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

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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=1.119–5.709, P=0.022; \( VEGFA \) +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=1.058–1.427, 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, P=0.001, 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 pigments) 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...
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 NlaIII 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 years).

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     | Direct sequencing       | Primer 1: CCG AGG TCC TTC AGG AAA TCT                  | 588            |
| CX3CR1 745G/A     |                          | Primer 2: TCA GCA TCA GGT TCA GGA ACA C                |                |
| PLEKHA1 958A/G    | Direct sequencing       | Primer 1: GGT CAT GAG TGA CTG ACC GT                   | 339            |
| (Thr320Ala)       |                          | 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 TGT GCA         |                |
| VEGFA +936C/T     | PCR-RFLP (cleaved      | Primer 1: AAG GAA GAG ACT CTG CGC AGA GC               | 208*           |
|                   | with NlaIII restriction enzyme) | Primer 2: TAA ATG TAT GTA TGT GGG TGG 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.

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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, respec-
tively. Genotyping of the polymorphisms was successfully
performed on all cases and controls. Genotype and allele fre-
quencies of the polymorphisms are shown in Table 3. All the
genotype distributions conformed to the Hardy-Weinberg equi-
librium in the controls (Table 4).

All polymorphisms showed statistically significant association
with AMD risk under additive model and at least 1 other ge-
etic 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 ad-
justed OR of 2.682 (95% CI=1.119–5.709) (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 un-
der the allele model using allele frequency, statistical signifi-
cance was not observed (P>0.05).

The CX3CR1 745G/A (Val249Ile) polymorphism showed a simi-
lar result (Table 3). Under the additive model, the mutant AA
genotype showed statistical significance with an adjusted OR
of 2.614 (95% CI=1.123–6.012) (P=0.020), while under the re-
cessive model, an adjusted OR of 2.340 (95% CI=1.231–5.993)
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 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 un-
der 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 high-
er risk of AMD with statistical significance under the additive
model, with the VEGFA +674C/T polymorphism showing an ad-
justed 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 recessive mod-
el (VEGFA +674C/T polymorphism: adjusted OR=1.287, 95%
CI=1.058–1.570, P<0.001; VEGFA +936C/T polymorphism: ad-
justed OR=1.207, 95% CI=1.031–1.514, P<0.001). The +936C/T
polymorphism also showed a statistically significant associ-
ation 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

| 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% |

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|                      | 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  | 0.272   | 2.682       | 1.119–5.709  | 0.022*  |
| TT vs. CT+CC (recessive model) | – | – | 2.868 | 1.125–7.312 | 0.272* | 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  | 0.493   |
| **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 |

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

| Allele | Cases | Controls | OR  | 95% CI       | P      | Adjusted OR | 95% CI       | P      |
|--------|-------|----------|-----|--------------|--------|-------------|--------------|--------|
| A      | 1245  | 1189     | Reference | –       | –       | Reference | –           | –      |
| G      | 409   | 465      | 0.840 | 0.720–0.981 | 0.027* | 0.883       | 0.763–0.992 | 0.014* |

**VEGFA +674C/T**

**Genotype frequency**

| Genotype | Cases | Controls | OR  | 95% CI       | P      | Adjusted OR | 95% CI       | P      |
|----------|-------|----------|-----|--------------|--------|-------------|--------------|--------|
| CC       | 254   | 307      | Reference | –       | –       | Reference | –           | –      |
| CT (additive model) | 392  | 389      | 1.218 | 0.980–1.514 | 0.076  | 1.195       | 0.991–1.478 | 0.101  |
| TT (additive model) | 181  | 131      | 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**

| Allele | Cases | Controls | OR  | 95% CI       | P      | Adjusted OR | 95% CI       | P      |
|--------|-------|----------|-----|--------------|--------|-------------|--------------|--------|
| C      | 900   | 1003     | Reference | –       | –       | Reference | –           | –      |
| T      | 754   | 651      | 1.291 | 1.124–1.482 | <0.001* | 1.220       | 1.118–1.427 | <0.001* |

**VEGFA +936C/T**

**Genotype frequency**

| Genotype | Cases | Controls | OR  | 95% CI       | P      | Adjusted OR | 95% CI       | P      |
|----------|-------|----------|-----|--------------|--------|-------------|--------------|--------|
| CC       | 456   | 505      | Reference | –       | –       | Reference | –           | –      |
| CT (additive model) | 299  | 273      | 1.213 | 0.986–1.492 | 0.068  | 1.185       | 0.988–1.442 | 0.103  |
| TT (additive model) | 72   | 49       | 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**

| Allele | Cases | Controls | OR  | 95% CI       | P      | Adjusted OR | 95% CI       | P      |
|--------|-------|----------|-----|--------------|--------|-------------|--------------|--------|
| C      | 1211  | 1283     | Reference | –       | –       | Reference | –           | –      |
| T      | 443   | 371      | 1.265 | 1.079–1.483 | 0.004* | 1.216       | 1.062–1.408 | <0.001* |

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

### 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       |
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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