615 and rs2268614) that showed significant association with nAMD and the *VEGFB* SNP rs594942 that showed association with PCV in the Hong Kong cohort were genotyped in an independent cohort of 907 unrelated participants recruited from the Joint Shantou International Eye Centre (JSIEC), Shantou, China, including 189 patients with nAMD, 187 with PCV, and 531 control subjects. The recruiting criteria were the same with that adopted for the Hong Kong sample.

Statistical Analysis

All SNPs were assessed for Hardy-Weinberg Equilibrium (HWE) using the exact test in PLINK (v1.07; in the public domain, http://pngu.mgh.harvard.edu/purcell/plink/).[25] Five SNPs, including four in *VEGFA* (rs699947, rs833061, rs833070, and rs1413711) and one in *PGF* (rs2268616), were excluded for further analysis because of deviation from HWE in the control group ($P < 0.05$). Allelic and genotypic distributions of each SNP among different study groups were compared by χ^2 test or Fisher's exact test in PLINK. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated with the nonrisk allele as reference. Logistic regression analysis was used to adjust the association for sex. Bonferroni correction was adopted to adjust the P values in multiple testing. A P value of less than 0.005 ($P = 0.05/10$, where 10 was the number of SNPs included in data analysis) was considered statistically significant.[26–28]

Table 1. Allelic Association of SNPs in the *VEGF-A, VEGF-B*, and *PGF* Genes With nAMD and PCV

				Risk Allele Frequency			Allelic Association			
							nAMD-Control		PCV-Control	
SNP	Gene	Nucleotide Change	Risk Allele	nAMD	PCV	Control	*P*	OR (95% CI)	*P*	OR (95% CI)
Hong Kong cohort				$n = 214$	$n = 236$	$n = 365$				
rs943080	*VEGFA*	g.43826627T>C	C	0.224	0.223	0.204	0.42	1.13 (0.84–1.51)	0.43	1.12 (0.84–1.49)
rs833069	*VEGFA*	c.658+450T>C	C	0.379	0.362	0.332	0.11	1.23 (0.96–1.58)	0.27	1.15 (0.90–1.46)
rs3025039	*VEGFA*	c.*237C>T	T	0.179	0.168	0.179	1	1.00 (0.74–1.38)	0.64	0.93 (0.69–1.27)
rs12366035	*VEGFB*	c.408C>T	T	0.094	0.081	0.071	0.16	1.36 (0.88–2.09)	0.55	1.14 (0.74–1.76)
rs4930152	*VEGFB*	c.545+285G>A	A	0.092	0.081	0.077	0.36	1.22 (0.80–1.87)	0.79	1.06 (0.69–1.63)
rs594942	*VEGFB*	c.*443C>T	T	0.229	0.286	0.234	0.87	0.97 (0.74–1.29)	0.038	1.31 (1.02–1.69)
rs11850328	*PGF*	c.-3764C>T	C	0.806	0.803	0.770	0.15	1.24 (0.93–1.67)	0.17	1.22 (0.92–1.62)
rs2268615	*PGF*	c.119-2161G>T	G	0.825	0.784	0.753	0.0047	1.54 (1.14–2.08)	0.22	1.19 (0.90–1.57)
rs2268614	*PGF*	c.315+60G>A	G	0.826	0.799	0.766	0.015	1.46 (1.07–1.97)	0.18	1.24 (0.92–1.61)
rs2359192	*PGF*	c.486-1087G>T	T	0.879	0.905	0.873	0.77	1.05 (0.73–1.52)	0.089	1.39 (0.95–2.02)
Shantou cohort				$n = 189$	$n = 187$	$n = 531$				
rs594942	*VEGFB*	c.*443C>T	T	0.298	0.235	0.272	0.33	1.14 (0.88–1.47)	0.17	0.83 (0.63–1.09)
rs2268615	*PGF*	c.119-2161G>T	G	0.779	0.711	0.739	0.12	1.25 (0.94–1.65)	0.29	0.87 (0.67–1.13)
rs2268614	*PGF*	c.315+60G>A	G	0.782	0.711	0.745	0.15	1.23 (0.93–1.63)	0.21	0.84 (0.65–1.10)

Pairwise LD and haplotype associations were assessed using Haploview[29] and PLINK. The variable-sized sliding-window method was used to capture the optimum markers in haplotype association.[30] Omnibus test for global haplotype association of each window was conducted using PLINK. The window that gave a smallest *P* value in the omnibus tests was considered the optimum window.[31,32] A haplotype-specific association test was then conducted for the haplotypes within that window. The permutation test was used to correct the *P* values for haplotype associations (number of iterations = 10,000).

To evaluate the effects of the *PGF*, *VEGFA*, and *VEGFB* SNPs in the context of other AMD genes and the genetic interaction among them, we obtained the genotypes of two major AMD and PCV-associated SNPs, *CFH* rs800292 and *HTRA1* rs11200638, from our previous studies.[10,15,33] The genetic effects of two significant *PGF* SNPs, rs2268615 and rs2268614, were estimated in the context of both *CFH* rs800292 and *HTRA1* rs11200638 in an additive model by using logistic regression analysis in PLINK. The *CFH* SNP rs1061170 was not involved in the logistic regression analysis since it is rare and not associated with nAMD in Chinese.[34,35] Furthermore, pairwise interaction analysis was conducted to detect epistatic effects among the SNPs in *VEGFA*, *VEGFB*, *PGF*, *CFH*, and *HTRA1* using the epistasis option in PLINK.

To combine the data of the two *PGF* SNPs from the Hong Kong and Shantou cohorts, we performed the Mantel-Haenszel χ^2 to obtain the pooled-OR and 95% CI using a fixed-effect model based on heterogeneity test results ($I^2 \leq 50\%$).[36,37] The test was performed using Review Manager (RevMan, version 5.2; The Cochrane Collaboration, Copenhagen, Denmark).

Results

Characteristics of Study Subjects

Supplementary Table S1 showed the characteristics of the study participants. There were more males in patient groups than in controls. Therefore, sex was adjusted by logistic regression in the association analysis. Because we purposely recruited subjects older than 60 years as controls, the age was not adjusted in the association analysis.

Individual SNP Association

The call rate of each SNP was greater than 99.7%. The G allele of the *PGF* SNP rs2268615 was overrepresented in nAMD, conferring a significant risk effect ($P = 0.0047$; OR = 1.54, 95% CI, 1.14–2.08; Table 1). Another SNP, *PGF* rs2268614, showed a similar trend of an association with nAMD (G allele, $P = 0.015$, OR = 1.46, 95% CI, 1.07–1.97). Logistic regression analysis revealed that these two SNPs, rs2268615 ($P = 0.0093$; OR = 1.50, 95% CI, 1.11–2.03) and rs2268614 ($P = 0.019$; OR = 1.46, 95% CI, 1.06–1.99), remained at similar levels of significance after adjusting for sex. These two *PGF* SNPs were not significantly associated with PCV, although they showed the same trend of effect as that in nAMD (Table 1). In addition, a borderline association was detected between *VEGFB* rs594942 and PCV ($P = 0.038$, OR = 1.31, 95% CI, 1.02–1.69; Table 1), but this association could not withstand multiple correction. The other two SNPs in *PGF*, two in *VEGFB* and three in *VEGFA* were not associated with nAMD or PCV. The five SNPs that were excluded due to deviation from HWE (i.e., *VEGFA* rs699947, rs833061, rs833070, and rs1413711, and *PGF* rs2268616) were not associated nAMD or PCV (data not shown).

To determine whether the effects of the *PGF* SNPs were independent of the two major AMD-associated SNPs, *CFH* rs800292 and *HTRA1* rs11200638, we assessed the associations in logistic regression models. *PGF* rs2268615 and rs2268614, respectively, remained associated with nAMD after conditioning on *CFH* rs800292, *HTRA1* rs11200638 and sex, indicating independent effects of the *PGF* SNPs (Supplementary Tables S2). In epistatic analysis, no significant SNP*SNP interaction was identified in either nAMD or PCV (data not shown).

Haplotype-Based Association Analysis

We performed a sliding-window haplotype association analysis of the *PGF* gene, with the window sizes ranging from two to

Table 2. Haplotype-Based Association of the *PGF* Gene in nAMD

	Haplotype		Frequency, %				
	rs2268615	rs2268614	Case	Control	*P*	OR (95% CI)	Permutation *P*
Hong Kong cohort							
1	T	A	15.8	23.0	0.0029	0.63 (0.41–0.97)	0.014
2	T	G	1.91	1.86	0.94	1.01 (0.30–3.40)	1.00
3	G	G	82.3	75.2	0.0042	1.54 (1.02–2.33)	0.020
Shantou cohort							
1	T	A	21.7	23.4	0.49	0.91 (0.69–1.21)	0.97
2	T	G	0	2.8	N/A	N/A	N/A
3	G	A	0	2.2	N/A	N/A	N/A
4	G	G	78.3	71.7	0.012	1.42 (1.08–1.88)	0.11

four. In nAMD, the most significant omnibus association was identified from a two-SNP window defined by rs2268615 and rs2268614 ($P_{omnibus} = 0.012$ at 2 degrees of freedom). Three haplotypes were detected in this window, of which two showed a significant association (Table 2). A haplotype G-G, presented in 82.3% of nAMD and 75.2% of controls, conferred an increased risk ($P = 0.0042$, permutation $P = 0.02$; OR = 1.54, 95% CI, 1.02–2.33), while the haplotype T-A, detected in 15.8% of nAMD and 23.0% of controls, was protective ($P = 0.0029$, permutation $P = 0.014$; OR = 0.63, 95% CI, 0.41–0.97; Table 2). In contrast, no significant omnibus association was identified for PCV (data not shown).

Replication Study and Pooled Analysis

In the Shantou Chinese samples, the two *PGF* SNPs, rs2268615 ($P = 0.12$, OR = 1.25, 95% CI, 0.94–1.65; Table 1) and rs2268614 (P = 0.15, OR = 1.23, 95% CI, 0.93–1.63; Table 1), did not show a statistically significant association with nAMD, but the ORs were toward the same trends as that in the Hong Kong sample. A significant omnibus association was detected for the haplotypes defined by rs2268615 and rs2268614 ($P_{omnibus} = 1.42 \times 10^{-4}$ at 3 degrees of freedom). The haplotype G-G, defined by the risk alleles, was associated with nAMD ($P = 0.012$, permutation $P = 0.11$; OR = 1.42, 95% CI, 1.08–1.88; Table 2). In contrast, no single SNP (including *VEGFB* rs594942) or haplotype was associated with PCV (Table 1).

By pooling the data of the two *PGF* SNPs and the haplotypes in the Hong Kong and Shantou samples, SNP rs2268615 ($P = 0.0022$; OR = 1.38, 95% CI, 1.12–1.69; $I^2 = 0\%$) and the haplotype G-G ($P = 0.0013$; OR = 1.46, 95% CI, 1.16–1.84; $I^2 = 0\%$) showed an enhanced, significant association with nAMD, while rs2268614 ($P = 0.0067$; OR = 1.33, 95% CI, 1.08–1.63; $I^2 = 0\%$) showed a similar trend towards an association (Fig.). In contrast, the two *PGF* SNPs and the haplotype G-G were not associated with PCV (Supplementary Fig. S1).

Discussion

In this study, we have, for the first time, identified a significant association between a haplotype-tagging SNP rs2268615 in the *PGF* gene and nAMD. Another *PGF* SNP rs2286614 also showed a trend toward an association with nAMD. Logistic regression suggested that the effects of rs2268615 and rs2268614 were independent of *CFH* rs800292 and *HTRA1* rs11200638. In addition, a significant omnibus haplotype association with nAMD was detected for a two-SNP window containing rs2268615 and rs2268614 in both the Hong Kong and Shantou cohorts. Finally, pooling the data of the Hong Kong and Shantou cohorts revealed enhanced associations of nAMD with the two *PGF* SNPs and the haplotype G-G defined by their risk alleles, with low intercohort heterogeneity. In contrast, no SNP or haplotype showed a significant association with PCV. Thus, our data indicates *PGF* as a susceptibility gene for nAMD, but not PCV.

The *PGF* gene, spanning a 13.94-kb region on chromosome 14, encodes the PGF, which is a member of the VEGF protein family and shares homology with VEGFA. Placental growth factor is involved in pathological angiogenesis via two receptors, Fms-related tyrosine kinase 1 and Neuropilin 1.[38] Placental growth factor directly stimulates the growth and migration of endothelial cells, and synergistically amplifies the action of VEGFA.[39] In the retina, PGF is proangiogenic on retinal endothelial cells.[40] It is also expressed in the intact choroid and could be upregulated during the course of laser-induced CNV in mice.[14] Furthermore, CNV could be prevented in mice by knocking out or knocking down PGF, or blocking the PGF receptor with a neutralizing antibody.[14,41]

In a previous study, a SNP rs1042886, located in the 3′-untranslated region of *PGF*, was associated with pre-eclampsia (OR for risk allele A: 1.5, $P = 0.010$).[42] In our present study, the *PGF* SNP rs2359192, also located in the 3′-untranslated region, was not associated with nAMD or PCV. Instead, we identified two *PGF* SNPs, rs2268615 (G; OR, 1.54) in intron 2 and rs2268614 (G; OR, 1.46) in intron 3, conferred increased risks to nAMD. To our knowledge, this is the first time that these two *PGF* SNPs are associated with a human disease. Notably, the AA genotype of *PGF* rs2268614 had been correlated with an elevated level of plasma PGF in a population-based sample of Caucasian origin.[18] The rs2268614 is located at the binding sequence of a transcription GA-binding factor.[18] Therefore, this SNP is likely to implicate in the regulation of PGF expression. In a recent study, a significant upregulation, instead of downregulation, of systemic PGF was detected in AMD patients treated with aflibercept.[43] This finding echoes to our finding that the *PGF* SNP rs2268614, which is reportedly correlated with a higher PGF plasma level, is protective for nAMD. Notably, in mice, knocking out or knocking down of *PGF* was found to prevent CNV formation.[14] Therefore, the roles of systemic PGF levels should be different between human nAMD and animal model of CNV.

We found no significant association of *VEGFA* and *VEGFB* with nAMD and PCV. Churchill et al.[44] first reported a SNP rs1413711 in *VEGFA* to associate with AMD in a Caucasian cohort. Later, some studies had found *VEGFA* as a risk factor for AMD,[23,45,46] whereas some others did not.[47–49] In a GWAS, the *VEGFA* SNP rs943080 was significantly associated with AMD in European populations ($P = 9 \times 10^{-16}$, OR = 0.87 for the minor

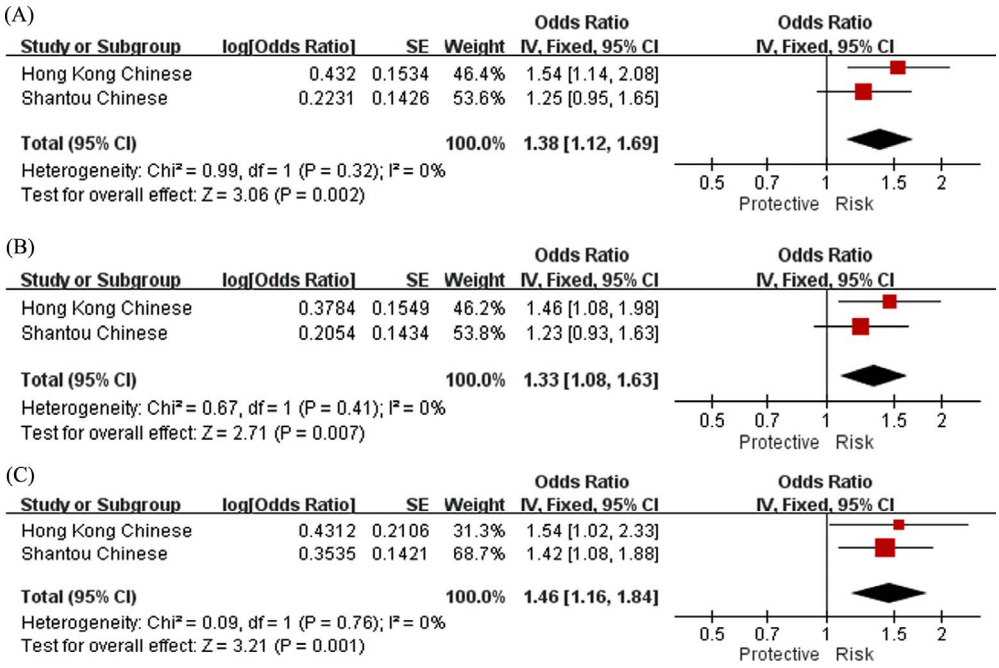

Figure. The forest plots of pooling the samples from Hong Kong and Shantou cohorts compared (**A**) rs2268615(G), (**B**) rs2268614(G), and (**C**) G-G defined by rs2268615 and rs2268614 between nAMD and control. *Squares* indicate the study-specific OR. The size of the *box* is proportional to the percent weight that each study contributed in the pooled OR. Horizontal lines indicate 95% CI. A *diamond* indicates the summary OR with its corresponding 95% CI.

allele C).[8] In contrast, a borderline association of rs943080-C with AMD was reported in Asians ($P = 0.041$, OR = 0.91).[19] In our present study, rs943080-C was not significantly associated with nAMD ($P = 0.42$, OR = 1.13) or PCV ($P = 0.43$, OR = 1.12) in the Hong Kong Chinese cohort (Table 1). We also studied other reported *VEGFA* SNPs of functional impacts. The *VEGFA* SNP rs833069 showed the same trend of effect (OR = 1.23 and 1.15 in nAMD and PCV, respectively) as that in a previous study,[23] but the associations were not statistically significant. This is likely due to the relatively small sample sizes in this study. Nevertheless, our data suggested that *VEGFA* is not a major genetic factor for nAMD and PCV in our study population.

There are several limitations in this study. First, the haplotype defined by rs2268615 and rs2268614 showed a borderline association with nAMD in the Shantou cohort. Also, the individual SNPs did not show a statistically significant association with nAMD. However, the ORs were toward the same trends as that in the Hong Kong sample. Hong Kong and Shantou, two different cities in southeast China about 400-km apart, share some genetic and social similarities. Approximately 1/6 of the Hong Kong residents are from the Chaoshan district, where Shantou is the biggest city (in the public domain, http://www.cafiu.org.cn/english/NewsInfo.asp?NewsId=1578). In our previous studies, we found similarities and disparities in the genetic associations of eye diseases between the Hong Kong and Shantou study populations. A common variant rs4236601 in the *CAV1/CAV2* locus was associated with POAG in both the Hong Kong (OR = 5.01) and Shantou (OR = 5.47) cohorts.[50] In contrast, *ABCC5* rs1401999, a SNP associated with PACG, showed different trends of effect (OR = 1.16 for Hong Kong and OR = 0.85 for Shantou).[51] Therefore, despite the Shantou cohort provided a positive replication in the present study, studies in other cohorts are warranted to verify the role of *PGF* in nAMD. Second, smoking status for a portion of our study subjects was not available; therefore smoking was not adjusted in the analysis.

In conclusion, we have identified *PGF* SNPs rs2268615 and rs2268614 as new susceptibility gene markers for nAMD in Chinese, indicating a biological role of the *PGF* gene in AMD mechanism. Further replication studies are warranted to extend our findings to other ethnic populations and verify the role of *PGF* in nAMD.

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References

1. Kawasaki R, Yasuda M, Song SJ, et al. The prevalence of age-related macular degeneration in Asians: a systematic review and meta-analysis. *Ophthalmology*. 2010;117:921–927.
2. Jager RD, Mieler WF, Miller JW. Age-related macular degeneration. *N Engl J Med*. 2008;358:2606–2617.
3. Spaide RF, Yannuzzi LA, Slakter JS, Sorenson J, Orlach DA. Indocyanine green videoangiography of idiopathic polypoidal choroidal vasculopathy. *Retina*. 1995;15:100–110.
4. Ciardella AP, Donsoff IM, Huang SJ, Costa DL, Yannuzzi LA. Polypoidal choroidal vasculopathy. *Surv Ophthalmol*. 2004;49:25–37.
5. Laude A, Cackett PD, Vithana EN, et al. Polypoidal choroidal vasculopathy and neovascular age-related macular degeneration: same or different disease? *Prog Retin Eye Res*. 2010;29:19–29.

6. Li Y, You QS, Wei WB, et al. Polypoidal choroidal vasculopathy in adult Chinese: the Beijing Eye Study. *Ophthalmology*. 2014; 121:2290–2291.
7. Ma L, Li Z, Liu K, et al. Association of genetic variants with polypoidal choroidal vasculopathy: a systematic review and updated meta-analysis. *Ophthalmology*. 2015;122:1854–1865.
8. Fritsche LG, Chen W, Schu M, et al. Seven new loci associated with age-related macular degeneration. *Nat Genet*. 2013;45: 433–439.
9. Lima LH, Schubert C, Ferrara DC, et al. Three major loci involved in age-related macular degeneration are also associated with polypoidal choroidal vasculopathy. *Ophthalmology*. 2010;117:1567–1570.
10. Liu K, Chen LJ, Lai TY, et al. Genes in the high-density lipoprotein metabolic pathway in age-related macular degeneration and polypoidal choroidal vasculopathy. *Ophthalmology*. 2014;121:911–916.
11. Traynor K. Aflibercept approved for macular degeneration. *Am J Health Syst Pharm*. 2012;69:6.
12. Schmidt-Erfurth U, Kaiser PK, Korobelnik JF, et al. Intravitreal aflibercept injection for neovascular age-related macular degeneration: ninety-six-week results of the VIEW studies. *Ophthalmology*. 2014;121:193–201.
13. Chang AA, Li H, Broadhead GK, et al. Intravitreal aflibercept for treatment-resistant neovascular age-related macular degeneration. *Ophthalmology*. 2014;121:188–192.
14. Rakic JM, Lambert V, Devy L, et al. Placental growth factor, a member of the VEGF family, contributes to the development of choroidal neovascularization. *Invest Ophthalmol Vis Sci*. 2003;44:3186–3193.
15. Liu K, Chen LJ, Tam PO, et al. Associations of the C2-CFB-RDBP-SKIV2L locus with age-related macular degeneration and polypoidal choroidal vasculopathy. *Ophthalmology*. 2013;120: 837–843.
16. Gomi F, Ohji M, Sayanagi K, et al. One-year outcomes of photodynamic therapy in age-related macular degeneration and polypoidal choroidal vasculopathy in Japanese patients. *Ophthalmology*. 2008;115:141–146.
17. Cackett P, Wong D, Yeo I. A classification system for polypoidal choroidal vasculopathy. *Retina*. 2009;29:187–191.
18. Sorice R, Ruggiero D, Nutile T, et al. Genetic and environmental factors influencing the placental growth factor (PGF) variation in two populations. *PLoS One*. 2012;7:e42537.
19. Cheng CY, Yamashiro K, Chen LJ, et al. New loci and coding variants confer risk for age-related macular degeneration in East Asians. *Nat Commun*. 2015;6:6063.
20. Huang C, Xu Y, Li X, Wang W. Vascular endothelial growth factor A polymorphisms and age-related macular degeneration: a systematic review and meta-analysis. *Mol Vis*. 2013;19:1211–1221.
21. Immonen I, Seitsonen S, Tommila P, et al. Vascular endothelial growth factor gene variation and the response to photodynamic therapy in age-related macular degeneration. *Ophthalmology*. 2010;117:103–108.
22. Hagstrom SA, Ying GS, Pauer GJ, et al. VEGFA and VEGFR2 gene polymorphisms and response to anti-vascular endothelial growth factor therapy: comparison of age-related macular degeneration treatments trials (CATT). *JAMA Ophthalmol*. 2014;132:521–527.
23. Galan A, Ferlin A, Caretti L, et al. Association of age-related macular degeneration with polymorphisms in vascular endothelial growth factor and its receptor. *Ophthalmology*. 2010; 117:1769–1774.
24. Al-Habboubi HH, Sater MS, Almawi AW, Al-Khateeb GM, Almawi WY. Contribution of VEGF polymorphisms to variation in VEGF serum levels in a healthy population. *Eur Cytokine Netw*. 2011;22:154–158.
25. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81:559–575.
26. de Jong EK, Breukink MB, Schellevis RL, et al. Chronic central serous chorioretinopathy is associated with genetic variants implicated in age-related macular degeneration. *Ophthalmology*. 2015;122:562–570.
27. Liu YH, Chen YJ, Wu HH, Wang TY, Tsai FJ. Single nucleotide polymorphisms at the PRR3, ABCF1, and GNL1 genes in the HLA class I region are associated with Graves' ophthalmopathy in a gender-dependent manner. *Ophthalmology*. 2014; 121:2033–2039.
28. Bland JM, Altman DG. Multiple significance tests: the Bonferroni method. *BMJ*. 1995;310:170.
29. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005; 21:263–265.
30. Guo Y, Li J, Bonham AJ, Wang Y, Deng H. Gains in power for exhaustive analyses of haplotypes using variable-sized sliding window strategy: a comparison of association-mapping strategies. *Eur J Hum Genet*. 2009;17:785–792.
31. Kondo N, Honda S, Kuno S, Negi A. Role of RDBP and SKIV2L variants in the major histocompatibility complex class III region in polypoidal choroidal vasculopathy etiology. *Ophthalmology*. 2009;116:1502–1509.
32. Chen ZT, Wang IJ, Shih YF, Lin LL. The association of haplotype at the lumican gene with high myopia susceptibility in Taiwanese patients. *Ophthalmology*. 2009;116:1920–1927.
33. Liu K, Lai TY, Chiang SW, et al. Gender specific association of a complement component 3 polymorphism with polypoidal choroidal vasculopathy. *Sci Rep*. 2014;4:7018.
34. Chen LJ, Liu DT, Tam PO, et al. Association of complement factor H polymorphisms with exudative age-related macular degeneration. *Mol Vis*. 2006;12:1536–1542.
35. Ng TK, Chen LJ, Liu DT, et al. Multiple gene polymorphisms in the complement factor h gene are associated with exudative age-related macular degeneration in Chinese. *Invest Ophthalmol Vis Sci*. 2008;49:3312–3317.
36. Kuritz SJ, Landis JR, Koch GG. A general overview of Mantel-Haenszel methods: applications and recent developments. *Annu Rev Public Health*. 1988;9:123–160.
37. Hayreh SS, Zimmerman MB. Nonarteritic anterior ischemic optic neuropathy: refractive error and its relationship to cup/disc ratio. *Ophthalmology*. 2008;115:2275–2281.
38. Otani A, Takagi H, Oh H, et al. Vascular endothelial growth factor family and receptor expression in human choroidal neovascular membranes. *Microvasc Res*. 2002;64:162–169.
39. Carmeliet P, Moons L, Luttun A, et al. Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. *Nat Med*. 2001;7:575–583.
40. Castellon R, Hamdi HK, Sacerio I, Aoki AM, Kenney MC, Ljubimov AV. Effects of angiogenic growth factor combinations on retinal endothelial cells. *Exp Eye Res*. 2002;74:523–535.
41. Nourinia R, Soheili ZS, Ahmadieh H, Akrami H, Rezaei Kanavi M, Samiei S. Knockdown of the placental growth factor gene inhibits laser induced choroidal neovascularization in a murine model. *J Ophthalmic Vis Res*. 2013;8:4–8.
42. Andraweera PH, Dekker GA, Dissanayake VH, Bianco-Miotto T, Jayasekara RW, Roberts CT. Vascular endothelial growth factor family gene polymorphisms in preeclampsia in Sinhalese women in Sri-Lanka. *J Matern Fetal Neonatal Med*. 2013;26: 532–536.
43. Zehetner C, Bechrakis NE, Stattin M, et al. Systemic counterregulatory response of placental growth factor levels to intravitreal aflibercept therapy. *Invest Ophthalmol Vis Sci*. 2015;56:3279–3286.
44. Churchill AJ, Carter JG, Lovell HC, et al. VEGF polymorphisms are associated with neovascular age-related macular degeneration. *Hum Mol Genet*. 2006;15:2955–2961.
45. Janik-Papis K, Zaras M, Krzyzanowska A, et al. Association between vascular endothelial growth factor gene polymor-

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phisms and age-related macular degeneration in a Polish population. *Exp Mol Pathol*. 2009;87:234–238.

46. Almeida LN, Melilo-Carolino R, Veloso CE, et al. Homozygosity for the +674C>T polymorphism on VEGF gene is associated with age-related macular degeneration in a Brazilian cohort. *Graefes Arch Clin Exp Ophthalmol*. 2012;250:185–189.
47. Boekhoorn SS, Isaacs A, Uitterlinden AG, et al. Polymorphisms in the vascular endothelial growth factor gene and risk of age-related macular degeneration: the Rotterdam Study. *Ophthalmology*. 2008;115:1899–1903.
48. Qu Y, Dai H, Zhou F, et al. Vascular endothelial growth factor gene polymorphisms and risk of neovascular age-related macular degeneration in a Chinese cohort. *Ophthalmic Res*. 2011;45:142–148.
49. McKay GJ, Silvestri G, Orr N, Chakravarthy U, Hughes AE. VEGF and age-related macular degeneration. *Ophthalmology*. 2009;116:1227, e1221–e1223.
50. Thorleifsson G, Walters GB, Hewitt AW, et al. Common variants near CAV1 and CAV2 are associated with primary open-angle glaucoma. *Nat Genet*. 2010;42:906–909.
51. Nongpiur ME, Khor CC, Jia H, et al. ABCC5, a gene that influences the anterior chamber depth, is associated with primary angle closure glaucoma. *PLoS Genet*. 2014;10:e1004089.