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Polymorphisms in Selected Genes and Their Association with Age-Related Macular Degeneration in a Chinese Population

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCDE Qing Huang
AEFG Yi Xiang

Department of Ophthalmology, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, P.R. China

Corresponding Author: Yi Xiang, e-mail: xiangyi145@hotmail.com
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Background: Increasing evidence shows that polymorphisms in a number of genes can influence age-related macular degeneration (AMD) risk. This study aimed to investigate the association of *CX3CR1* 839C/T, *CX3CR1* 745G/A, *PLEKHA1* 958A/G, *VEGFA* +674C/T, and *VEGFA* +936C/T polymorphisms with AMD risk among Chinese.

Material/Methods: The polymorphisms were genotyped on 827 AMD patients and 827 controls, and the odds ratios (ORs) were calculated under allele, additive, recessive, and dominant genetic models. Logistic regression analysis was performed to control for potential confounders (age, sex, and smoking status).

Results: We showed that all the 5 polymorphisms showed a significant association with AMD risk under the additive model (for homozygous mutant genotype) and at least 1 other genetic model, both before and after adjustment for the potential confounders. *PLEKHA1* 958A/G polymorphism was associated with a decreased AMD risk (additive model: aOR=0.722, 95% CI=0.450–0.979, P=0.019; allele model: aOR=0.883, 95% CI=0.736–0.992, P=0.014), while all other polymorphisms were associated with an increased AMD risk (*CX3CR1* 839C/T, additive model: aOR=2.682, 95% CI=1.119-5.709, P=0.022, recessive model: aOR=2.729, 95% CI=1.141–6.048, P=0.010; *CX3CR1* 745G/A, additive model: aOR=2.614, 95% CI=1.231–6.012, P=0.020, recessive model: aOR=2.340, 95% CI=1.227–5.993, P=0.011; *VEGFA* +674C/T, additive model: aOR=1.601, 95% CI=1.253–2.179, P<0.001, dominant model: aOR=1.287, 95% CI=1.058–1.570, P<0.001, allele model: OR=1.220, 95% CI=1.118–1.427, P<0.001; *VEGFA* +936C/T, additive model: aOR=1.509, 95% CI=1.105–2.311, P<0.001, recessive model: aOR=1.432, 95% CI=1.027–2.192, P=0.009, dominant model: aOR=1.207, 95% CI=1.031–1.514, P0.001, allele model: aOR=1.216, 95% CI=1.062–1.408, P<0.001).

Conclusions: We conclude that the 5 polymorphisms could serve as biomarkers for AMD susceptibility.

MeSH Keywords: Genetic Predisposition to Disease • Genotyping Techniques • Macular Degeneration • Odds Ratio • Polymorphism, Genetic

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Background

Age-related macular degeneration (AMD) is a common ophthalmologic disorder which results in a slow and progressive damage to the central vision among the elderly, which eventually leads to irreversible blindness. There are 2 types of AMD: dry and wet types. Dry AMDs are characterized by gradual wasting of the retinal pigment epithelium and photoreceptors, while wet AMDs involve abnormal neovascularization which damages the retina. Although wet AMDs are less common than dry AMDs, the former contribute to the vast majority of AMD-related vision loss [1]. Globally, AMD has a prevalence of 8.69%, and the number of individuals with the disorder is expected to rise tremendously in the next few decades [2]. AMD is multifactorial in origin, with the most important risk factor being aging, although other factors are thought to play an equally important etiological role [3]. An increasing body of evidence now suggests that genetic factors are exceedingly important for the development of AMD [3]. As such, a number of genetic polymorphisms have been commonly associated with the susceptibility to AMD [4–7].

Recently, Gupta et al. [4] investigated the association of 5 polymorphisms in *CX3CR1*, *PLEKHA1,* and *VEGFA* genes with AMD risk in an Indian population. The authors observed a lack of statistically significant association between *CX3CR1* polymorphisms and AMD risk, although several small-scale studies in the Chinese population showed otherwise [8,9]. Besides, while Gupta et al. [4] showed a significant association of *PLEKHA1* and *VEGFA* polymorphisms with AMD risk, these polymorphisms have not been extensively investigated in the Chinese population. The influence of genetic polymorphisms is known to vary from populations to populations due to the different allele and genotype frequencies across diverse geographical regions. Therefore, it would be interesting to examine the influence of the polymorphisms in these genes on AMD risk among Chinese.

CX3CR1 encodes the CX3C chemokine receptor 1, which plays an important role in facilitating the migration and accumulation of microglia cells at the site of macular damage [10]. This process of microglia cell accumulation is thought to serve as an important contributory factor for the breakdown of the macula in AMD [10]. The role of *CX3CR1* on microglia cell migration is heavily dependent on its expression level [10]. The *CX3CR1* gene contains 2 polymorphisms – 839C/T (Thr280Met) and 745G/A (Val249Ile) polymorphisms – which have been shown to influence the expression level of the *CX3CR1* gene and susceptibility to AMD [11].

PLEKHA1 encodes the pleckstrin homology domain-containing family A member 1 protein, which plays a role in the process of cellular signalling. The exact role of *PLEKHA1* in AMD pathogenesis is poorly understood. However, sequence variations of *PLEKHA1*, which was originally identified from a genome-wide screening [12], has been frequently implicated in the mediation of AMD susceptibility [4,5,13,14]. One of the *PLEKHA1* sequence variations which has been found to be associated with AMD risk is the 958A/G (Thr320Ala) polymorphism [4,5]. However, the involvement of the *PLEKHA1* 958A/G (Thr320Ala) polymorphism in AMD susceptibility has not been investigated previously in the Chinese population.

VEGFA encodes the vascular endothelial growth factor A protein, which is involved in neovascularization. As mentioned above, abnormal neovascularization can result in wet AMD, which causes most of the AMD-related vision loss. An increased VEGFA expression has been thought to be an important mechanism leading to wet AMD [15]. Therefore, polymorphisms in *VEGFA* gene which could influence its expression level are ideal candidates for genetic association studies of AMD. The *VEGFA* +674C/T and +936C/T polymorphisms, located respectively at the intronic and 3'-UTR regions of the gene, are 2 such polymorphisms which have been commonly evaluated in AMD. The influence of *VEGFA* +674C/T and +936C/T polymorphisms on risk of AMD has not been extensively studied in the Chinese population.

This study aimed to investigate the association of *CX3CR1* 839C/T (Thr280Met), *CX3CR1* 745G/A (Val249Ile), *PLEKHA1* 958A/G (Thr320Ala), *VEGFA* +674C/T, and *VEGFA* +936C/T polymorphisms with risk of AMD in a Chinese population.

Material and Methods

Ethics and informed consent

The study was approved by the Ethics Review Board of Tongji Medical College, Huazhong University of Science and Technology. The study was conducted in accordance with the Declaration of Helsinki. All study subjects gave written informed consent before enrolment into the study.

Cases and controls

Cases and controls were recruited from the Central Hospital of Wuhan between November 2014 and November 2016. Cases comprised 827 clinically diagnosed AMD patients, while controls comprised the same number of elderly (≥70 years old) individuals without AMD or ophthalmologic abnormalities associated with AMD (e.g., drusen or abnormal RPE pigmentations) or other eye diseases. All subjects were Han Chinese. Ophthalmological evaluations, including (i) acuity measurement utilizing the Snellen chart method, (ii) slit-lamp biomicroscopy, and (iii) fundoscopy in dilated pupils, were performed

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Table 1. Methods, primer sequences and band sizes obtained for genotyping of the polymorphisms.

Polymorphism	Genotyping method	Primer sequences (5'→3')	Band size (bp)
CX3CR1 839C/T (Thr280Met) and CX3CR1 745G/A (Val249Ile)	Direct sequencing	Primer 1: CCG AGG TCC TTC AGG AAA TCT	588
		Primer 2: TCA GCA TCA GGT TCA GGA ACA C	
PLEKHA1 958A/G (Thr320Ala)	Direct sequencing	Primer 1: GGT CAT GAG TGA CTG ACC GT	339
		Primer 2: GCT CGC ATC GTC CAA GTC TA	
VEGFA +674C/T	ARMS-PCR	Forward primer 1 (C allele): AAC CGC CCC TCC TGT GCC	118
		Forward primer 2 (T allele): AAC CGC CCC TCC TGT GCT	
		Common reverse primer: CCT GCC TTC CCC CTG ACA	
VEGFA +936C/T	PCR-RFLP (cleaved with NlaIII restriction enzyme)	Primer 1: AAG GAA GAG GAG ACT CTG CGC AGA GC	208*
		Primer 2: TAA ATG TAT GTA TGT GGG TGG GTG TGT CTA CAG G	

* Following restriction enzyme cutting, the PCR product of the mutant *VEGFA* +936 T allele was cleaved into two fragments of 122 and 86 bp.

on all subjects, and AMD diagnoses were ascertained via fluorescein angiography and time-domain optical coherence tomography. All examinations were conducted by qualified and certified ophthalmologists. All subjects were unrelated to one another and did not have any family history of AMD or other eye diseases.

Genotyping

Genotyping of *CX3CR1* 839C/T (Thr280Met), *CX3CR1* 745G/A (Val249Ile), *PLEKHA1* 958A/G (Thr320Ala), *VEGFA* +674C/T, and *VEGFA* +936C/T polymorphisms was performed on genomic DNA isolated from peripheral blood samples of all subjects, based on the methods described by Gupta et al. [4]. Isolation of DNA was performed by using the TIANamp Blood DNA Kit (Tiangen, China). Genotypes of the 2 *CX3CR1* polymorphisms were determined by sequencing the gene using the primers listed in Table 1. Similarly, *PLEKHA1* 958A/G (Thr320Ala) was also genotyped by direct sequencing of a fragment containing the polymorphism (Table 1). Genotypes of the *VEGFA* +674C/T polymorphism were determined by ARMS-PCR, where a unique forward primer was used to amplify each allele and a common reverse primer was used to amplify both alleles (Table 1). Finally, *VEGFA* +936C/T polymorphisms were genotyped via PCR-RFLP, in which cleaving was performed by using *Nla*III restriction enzyme at 37°C (Table 1). Following restriction enzyme cutting, the PCR product of the wild type *VEGFA* +936 C allele remained intact at 208 bp, while the PCR product of the mutant T allele was cleaved into 2 fragments of 122 and 86 bp. Genotypes of both *VEGFA* polymorphisms were re-confirmed by direct sequencing of 15% of the samples using the same primers listed in Table 1. All sequencing reactions were performed with BigDye Terminator v3.1 Cycle Sequencing Kit on a Thermo Fisher 310 Genetic Analyzer, while all PCR reactions above were performed by using Taq Plus DNA mix (Tiangen, China) on a Life Express thermocycler (Bioer, China).

Statistical analysis

Frequencies of each allele and genotype were determined by manual counting. Linkage disequilibrium (LD) test was performed as previously described [16]. The genotype distributions among the controls were tested for deviation from Hardy-Weinberg equilibrium using a chi-squared test. Odds ratio (OR) and its 95% confidence interval were calculated using the MedCalc statistical software. The association between the polymorphisms and AMD risk was assessed under additive model ("heterozygous *vs.* wild type" and "mutant *vs.* wild type"), recessive model ("mutant *vs.* wild type + heterozygous"), dominant model ("heterozygous + mutant *vs.* wild type"), and allele model ("mutant allele *vs.* wild type allele"). Logistic regression analysis was run to control/adjust for 3 potential confounders (age, sex, and smoking status of the subjects). Statistical significance was set at $P<0.05$.

Results

We successfully recruited 827 AMD patients and 827 controls into the study (Table 2), and both cases and controls were sex-matched to each other. In each group of subjects, 489 (59.1%) individuals were males and 338 (40.9%) were females. Ages of the cases ranged from 62 to 94 years (mean=77.5 years) while controls ranged from 70 to 89 years old (mean=79.8

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Table 2. Description of cases and controls.

	Cases	Control
Total subject	827	827
Age		
Range	62–94	70–89
Mean	77.5	79.8
Sex		
Male	489 (59.1%)	489 (59.1%)
Female	338 (40.9%)	338 (40.9%)
Smoking status		
Ever smoker	294 (35.6%)	216 (26.1%)
Never smoker	533 (64.4%)	611 (73.9%)
Genotyping success rate	100%	100%

years). Among the cases, 294 (35.6%) were ever smokers and 533 (64.4%) were never smokers, while 216 (26.1%) and 611 (73.9%) of the controls were ever and never smokers, respectively. Genotyping of the polymorphisms was successfully performed on all cases and controls. Genotype and allele frequencies of the polymorphisms are shown in Table 3. All the genotype distributions conformed to the Hardy-Weinberg equilibrium in the controls (Table 4).

All polymorphisms showed statistically significant association with AMD risk under additive model and at least 1 other genetic model, both before and after adjustment for age, sex, and smoking status (Table 3). For *CX3CR1* 839C/T (Thr280Met) polymorphism, the mutant TT genotype was present in 2.1% of the cases and 0.7% of the controls, which resulted in an adjusted OR of 2.682 (95% CI=1.119–5.709), which was statistically significant under the additive model (P=0.022). A similar observation was seen under the recessive model (OR=2.729, 95% CI=1.141–6.048, P=0.010), but not the dominant model (P>0.05). Likewise, when the association was investigated under the allele model using allele frequency, statistical significance was not observed (P>0.05).

The *CX3CR1* 745G/A (Val249Ile) polymorphism showed a similar result (Table 3). Under the additive model, the mutant AA genotype showed statistical significance with an adjusted OR of 2.614 (95% CI=1.231–6.012) (P=0.020), while under the recessive model, an adjusted OR of 2.340 (95% CI=1.227–5.993) was observed (P=0.011). However, analyses under dominant and allele models did not reveal any significant association (P>0.05). Linkage disequilibrium (LD) test was performed on the 2 *CX3CR1* polymorphisms. A strong LD was observed for the 2 polymorphisms, with a D' value of 0.968 (data not shown).

For *PLEKHA1* 958A/G (Thr320Ala) polymorphism (Table 3), the mutant G allele was found to be associated with a lower risk of AMD at an adjusted of OR=0.883 (95% CI=0.736-0.992) (P=0.014). Thus, homozygosity of the mutant allele (the GG genotype) also showed a significantly lower risk association under additive model (adjusted OR=0.722, 95% CI=0.450-0.979) (P=0.019). However, no significant associations were observed under recessive and dominant models (P>0.05).

Similarly, the mutant T alleles of both the *VEGFA* +674C/T and +936C/T polymorphisms also showed statistically significant associations with AMD risk under the allele model (Table 3). The former had an adjusted OR of 1.220 (95% CI=1.118–1.427) (P<0.001), while the latter showed an adjusted OR of 1.216 (95% CI=1.062–1.408) (P<0.001). Hence, homozygosity of the respective mutant alleles (the TT genotype) resulted in a higher risk of AMD with statistical significance under the additive model, with the *VEGFA* +674C/T polymorphism showing an adjusted OR of 1.601 (95% CI=1.253–2.179) (P<0.001) and the +936C/T polymorphism showing an adjusted OR of 1.509 (95% CI=1.105–2.311) (P<0.001). Both polymorphisms also showed a statistically significant association under the dominant model (*VEGFA* +674C/T polymorphism: adjusted OR=1.287, 95% CI=1.058–1.570, P<0.001; *VEGFA* +936C/T polymorphism: adjusted OR=1.207, 95% CI=1.031–1.514, P<0.001). The +936C/T polymorphism also showed a statistically significant association under the recessive model (adjusted OR=1.432, 95% CI=1.027–2.192, P=0.009).

Discussion

In this study, we examined the relationship between *CX3CR1* 839C/T (Thr280Met), *CX3CR1* 745G/A (Val249Ile), *PLEKHA1* 958A/G (Thr320Ala), *VEGFA* +674C/T, and *VEGFA* +936C/T

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Table 3. Association of the polymorphisms with AMD risk.

	Cases	Controls	OR	95% CI	P	Adjusted OR	95% CI	P
CX3CR1 839C/T (Thr280Met)								
Genotype frequency								
CC	648 (78.3%)	655 (79.2%)	Reference	–	–	Reference	–	–
CT (additive model)	162 (19.6%)	166 (20.1%)	0.986	0.774–1.257	0.912	0.986	0.781–1.183	0.793
TT (additive model)	17 (2.1%)	6 (0.7%)	2.864	1.122–7.310	0.028*	2.682	1.119–5.709	0.022*
TT *vs.* CT+CC (recessive model)	–	–	2.868	1.125–7.312	0.027*	2.729	1.141–6.048	0.010*
TT+CT *vs.* CC (dominant model)	–	–	1.058	0.836–1.339	0.640	1.033	0.867–1.175	0.493
Allele frequency								
C	1458 (88.1%)	1476 (89.2%)	Reference	–	–	Reference	–	–
T	196 (11.9%)	178 (10.8%)	1.115	0.899–1.383	0.323	1.097	0.912–1.312	0.239
CX3CR1 745G/A (Val249Ile)								
Genotype frequency								
GG	611 (73.88%)	631 (76.30%)	Reference	–	–	Reference	–	–
GA (additive model)	194 (23.46%)	188 (22.73%)	1.066	0.847–1.340	0.587	1.066	0.866–1.319	0.471
AA (additive model)	22 (2.66%)	8 (0.97%)	2.840	1.255–6.428	0.012*	2.614	1.231–6.012	0.020*
AA *vs.* GA+GG (recessive model)	–	–	2.798	1.238–6.321	0.013*	2.340	1.227–5.993	0.011*
AA+GA *vs.* GG (dominant model)	–	–	1.138	0.911–1.423	0.256	1.112	0.917–1.681	0.249
Allele frequency								
G	1416 (85.6%)	1450 (87.7%)	Reference	–	–	Reference	–	–
A	238 (14.4%)	204 (12.3%)	1.195	0.977–1.460	0.083	1.129	0.968–1.327	0.062
PLEKHA1 958A/G (Thr320Ala)								
Genotype frequency								
AA	466 (56.35%)	429 (51.87%)	Reference	–	–	Reference	–	–
AG (additive model)	313 (37.85%)	331 (40.02%)	0.871	0.711–1.066	0.180	0.902	0.732–1.041	0.219
GG (additive model)	48 (5.80%)	67 (8.10%)	0.660	0.445–0.977	0.038*	0.722	0.450–0.979	0.019*
GG *vs.* AG+AA (recessive model)	–	–	0.699	0.476–1.026	0.067	0.708	0.503–1.018	0.083

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Table 3 continued. Association of the polymorphisms with AMD risk.

	Cases	Controls	OR	95% CI	P	Adjusted OR	95% CI	P
GG+AG *vs.* AA (dominant model)	–	–	0.835	0.688–1.013	0.068	0.916	0.716–1.009	0.109
Allele frequency								
A	1245 (75.3%)	1189 (71.9%)	Reference	–	–	Reference	–	–
G	409 (24.7%)	465 (28.1%)	0.840	0.720–0.981	0.027*	0.883	0.763–0.992	0.014*
VEGFA +674C/T								
Genotype frequency								
CC	254 (30.71%)	307 (37.12%)	Reference	–	–	Reference	–	–
CT (additive model)	392 (47.40%)	389 (47.04%)	1.218	0.980–1.514	0.076	1.195	0.991–1.478	0.101
TT (additive model)	181 (21.89%)	131 (15.84%)	1.670	1.263–2.209	<0.001*	1.601	1.253–2.179	<0.001*
TT *vs.* CT+CC (recessive model)	–	–	0.847	0.655–1.096	0.206	0.868	0.692–1.079	0.302
TT+CT *vs.* CC (dominant model)	–	–	1.332	1.086–1.634	0.006*	1.287	1.058–1.570	<0.001*
Allele frequency								
C	900 (54.4%)	1003 (60.6%)	Reference	–	–	Reference	–	–
T	754 (45.6%)	651 (39.4%)	1.291	1.124–1.482	<0.001*	1.220	1.118–1.427	<0.001*
VEGFA +936C/T								
Genotype frequency								
CC	456 (55.14%)	505 (61.06%)	Reference	–	–	Reference	–	–
CT (additive model)	299 (36.15%)	273 (33.01%)	1.213	0.986–1.492	0.068	1.185	0.988–1.442	0.103
TT (additive model)	72 (8.71%)	49 (5.93%)	1.627	1.108–2.390	0.013*	1.509	1.105–2.311	<0.001*
TT *vs.* CT+CC (recessive model)	–	–	1.514	1.039–2.207	0.031*	1.432	1.027–2.192	0.009*
TT+CT *vs.* CC (dominant model)	–	–	1.276	1.049–1.552	0.015*	1.207	1.031–1.514	<0.001*
Allele frequency								
C	1211 (73.2%)	1283 (77.6%)	Reference	–	–	Reference	–	–
T	443 (26.8%)	371 (22.4%)	1.265	1.079–1.483	0.004*	1.216	1.062–1.408	<0.001*

Adjusted OR – adjusted for age, sex, smoking status.

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Table 4. Deviation from Hardy-Weinberg equilibrium.

Polymorphism	HWE P-value
CX3CR1 839C/T (Thr280Met)	0.195
CX3CR1 745G/A (Val249Ile)	0.141
PLEKHA1 958A/G (Thr320Ala)	0.778
VEGFA +674C/T	0.647
VEGFA +936C/T	0.140

polymorphisms and risk of AMD among Chinese. The same 5 polymorphisms have been studied previously in an Indian population [4], but since the impact of polymorphisms on disease risk is known to differ from population to population, the effects of these polymorphisms on AMD risk remained poorly understood among the Chinese. There were a few previous studies on the association of *CX3CR1* and *VEGFA* polymorphisms on AMD risk in the Chinese population, but these studies had small sample sizes and thus were underpowered [8,9,17–19]. The present study performed genetic risk analysis of these polymorphisms on a large sample size of 1654 subjects (827 cases and 827 controls), which provided sufficient statistical power for the study. The present work was also the first study to investigate the association of *PLEKHA1* 958A/G (Thr320Ala) polymorphism and AMD susceptibility among Chinese. In this work, we included patients of both wet and dry AMD types and analyzed them together. This is because the aim of this study was to identify a general biomarker which can be used to detect *any* type of AMD, regardless of whether it is dry type or wet type. The mean age of the controls recruited was slightly higher than the cases, because we included only controls of ≥70 years old to minimize false-negatives (since some AMDs develop late in life).

We found that all the 5 polymorphisms studied were associated with AMD risk under at least 2 genetic models. For the 2 *CX3CR1* polymorphisms, statistically significant associations were not observed under the allele model, but were observed for the respective homozygous mutant genotypes under the additive model. This suggests that the presence of a single mutant allele was not sufficient for the polymorphisms to increase the risk of AMD significantly, and homozygosity for the mutant allele is necessary for AMD risk increment. There were 2 previous studies which examined the association of the 2 *CX3CR1* polymorphisms with AMD risk in the Chinese population [8,9]. In both studies, a statistically significant association was observed under the allele model. One previous study [8] also showed that the association with AMD risk was statistically significant under the dominant model, which was contrary to our findings. We believe that the discrepancy between our study and these previous studies was due to the sample size employed. Our study used a larger sample size than both these studies combined, which gives a higher statistical power of analysis. Despite the differences, the present and previous studies all suggest that the 2 polymorphisms could be associated with an increased AMD risk among Chinese, potentially by influencing the function of the protein product (since both polymorphisms result in amino acid changes) [20], or by altering its expression level [11]. In addition, similar to a previous study [8], the present study also showed that the 2 polymorphisms were in strong linkage disequilibrium. Thus, future studies can probably focus on just one of these polymorphisms to save experimental expenses.

We also showed in this work that the mutant allele of *PLEKHA1* 958A/G (Thr320Ala) polymorphism demonstrated a protective effect against AMD. Similar to the present work, Gupta et al. [4] also found a significantly lowered risk of AMD associated with the mutant *PLEKHA1* allele under the allele model and additive model (although the authors erroneously concluded a lack of significant association in their conclusions). However, a study in a German population noted the overrepresentation of the mutant *PLEKHA1* allele among AMD cases, suggesting that the polymorphism was associated with increased AMD risk [5]. This discrepancy was probably due to the different ethnicities (Asians *vs.* whites) of the subjects investigated. Understanding the functions and role of PLEKHA1 in AMD, as well as the effect of the 958A/G (Thr320Ala) polymorphism, will help to clarify how the polymorphism results in associations of opposite magnitudes in different populations. Currently, although *PLEKHA1* is not an uncommonly studied gene in AMD, there is a limited understanding on the above matters. *PLEKHA1* gained popularity in AMD studies as it was identified from a genome-wide screening [12] and subsequently was found to be frequently involved in the disorder [13,14].

In the present study, we also found that *VEGFA* +674C/T and +936C/T polymorphisms were significantly associated with an increased AMD risk under allele, additive, and dominant models. In addition, the +936C/T polymorphism also showed a statistically significant association with increased AMD risk under the recessive model. *VEGFA* is involved in neovascularization, improper regulation of which could result in abnormal blood vessel formation in the eyes that causes retinal damage and subsequently AMD [15]. Hence, polymorphisms in the *VEGFA* gene, which could enhance its expression levels, such as the +674C/T and +936C/T polymorphisms, can potentially increase the susceptibility to wet AMD. In agreement with this, a previous study from the Chinese population showed that the +936C/T polymorphism was significantly associated with an increased risk of wet AMD [18]. However, another study showed no association between both polymorphisms and AMD risk among Chinese [20]. In addition, the association between the 2 polymorphisms and AMD risk has also been investigated in non-specific AMDs (regardless of whether it is of

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dry type or wet type) and 1 study showed that the +936C/T polymorphism was associated with an increased risk of the disorder among Chinese [19], although the reason underlying such an association remained unexplained. However, another study reported a lack of significant association between the *VEGFA* +674C/T polymorphism and AMD risk in the Chinese population [18], which contradicts the findings of the present study. However, our analysis was performed on a much larger sample size compared to these previous reports, which could potentially yield a more reliable result.

Conclusions

We found that the *CX3CR1* 839C/T (Thr280Met), *CX3CR1* 745G/A (Val249Ile), *VEGFA* +674C/T, and *VEGFA* +936C/T polymorphisms were associated with an increased risk of AMD in a Chinese population, while the *PLEKHA1* 958A/G (Thr320Ala) was associated with a decreased AMD risk.

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Conflict of interest

None.

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