# Associations Between the T280M and V249I SNPs in CX3CR1 and the Risk of Age-Related Macular Degeneration

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**Purpose.** Two common single nucleotide polymorphisms (SNPs) in the *CX3CR1* gene, T280M and V249I, have been reported to affect the risk of age-related macular degeneration (AMD) in several studies. The aim of the present study was to combine all published data on the relationship between these two variants and AMD susceptibility in a meta-analysis to clarify this association.

**Methods.** MEDLINE, EMBASE, and ISI Web of Science were searched for all eligible studies on the relationship between AMD and T280M and V249I variants. The pooled odds ratio (OR) with 95% confidence intervals (CIs) for each SNP in the allele frequency, homozygote, second codominant genotype, and dominant genotype models were calculated to evaluate the strength of this association.

**Results.** A total of 3017 AMD cases and 4096 controls from eight studies were involved in this meta-analysis. Both T280M and V249I SNPs exhibited significant associations with increased risk of AMD in the allele (T versus C: OR = 1.43, 95% CI: 1.06–1.91; A versus G: OR = 1.25, 95% CI: 1.01–1.55) and homozygous models (TT versus CC: OR = 2.11, 95% CI: 1.00–4.43; AA versus GG: OR = 1.27, 95% CI: 1.00–1.61), while no significance association was observed for the codominant genotype model. Moreover, studies showing high linkage disequilibrium between these two variants demonstrated a significantly stronger connection between these SNPs and AMD risk, compared with the moderate linkage disequilibrium group.

**Conclusions.** Significant evidence for a relationship between T280M and V249I variants in CX3CR1 in the homozygote state with increased susceptibility to AMD was reported. Further studies are needed to confirm these findings.

Keywords: age-related macular degeneration, CX3CR1, SNP

Age-related macular degeneration (AMD) is the most common cause of visual impairment and blindness among elderly people in developed countries.[1] The pathologic hallmarks of AMD, such as drusen formation, inflammatory products, cell debris, lipoprotein aggregates, and oxysterols, primarily deposit in the extracellular space between the retinal pigment epithelium (RPE) and Bruch's membrane, and these aggregates gradually result in the formation of geographic atrophy (GA) and choroidal neovascularization (CNV).[2] Although the etiology of AMD remains elusive, AMD has been generally considered a complex multivariable disease with significant contribution from genetic factors.[3] Indeed, single nucleotide polymorphisms (SNPs) in several genes, such as *CFH* and *ARMS2/HTRA1*, are closely associated with the development of this disease.[4,5]

Chemokines, as important cytokines, play a central role in the migration of immune cells to inflamed tissues.[6] The normal chemoattractant function of these compounds has been identified as a protective factor against AMD development in animal experiments.[7] The *CX3CR1* gene encodes a specific receptor for fractalkine (CX3CL1), which is expressed in a variety of inflammatory cells in the brain and eye, including neutrophils, monocytes, microglia, T lymphocytes, and solid organs.[8,9] Two common nonsynonymous SNPs in the *CX3CR1* gene, T280M (rs3732378: C>T) and V249I (rs3732379: G>A), have been associated with several age-related chronic degenerative diseases, such as arteriosclerosis and coronary artery disease,[10,11] which share similar pathology mechanisms with AMD, particularly with CNV. The epigenetic changes in the *CX3CR1* gene might lead to a decreased number of fractalkine receptor binding sites, thereby reducing the binding affinity of the receptor to fractalkine, which partially explains the individual differences in vascular disease risks.[12] Recently, several studies[13–15] have shown that the two variants of the *CX3CR1* gene might be associated with an increased risk of AMD, but these results are inconsistent and inconclusive. Additional studies[16–18] have also shown that the frequency of V249I and T280M exhibits ethnicity specificity, with a dose-response relationship between the frequency of these mutations and AMD; however, whether these factors significantly alter this association remains unclear.

Accordingly, we performed a meta-analysis of the available studies to assess the association between these two SNPs with the risk of AMD and explored the sources of heterogeneity.

## Methods

### Literature Search

To identify all published articles reporting on the V249I and T280M variants in the *CX3CR1* gene associated with the risk of AMD, we performed a comprehensive search of PubMed, Embase, and Web of Science databases (up to October 2014), using different combinations of the following terms: "CX3CR1" or "chemokine receptor 1" or "C-X3-C motif receptor 1" or "CCRL1" or "V28" or "CCRL1" or "GPR13" or "CMKDR1" or "GPRV28" or "CMKBRL1" and "AMD" or "age-related maculopathy" or "age-related macular degeneration" or "choroidal neovascularization" or "geographic atrophy." No language filter was applied. Additional strategies included a manual review of the bibliographic references cited in the retrieved articles and Web-based searches to identify relevant publications for additional potential studies. Moreover, the supplementary material was perused for missing data points.

### Selection of Studies

To identify all eligible publications, a two-step search strategy was adopted. First, an initial screen of the identified abstracts and titles for all of the relevant articles was conducted to determine whether a citation met the inclusion criteria. Second, full-text versions of the remaining articles were further examined for eligibility for the present meta-analysis. As no cohort studies were observed, the present meta-analysis was limited to case-control or cross-sectional studies. For inclusion, the following screening criteria were used to determine qualitative eligibility: (1) the major objective was to evaluate the relationship between V249I or T280M polymorphisms and AMD risk; (2) studies must provide sufficient original data on the frequencies of SNPs for calculating odds ratios (ORs) with corresponding 95% confidence intervals (95% CIs); and (3) the genotype distribution of the control population must be in Hardy-Weinberg equilibrium (HWE). When multiple publications were reported on the same or overlapping data, the publication with the largest sample size was selected. Two authors (RZ and L-YW) independently scanned all of the relevant publications identified through the search, and a third author (LM) resolved any disagreements in opinion.

### Data Extraction and Quality Assessment

The following information was extracted from each eligible article: author names, year of publication, study design, number of AMD cases and controls, ethnicity, mean age and sex ratio of the subjects, AMD type, control sources, diagnostic methods, classification criteria, genotyping methods, magnitude of linkage disequilibrium (LD) for these two loci, the distribution of genotypes and alleles for each polymorphism, and more. The definition of AMD varied between studies and was based on various AMD diagnostic criteria (fundus photography and medical records review of visual acuity). Early AMD was defined as the presence of drusen and pigmentary abnormalities in the RPE or both; and late AMD, including CNV and GA, was defined as the presence of neovascularization, detachment of RPE, or geographic atrophy. The term "AMD" represents the combination of both early and late AMD combined, unless otherwise indicated. When studies provided subtypes of AMD disease, the association of these two SNPs with each AMD subtype was investigated from all retrieved articles. The magnitude of LD was measured as $r^2$, where $r^2 > 0.8$ suggests high LD and $0.5 < r^2 < 0.8$ suggests moderate LD.[19,20] The Newcastle-Ottawa quality scale (NOS) was used to assess the quality of each study, comprising three broad perspectives containing subject selection (0–4 scores), comparability (0–2 scores), and exposure (0–3 scores).[21] A quality score was finally obtained after summing each component; and scores of 0 to 4; 5 and 6; and 7 to 9 were defined as low-, moderate-, and high-quality, respectively.[22] To guarantee the accuracy of the data, two investigators (RZ and L-YW) independently extracted all information, and a third author (LM) adjudicated any discrepancies.

### Statistical Analysis

For each study, the HWE of the control group was initially evaluated by using the $\chi^2$ test (cutoff point: $P < 0.05$). The strength of the association between the SNPs V249I and T280M within the *CX3CR1* gene and AMD susceptibility was measured as the OR with 95% CI, using four genetic models of analysis: allele frequency, homozygote, second codominant genotype, and dominant genotype models. Between-study heterogeneity was evaluated by using Cochran's $\chi^2$-based $Q$ statistic, followed by the $I^2$ statistic test. A $P$ value of $<0.1$ was considered significant for heterogeneity between the data sets for the $Q$ statistic. The $I^2$ value describes the percentage of variation on a scale of 0% to 100%, where an $I^2$ less than or equal to 25%, 50%, and 75% represents low, moderate, and high heterogeneity, respectively. Subgroup analysis was conducted to test the individual association of several covariates with pooled estimates, when the data permitted, evaluating the type of AMD (early AMD versus late AMD [GA versus CNV]), ethnicity (Caucasians versus Asians), source of control (population based versus hospital based), mean age of AMD patients ($\geq$75 vs. $<$75 years), diagnostic method (fundus photography versus visual acuity criteria), the magnitude of LD for the two loci (high LD versus moderate LD), genotyping method (polymerase chain reaction–restriction fragment length polymorphism [PCR-RFLP] versus TaqMan SNP genotyping assay [TaqMan] versus sequencing), classification criteria (Age-Related Eye Disease Study [AREDS] versus International Classification and Grading System [ICGS]), and NOS quality scores (low quality versus moderate quality versus high quality). When significant statistical heterogeneity was detected ($I^2 > 50\%$), a sensitivity analysis was performed to evaluate the stability of the pooled results by removing one study at a time and subsequently recalculating a pooled estimation for the remaining data sets. Moreover, Begg's funnel plot and Egger's regression test ($P < 0.05$ was considered statistically significant) were used to determine potential publication bias.[23,24] All statistical analyses were conducted by using Stata version 11.0 software (StataCorp, College Station, TX, USA).

## Results

### Study Selection and Characteristics

The combined search yielded a total of 667 potentially relevant citations. After duplicate references were removed, 546 references were screened after reading the titles and abstracts, and 44 articles were selected for detailed assessment (Fig. 1). Among these, the remaining eight articles were included in this meta-analysis.[13–16,18,25–27]

The characteristics of the included studies are presented in Table 1. Among the eligible studies, six studies presented data on Caucasian subjects, and two studies presented data on Asian populations. The number of subjects ranged from 137 to 3642, comprising a total of 3017 AMD cases and 4096 controls. Two studies used hospital-based controls, and the remaining studies were performed by using population-based controls. The AMD

**Figure 1.** Flowchart showing study selection procedure.

diagnostic in six studies was based on fundus photography, while the other diagnostics included confirmed cases of AMD based on the medical records review of visual acuity. AREDS and ICGS criteria were applied in four and two studies, respectively, whereas the remaining studies did not report a comprehensive classification criterion. Most studies (50%) determined the variants through PCR-RFLP, while the TaqMan assay and sequencing were applied in two studies. Three of the six studies conclusively showed high LD, and two studies indicated moderately strong LD between the two loci. For each study, both genotype distributions of the *CX3CR1* gene were consistent with HWE. Furthermore, the NOS quality scores ranged from 6 to 9, with 75% of the studies classified as high quality.

## T280M Polymorphism and AMD Risk

All of the included studies contributed to the analysis of the T280M polymorphism and the determination of AMD susceptibility. Six studies established a positive correlation between the T280M variant and the risk of AMD, and four studies reported a statistically significant relationship. With significant heterogeneity ($I^2$ range, 65.0%–83.2%; all $P < 0.05$) between the data sets, the random-effects model was used to calculate the pooled OR. The pooled estimate showed a significantly increased AMD risk with the T280M variant, with a summary OR of 1.43 under the allele model (T versus C: 95% CI: 1.06–1.91), 2.11 under the homozygous model (TT versus CC: 95% CI: 1.00–4.43), and 1.47 under the dominant model (CT+TT versus CC: 95% CI: 1.03–2.08); however, no significant association was observed for the second codominant model

**Table 1.** Characteristics of the Included Studies in the Meta-Analysis

| First Author (y) | Country of Origin | Study Type | Ethnicity | Variation Location | Mean Age, Case/ Control, y | Sex Ratio of Case, M/F | Sex Ratio of Control, M/F | Sample Size, Case/ Control | Type of AMD | Source of Controls | Diagnostic Criteria | Classification Criteria | Genotyping Methods | LD* | Quality Score† | HWE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tuo (2004) | USA | C-C | Caucasians | T280M, V249I | 79.1/68.2 | 40/45 | 55/50 | 85/105 | Late AMD (GA/CNV) | PB | FP | AREDS | PCR-RFLP | H | 7 | Yes |
| Chan (2005) | USA | C-C | Caucasians | T280M | 82.8/68.2 | NR | 55/50 | 32/105 | Late AMD (GA/CNV) | PB | FP | AREDS | PCR-RFLP | NA | 6 | Yes |
| Combadière (2007) | France | C-C | Caucasians | T280M, V249I | NR | 82/202 | 291/236 | 284/520 | AMD | HB | BCVA | NR | PCR-RFLP | H | 6 | Yes |
| Yang (2010) | China | C-C | Asians | T280M, V249I | 65.8/64.1 | 45/64 | 76/74 | 109/150 | Late AMD (CNV) | PB | FP | AREDS | TaqMan | H | 8 | Yes |
| Zerbib (2011) | France | C-C | Caucasians | T280M | 79.0/67.9 | 370/723 | 159/237 | 1093/396 | Late AMD (CNV) | HB | FP | ICGS | TaqMan | NA | 8 | Yes |
| Anastasopoulos (2012) | Greece | C-S | Caucasians | T280M, V249I | 73.7/69.9 | 103/80 | 110/78 | 183/188 | AMD | PB | FP | ICGS | PCR-RFLP | M | 8 | Yes |
| Schaumberg (2014) | USA | C-C | Caucasians | T280M, V249I | 62.4/62.4 | 524/586 | 1217/1315 | 1110/2532 | AMD | PB | BCVA | AREDS | Sequencing | M | 8 | Yes |
| Gupta (2014) | India | C-C | Asians | T280M, V249I | 70.40/74.42 | 74/47 | 71/29 | 121/100 | AMD | PB | FP | NR | Sequencing | NR | 7 | Yes |

BCVA, best-corrected visual acuity; C-C, case-control; C-S, cross-sectional; FP, fundus photography; H, high linkage disequilibrium between T280M and V249I; HB, hospital based; M, moderate linkage disequilibrium between T280M and V249I; NA, not applicable; NR, not reported; PB, population based; Sequencing, direct sequencing; TaqMan, TaqMan SNP genotyping assay.

* Linkage disequilibrium was measured by $r^2$.

† Study quality was judged by the Newcastle-Ottawa Scale.

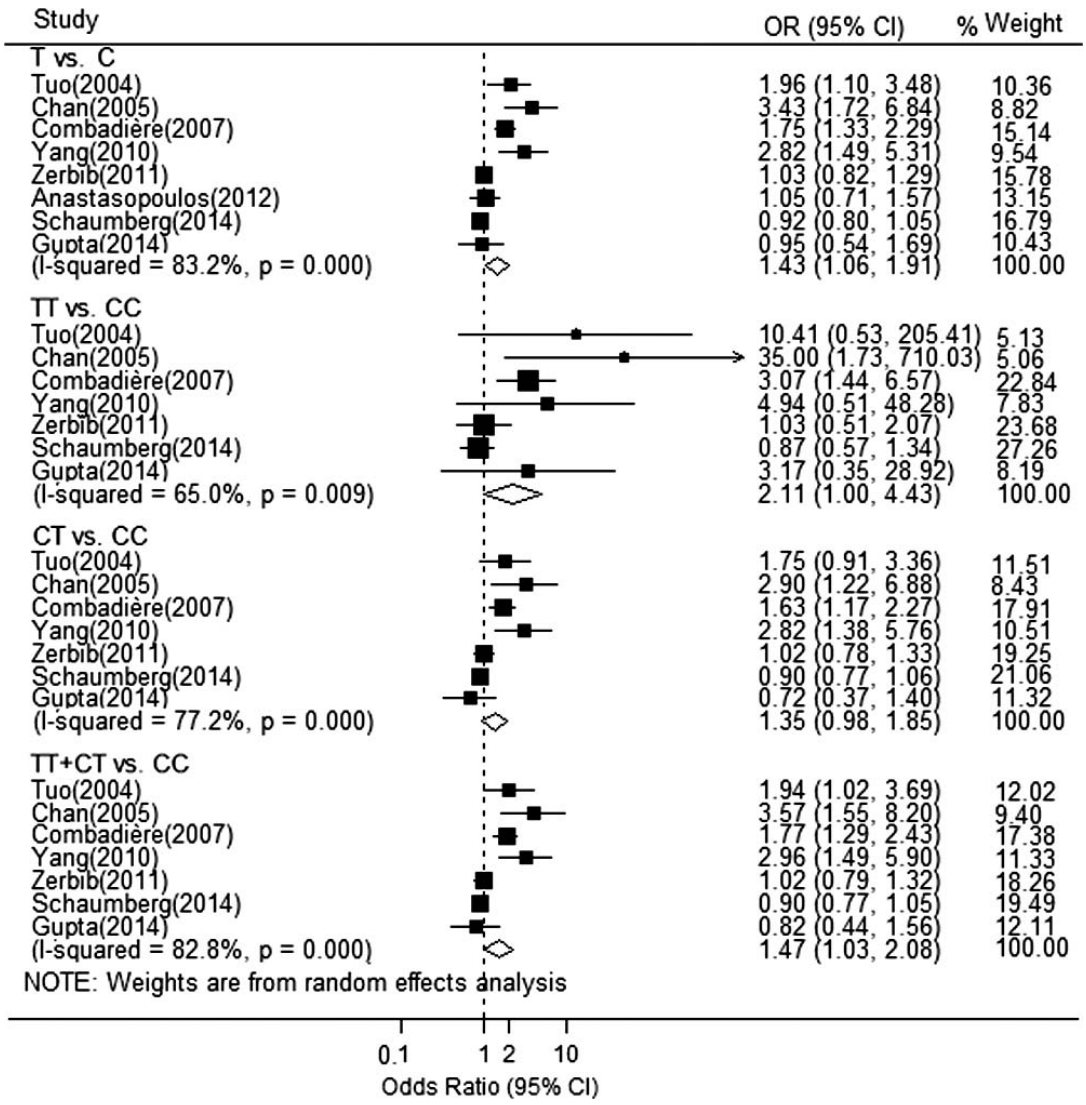


**Figure 2.** Forest plot on the association between the T280M polymorphism and AMD risk under four genetic models: allele frequency (T versus C), homozygote (TT versus CC), second codominant genotype (CT versus CC), and dominant genotype models (CT+TT versus CC). For each study, the estimation of OR and its 95% CI are plotted with a *box* and a *horizontal line*. The pooled OR is represented by a *diamond*. The area of the *gray squares* reflects the weight of the study in the meta-analysis.

(CT versus CC: OR = 1.35; 95% CI: 0.98–1.85; Fig. 2). In the subgroup analyses, according to the magnitude of LD between these two variants, studies with high LD showed a significantly stronger association between T280M and AMD risk (T versus C: OR = 1.89, 95% CI: 1.51–2.38, $P = 0.39$ for heterogeneity; TT versus CC: OR = 3.44, 95% CI: 1.71– 6.93, $P = 0.70$ for heterogeneity; CT versus CC: OR = 1.79, 95% CI: 1.36–2.35, $P = 0.39$ for heterogeneity; CT+TT versus CC: OR = 1.94, 95% CI: 1.49–2.52, $P = 0.41$ for heterogeneity), whereas this association was not demonstrated in the moderate LD group (Table 2). We subsequently analyzed the AMD subcategories separately and observed that the pooled OR remained similar in the direction and magnitude of the overall effects. Stratified analyses using other characteristics also did not substantially alter the association. Although the present meta-analyses showed relatively high heterogeneity, the sensitivity analysis showed minimal influence on the overall pooled results after the removal of each individual study. Furthermore, the Begg's funnel plots were symmetric upon visual inspection, and Egger's tests also indicated no evidence of publication bias ($P > 0.05$).

## V249I Polymorphism and AMD Risk

Six studies[14–16,18,25,27] focused on the relationship between V249I polymorphism and AMD susceptibility; and three of these studies revealed a statistically significant association between this variant and increased risk of AMD. For the homozygous model, there was no significant heterogeneity across the studies ($I^2 = 24.1\%$; $P = 0.26$ for heterogeneity), and the fixed-effects pooled OR revealed significant evidence for a relationship between the AA genotype and susceptibility to AMD (AA versus GG: OR = 1.27, 95% CI: 1.00–1.61; Fig. 3). Given the evidence of high heterogeneity across studies under other genetic models ($I^2$ range, 63.3%–68.7%; all $P < 0.05$), the random-effects model was therefore applied. The pooled OR showed a significant detrimental effect of the A allele on the risk of AMD under the allele model (A versus G: OR = 1.25, 95% CI: 1.01–1.55), but no significant association for the two additional genotype models (GA versus GG: OR = 1.20, 95% CI: 0.89–1.61; GA+AA versus GG: OR = 1.27, 95% CI: 0.93–1.73; Fig. 3). Similar to the T280M variant, the magnitude of LD between these two variants was slightly relevant to these

**Table 2.** Stratified Analysis of the Association Between the T280M Polymorphism and AMD

| | Allele Model | | | | | Genotype Model | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T vs. C | | | | | TT vs. CC | | | | | CT vs. CC | | | | CT+TT vs. CC | | | |
| Subgroup | *N* | OR (95% CI) | $I^2$, % | $P_z$ | $P_b$ | *N* | OR (95% CI) | $I^2$, % | $P_z$ | $P_b$ | OR (95% CI) | $I^2$, % | $P_z$ | $P_b$ | OR (95% CI) | $I^2$, % | $P_z$ | $P_b$ |
| Late AMD | 6 | 1.50 (1.04–2.17) | 78.9 | <0.01 | NA | 5 | 1.97 (0.83–4.68) | 52.2 | 0.08 | NA | 1.46 (0.98–2.18) | 73.8 | <0.01 | NA | 1.60 (1.04–2.47) | 80.0 | <0.01 | NA |
| CNV | 4 | 1.18 (0.85–1.66) | 69.3 | 0.02 | | 3 | 1.21 (0.76–1.92) | 0 | 0.43 | | 1.20 (0.79–1.83) | 75.7 | 0.02 | | 1.24 (0.81–1.91) | 78.7 | <0.01 | |
| GA | 1 | 1.62 (0.73–3.59) | NA | NA | | 0 | NA | NA | NA | | NA | NA | NA | | NA | NA | NA | |
| Ethnicity | | | | | | | | | | | | | | | | | | |
| Caucasians | 6 | 1.38 (1.01–1.88) | 84.8 | <0.01 | 0.79 | 6 | 1.90 (0.82–4.44) | 73.4 | <0.01 | 0.56 | 1.31 (0.94–1.82) | 77.5 | <0.01 | 0.83 | 1.43 (0.98–2.09) | 84.6 | <0.01 | 0.96 |
| Asians | 2 | 1.62 (0.56–4.70) | 83.2 | <0.01 | | 2 | 3.93 (0.80–19.22) | 0 | 0.784 | | 1.42 (0.37–5.39) | 86.7 | <0.01 | | 1.55 (0.44–5.44) | 86.0 | <0.01 | |
| Source of controls | | | | | | | | | | | | | | | | | | |
| PB | 6 | 1.51 (0.99–2.32) | 82.8 | <0.01 | 0.78 | 5 | 3.69 (0.86–15.87) | 63.9 | 0.03 | 0.57 | 1.47 (0.85–2.52) | 79.1 | <0.01 | 0.96 | 1.63 (0.90–2.93) | 83.8 | <0.01 | 0.72 |
| HB | 2 | 1.34 (0.80–2.24) | 88.4 | <0.01 | | 2 | 1.76 (0.60–5.15) | 76.9 | 0.04 | | 1.28 (0.81–2.02) | 78.7 | 0.03 | | 1.33 (0.78–2.29) | 85.9 | <0.01 | |
| Age of case, y | | | | | | | | | | | | | | | | | | |
| <75 | 4 | 1.18 (0.80–1.74) | 85.0 | <0.01 | 0.42 | 3 | 1.50 (0.50–4.54) | 39.4 | 0.19 | 0.60 | 1.17 (0.61–2.26) | 79.8 | <0.01 | 0.73 | 1.24 (0.63–2.42) | 82.0 | <0.01 | 0.55 |
| ≥75 | 3 | 1.80 (0.87–3.73) | 74.3 | <0.01 | | 3 | 5.06 (0.45–56.32) | 71.7 | 0.03 | | 1.56 (0.85–2.86) | 70.1 | 0.04 | | 1.77 (0.85–3.68) | 80.5 | <0.01 | |
| Classification criteria | | | | | | | | | | | | | | | | | | |
| AREDS | 4 | 1.96 (0.94–4.07) | 86.6 | <0.01 | 0.26 | 5 | 4.33 (0.68–27.72) | 70.4 | 0.02 | 0.47 | 1.77 (0.90–3.50) | 83.5 | <0.01 | 0.52 | 1.96 (0.92–4.19) | 87.6 | <0.01 | 0.43 |
| ICGS | 2 | 1.04 (0.85–1.26) | 0 | 0.93 | | 1 | 1.03 (0.51–2.07) | NA | NA | | 1.02 (0.78–1.33) | NA | NA | | 1.02 (0.79–1.32) | NA | NA | |
| Linkage disequilibrium | | | | | | | | | | | | | | | | | | |
| High | 3 | 1.89 (1.51–2.38) | 0 | 0.39 | 0.01 | 3 | 3.44 (1.71–6.93) | 0 | 0.70 | 0.08 | 1.79 (1.36–2.35) | 0 | 0.39 | 0.18 | 1.94 (1.49–2.52) | 0 | 0.41 | 0.04 |
| Moderate | 2 | 0.93 (0.82–1.06) | 0 | 0.52 | | 1 | 0.87 (0.57–1.34) | NA | NA | | 0.91 (0.77–1.06) | NA | NA | | 0.91 (0.77–1.05) | NA | NA | |
| Diagnostic criteria | | | | | | | | | | | | | | | | | | |
| FP | 6 | 1.54 (1.04–2.29) | 76.5 | <0.01 | 0.62 | 5 | 3.66 (0.97–13.73) | 53.3 | 0.07 | 0.45 | 1.51 (0.91–2.48) | 72.3 | 0.01 | 0.74 | 1.67 (0.97–2.85) | 77.8 | <0.01 | 0.57 |
| BCVA | 2 | 1.25 (0.67–2.35) | 94.3 | <0.01 | | 2 | 1.57 (0.46–5.39) | 87.5 | <0.01 | | 1.19 (0.67–2.12) | 89.9 | <0.01 | | 1.25 (0.64–2.41) | 93.0 | <0.01 | |
| Genotyping methods | | | | | | | | | | | | | | | | | | |
| PCR-RFLP | 4 | 1.76 (1.17–2.64) | 69.2 | 0.02 | 0.14 | 3 | 5.60 (1.45–21.66) | 30.5 | 0.24 | 0.09 | 1.75 (1.32–2.32) | 0 | 0.48 | 0.28 | 1.93 (1.48–2.52) | 15.7 | 0.31 | 0.05 |
| TaqMan | 2 | 1.63 (0.61–4.34) | 88.3 | <0.01 | | 2 | 1.53 (0.40–5.83) | 40.1 | 0.20 | | 1.60 (0.60–4.32) | 85.4 | 0.01 | | 1.65 (0.59–4.68) | 87.6 | <0.01 | |
| Sequencing | 2 | 0.92 (0.81–1.05) | 0 | 0.90 | | | 1.03 (0.44–2.44) | 20.7 | 0.26 | | 0.90 (0.77–1.04) | 0 | 0.52 | | 0.90 (0.77–1.04) | 0 | 0.79 | |

$P_b$, *P* for between-study heterogeneity; $P_z$, *P* for *z* test.

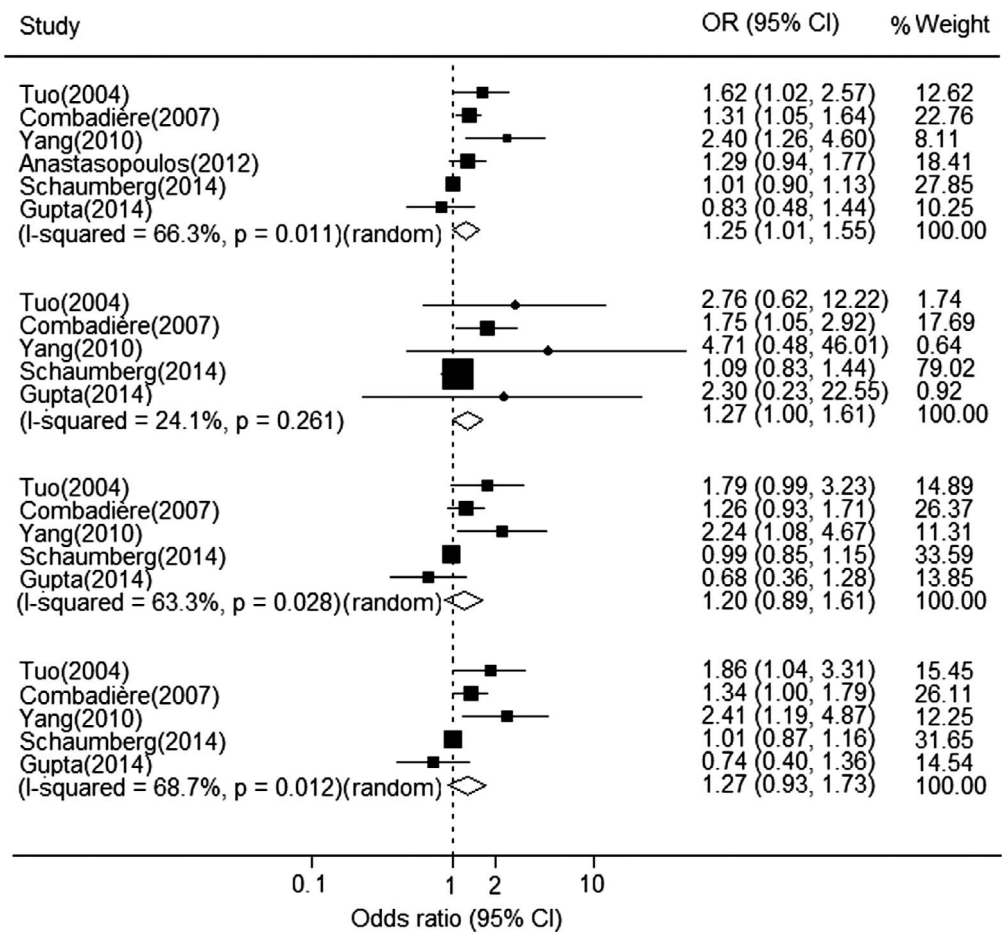


**Figure 3.** Forest plot on the association between the V249I polymorphism and AMD risk under four genetic models: allele frequency (A versus G), homozygote (AA versus GG), second codominant genotype (GA versus GG), and dominant genotype models (GA+AA versus GG). For each study, the estimation of OR and its 95% CI are plotted with a *box* and a *horizontal line*. The pooled OR is represented by a *diamond*. The area of the *gray squares* reflects the weight of the study in the meta-analysis.

results. Compared with a wider confidence interval for the moderate LD group, studies with high LD showed a significant association between V249I SNPs and increased AMD risk (Table 3). The relationship between V249I and AMD showed no obvious distinction among different subtypes of AMD. Other stratified analyses were conducted across participant characteristics, with no essential changes observed in the present study (Table 3). The sensitivity analysis did not yield a significant difference when any single study was excluded. Moreover, neither the Egger's test ($P > 0.05$) nor the Begg's test ($P > 0.05$) revealed significant publication bias for each allele and genetic model.

## Discussion

Evidence from the present meta-analysis showed that both the T280M and V249I polymorphisms in the *CX3CR1* gene were significantly associated with increased susceptibility of AMD. However, no significant effect on AMD risk was observed for individuals carrying only one risk allele. Additionally, the subgroup analysis indicated that these two risk SNPs were strongly associated with AMD events in the high LD group compared with the moderate LD group.

An increased amount of deposit in Bruch's membrane has been associated with the presence and severity of AMD.[28,29] Under normal circumstances, the continuous generation of macular deposition can be eliminated through inflammatory cells attracted to this site through chemokines to achieve a dynamic balance.[30] CX3CL1, the only identified CX3C chemokine, is widely expressed in the eye tissues. As the sole receptor for this protein, CX3CR1 is constitutively expressed in the retina and other ocular tissues, where this receptor protein mediates the migration of macrophages and microglia cells (MCs) to clear accumulated deposits.[31,32] The altered functions of inflammatory cells might be involved in the pathogenesis of AMD. Recently, several studies[14,15] have suggested that T280M and V249I, two common nonsynonymous SNPs located in *CX3CR1*, might be associated with the risk for AMD; but these results are inconsistent. The results of the present meta-analysis showed that both of these SNPs were significantly associated with the increased risk of AMD. Several functional studies[12,33] have suggested that these two loci decrease the number of fractalkine receptor binding sites and the binding affinity of the receptor to fractalkine, thus crippling chemokine activity. The inhibition of chemokine activity might in turn lead to the inadequate recruitment of inflammatory cells, such as macrophages and MCs, to ocular tissues where the age-related deposits are progressively accumulated.[31,34] These deposits play a specific role in the development of drusen and CNV, which are the chief characteristics of AMD.[35,36] Moreover, these accumulated deposits might affect the transport of macromolecules, such as oxygen, between the retina and the choroidal vessels, and injure RPE and photoreceptor cells,

**Table 3.** Stratified Analysis of the Association Between the V249I Polymorphism and AMD

| | Allele Model | | | | | Genotype Model | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A vs. G | | | | | AA vs. GG | | | | | GA vs. GG | | | | GA+AA vs. GG | | | |
| Subgroup | *N* | OR (95% CI) | $I^2$, % | $P_z$ | $P_b$ | *N* | OR (95% CI) | $I^2$, % | $P_z$ | $P_b$ | OR (95% CI) | $I^2$, % | $P_z$ | $P_b$ | OR (95% CI) | $I^2$, % | $P_z$ | $P_b$ |
| Late AMD | 4 | 1.55 (0.98–2.45) | 79.6 | <0.01 | NA | 3 | 1.55 (0.57–4.21) | 45.9 | 0.16 | NA | 1.46 (0.84–2.52) | 70.9 | 0.03 | NA | 1.52 (0.83–2.78) | 77.5 | 0.01 | NA |
| CNV | 3 | 1.41 (0.81–2.46) | 75.4 | 0.02 | | 2 | 1.39 (0.33–5.86) | 48.7 | 0.16 | | 1.38 (0.62–3.05) | 77.1 | 0.04 | | 1.43 (0.59–3.47) | 82.7 | 0.02 | |
| GA | 1 | 2.62 (1.35–5.06) | NA | NA | | 0 | NA | NA | NA | | NA | NA | NA | | NA | NA | NA | |
| Ethnicity | | | | | | | | | | | | | | | | | | |
| Caucasians | 4 | 1.22 (0.99–1.49) | 64.0 | 0.04 | 0.72 | 3 | 1.24 (0.98–1.57) | 46.0 | 0.16 | 0.70 | 1.18 (0.89–1.57) | 60.0 | 0.08 | 0.74 | 1.24 (0.92–1.69) | 68.8 | 0.04 | 0.74 |
| Asians | 2 | 1.40 (0.49–3.93) | 83.2 | 0.02 | | 2 | 3.28 (0.65–16.48) | 0 | 0.66 | | 1.22 (0.38–3.91) | 82.2 | 0.02 | | 1.32 (0.41–4.19) | 83.8 | 0.01 | |
| Source of controls | | | | | | | | | | | | | | | | | | |
| PB | 5 | 1.25 (0.95–1.66) | 67.4 | 0.02 | 1.00 | 4 | 1.30 (0.81–2.07) | 9.1 | 0.35 | 0.49 | 1.22 (0.78–1.89) | 68.6 | 0.02 | 0.99 | 1.28 (0.82–2.01) | 71.8 | 0.01 | 0.98 |
| HB | 1 | 1.31 (1.05–1.64) | NA | NA | | 1 | 1.75 (1.05–2.92) | NA | NA | | 1.26 (0.93–1.71) | NA | NA | | 1.34 (1.00–1.79) | NA | NA | |
| Age of case, y | | | | | | | | | | | | | | | | | | |
| <75 | 4 | 1.19 (0.88–1.60) | 67.0 | 0.03 | 0.68 | 4 | 1.24 (0.98–1.58) | 28.3 | 0.24 | 0.64 | 1.11 (0.82–1.51) | 61.9 | 0.05 | 0.66 | 1.18 (0.86–1.62) | 68.5 | 0.02 | 0.68 |
| ≥75 | 1 | 1.62 (1.02–2.57) | NA | NA | | 1 | 2.76 (0.62–12.32) | NA | NA | | 1.79 (0.99–3.23) | NA | NA | | 1.86 (1.04–3.31) | NA | NA | |
| Linkage disequilibrium | | | | | | | | | | | | | | | | | | |
| High | 3 | 1.54 (1.14–2.09) | 38.9 | 0.19 | 0.24 | 3 | 1.91 (1.19–3.07) | 0 | 0.62 | 0.38 | 1.51 (1.09–2.10) | 24.2 | 0.27 | 0.28 | 1.61 (1.16–2.24) | 29.2 | 0.24 | 0.29 |
| Moderate | 2 | 1.09 (0.87–1.37) | 52.8 | 0.15 | | 1 | 1.09 (0.83–1.44) | NA | NA | | 0.99 (0.85–1.15) | NA | NA | | 1.01 (0.87–1.16) | NA | NA | |
| Classification criteria | | | | | | | | | | | | | | | | | | |
| AREDS | 3 | 1.46 (0.88–2.45) | 80.1 | <0.01 | 0.78 | 3 | 1.52 (0.70–3.23) | 32.3 | 0.23 | NA | 1.45 (0.84–2.53) | 74.3 | 0.02 | NA | 1.53 (0.85–2.75) | 78.4 | 0.01 | NA |
| ICGS | 1 | 1.29 (0.94–1.77) | NA | NA | | 0 | NA | NA | NA | | NA | NA | NA | | NA | NA | NA | |
| Diagnostic criteria | | | | | | | | | | | | | | | | | | |
| FP | 4 | 1.39 (0.98–1.98) | 54.8 | 0.08 | 0.49 | 3 | 3.01 (1.01–8.97) | 0 | 0.90 | 0.55 | 1.38 (0.68–2.82) | 72.3 | 0.03 | 0.52 | 1.47 (0.73–2.96) | 72.8 | 0.03 | 0.52 |
| BCVA | 2 | 1.13 (0.88–1.45) | 76.2 | 0.04 | | 2 | 1.21 (0.95–1.54) | 60.9 | 0.11 | | 1.07 (0.86–1.34) | 48.2 | 0.17 | | 1.13 (0.86–1.48) | 66.9 | 0.08 | |
| Genotyping methods | | | | | | | | | | | | | | | | | | |
| PCR-RFLP | 3 | 1.34 (1.13–1.59) | 3.8 | 0.35 | 0.23 | 2 | 1.84 (1.14–2.98) | 0 | 0.57 | 0.37 | 1.36 (1.02–1.81) | 4.8 | 0.31 | 0.34 | 1.43 (1.10–1.85) | 0 | 0.89 | 0.35 |
| TaqMan | 1 | 2.40 (1.26–4.60) | NA | NA | | 1 | 4.71 (0.48–46.01) | NA | NA | | 2.24 (1.08–4.67) | NA | NA | | 2.41 (1.19–4.87) | NA | NA | |
| Sequencing | 2 | 1.00 (0.90–1.12) | 0 | 0.50 | | 2 | 1.10 (0.84–1.45) | 0 | 0.53 | | 0.94 (0.72–1.22) | 22.0 | 0.26 | | 0.99 (0.86–1.14) | 0 | 0.33 | |

which enhance the progression and severity of AMD.[37] On the other hand, a lower level of CX3CR1 expression is detected in the macular area than in the peripheral retina of AMD eyes, while control subjects exhibit almost identical CX3CR1 expression in both macular and peripheral retina.[13,16] Thus, relatively lower CX3CR1 expression levels in the cells from AMD patients might reflect the observed AMD susceptibility. However, it is impossible that these two variants directly affect the levels of transcription. DeVries et al.[38] have suggested a moderately strong LD between both these two SNPs in *CX3CR1* and the −15430G → C SNP located in the *CX3CR1* promoter region. This special relationship might affect the transcriptional level of CX3CR1 proteins, thereby contributing to the risk of AMD.

In contrast with the significant association between homozygosity and the increased occurrence of AMD, no significant effect on AMD risk was observed in individuals carrying only one risk allele, suggesting a dose–response relationship between the frequency of these mutations and AMD. Furthermore, studies with high LD showed a stronger association between these two variants and AMD risk, whereas this association was not demonstrated in the moderate LD group. Differences in the distribution of AMD-susceptible SNPs suggested that these two loci likely contribute to the risk of AMD through the haplotype containing both M280 and I249, which has been associated with many other diseases, such as HIV and atherosclerosis.[39,40] Owing to the high LD between these two variants, individuals homozygous for the M280 or I249 allele were more likely to be carriers containing both of these two SNPs. Furthermore, the possibility of the simultaneous occurrence of these two variants was increased along with the degree of LD between them. Therefore, the association observed in the present study might be associated with the potential effect of the haplotype of I249-M280 on the risk of AMD. However, further studies are required to assess the effects of M280-I249 haplotype on AMD risk.

There are several limitations to the present study that should be considered when interpreting these findings. First, the relatively small sample sizes might reduce the statistical power to assess the association between the two SNPs and susceptibility to AMD. Although some genome-wide association studies have investigated the association of these two variants with AMD, no studies have provided the relevant statistics. Hence, further studies with detailed information should be performed to validate this association. Second, some AMD susceptibility genes, such as *CFH* Y402H and *ARMS2* A69S, might affect the relationship of these two variants with AMD risk.[5] However, most of the included studies did not provide sufficient information to adjust for these genes, and the present study was primarily based on unadjusted estimates. Therefore, the effects of these AMD susceptibility genes on the relationship between T280M and V249I variants and AMD risk cannot be fully ruled out. Third, only a few studies have reported the frequency of smoking, obesity, and ω-3 fatty acid intake in AMD cases and control subjects. Therefore, we were unable to include these important covariates in this model, and subsequently could not assess the influence of gene–environment interactions owing to insufficient data in these areas. Fourth, the included studies were limited to observational studies in which selection bias might exist. Moreover, although no significant publication bias was detected, the potential bias could not be completely ruled out.

In conclusion, the findings of this present meta-analysis suggested that both T280M and V249I polymorphisms in the heterozygote state play an important role in the development of AMD. In addition, this association might be attributed to the haplotype of 249I-280M. Additional studies considering gene–gene and gene–environment interactions are still required to confirm these findings.

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