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# TNF-α -308 A/G and -238 A/G polymorphisms and susceptibility to glaucoma: a meta-analysis

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**ABSTRACT.** The purpose of this study was to examine whether tumor necrosis factor-α (TNF-α) -308 A/G and -238 A/G polymorphisms confer susceptibility to glaucoma. A meta-analysis was conducted examining the association between TNF-α -308 A/G and -238 A/G polymorphisms and glaucoma. A total of 13 studies on TNF-α -308 A/G and 238 A/G polymorphisms were included in this meta-analysis. The meta-analysis revealed no association between the TNF-α -308 A allele and glaucoma [odds ratio (OR) = 1.403, 95% confidence interval (CI) = 0.784-2.513, P = 0.254]. Subgroup analysis by disease type revealed no association between the TNF-α -308 A allele and glaucoma. The meta-analysis revealed no significant association between the TNF-α -238 A allele and glaucoma (OR = 1.120, 95%CI = 0.708-1.773, P = 0.628). This meta-analysis showed no association between the A alleles of the TNF-α -308 A/G or -238 A/G polymorphisms and glaucoma.

**Key words:** TNF-α; Meta-analysis; Polymorphism; Glaucoma

 

## INTRODUCTION

Glaucoma is a complicated disease in which damage to the optic nerve leads to progressive, irreversible vision loss; it is the second most prevalent cause of blindness. Although the etiology of glaucoma is not fully understood, genetic factors have been implicated (Burdon, 2012).

Tumor necrosis factor-α (TNF-α) is a potent pro-inflammatory cytokine that plays an important role in inflammatory and immune responses. TNF-α stimulates cytokine production, enhancing the expression of adhesion molecules and increasing neutrophil activation. The level of TNF-α is increased in the retina of glaucomatous eyes and it has been suggested that it plays an important role in glaucomatous degeneration and progression (Tezel et al., 2001). The *TNF* gene is located on chromosome 6, within the class III region of human leukocyte antigen (HLA), and several single nucleotide polymorphisms have been identified in its promoter (Allen, 1999). Of these polymorphisms, G-to-A substitutions at positions -308 (rs1800629) and -238 (rsrs361525) have been intensively studied, and some have suggested that these allelic variations could be of functional significance (D'Alfonso and Richiardi, 1994). However, published results are inconsistent (Wilson et al., 1997). Several studies have examined the potential contributions made by TNF-α promoter polymorphisms to glaucoma susceptibility (Lin et al., 2003; Funayama et al., 2004; Tekeli et al., 2008; Khan et al., 2009; Mossböck et al., 2006, 2009; Razeghinejad et al., 2009; Fan et al., 2010; Bozkurt et al., 2012; Buentello-Volante et al., 2013), but the findings of these studies are mixed, probably owing to small sample sizes and low statistical power.

Individual studies with small sample sizes have insufficient power to detect a positive association and lack the power to demonstrate an absence of association. Meta-analysis is a powerful method that can be used to overcome the problem of small sample sizes and inadequate statistical power in genetic studies of complex traits (Nath et al., 2005; Choi et al., 2006; Lee et al., 2006b). Accordingly, we conducted a meta-analysis to determine whether TNF-α -308 A/G and -238 A/G polymorphisms contribute to susceptibility to glaucoma.

## MATERIAL AND METHODS

### Identification of eligible studies and data extraction

A literature search for studies that examined the association between the TNF-α -308 A/G and -238 A/G polymorphisms and glaucoma was conducted. We utilized the MEDLINE and EMBASE citation indices to identify articles published in January 2013 in which the TNF-α -308 A/G and -238 A/G polymorphisms were identified in glaucoma patients and controls. Additionally, all references in the identified articles were reviewed to identify studies not indexed by MEDLINE and EMBASE. The following key words and subject terms were used: "tumor necrosis factor"; "TNF-α"; and "glaucoma". Studies were included in the analysis if they: 1) were case control studies; 2) contained genotype data; and 3) contained sufficient data to calculate odds ratios (ORs). No language restriction was applied. We excluded the following: 1) studies containing overlapping data; 2) studies in which the number of null and wild genotypes could not be ascertained; and 3) studies in which family members had been studied, as these analyses are based on linkage considerations. The following information was extracted from each identified study: author, year of publication, ethnicity of the study population, demographics, subtype of glaucoma, number of cases and controls, and the frequency of

the genotypes and alleles of the TNF-α polymorphisms. Glaucoma was categorized into three subgroups: primary open-angle glaucoma (POAG), pseudoexfoliation glaucoma (PEXG), and others such as exfoliation or chronic angle closure glaucoma. Two independent reviewers extracted data from original studies and any discrepancies between the reviewers were resolved by consensus or by a third reviewer.

### Evaluation of publication bias and study quality

The chi-square test was used to determine if the observed genotype frequencies in controls conformed to Hardy-Weinberg expectations. Funnel plots are typically used to detect publication bias, but they require a range of studies of varying sizes and subjective judgments. Thus, we evaluated publication bias using the Egger linear regression test (Egger et al., 1997a). The Egger linear regression test measures funnel plot asymmetry on a natural logarithmic scale of ORs.

### Evaluation of statistical associations

We performed meta-analyses using 1) allelic contrast, and 2) the dominant model, because the frequency of the AA genotype of the polymorphisms was too low to perform meta-analyses using the recessive model and homozygote contrast. Point estimates of risks, ORs, and 95% confidence intervals (CI) were estimated for each study. Additionally, within- and between-study variations and heterogeneities were assessed using Cochran's Q-statistic. The Cochran Q-statistic test assesses the null hypothesis that all the studies evaluated have the same effect. The effect of heterogeneity was quantified using $I^2$, with a range of 0-100%, which represents the proportion of between-study variability attributable to heterogeneity rather than chance (Higgins and Thompson, 2002). $I^2$ values of 25, 50, and 75% were nominally assigned as low, moderate, and high estimates, respectively. The fixed-effect model assumes that a genetic factor has a similar effect on disease susceptibility across all studies investigated and that observed variations among studies are caused by chance alone (Egger et al., 1997b). The random-effect model assumes that different studies show substantial diversity and assesses both within-study sampling error and between-study variance (DerSimonian and Laird, 1986). When study groups are homogeneous, the two models are similar. If the study groups lack homogeneity, the random-effect model usually provides wider CIs than the fixed-effect model. The random-effect model is most appropriate in the presence of significant between-study heterogeneity (DerSimonian and Laird, 1986). Statistical manipulations were performed using the Comprehensive Meta-Analysis program (Biostat, Englewood, NJ, USA). The power of each study was computed as the probability of detecting an association between the TNF-α polymorphisms and glaucoma using a significance level of 0.05 and assuming an OR of 1.5 (small effect size). Power analysis was performed using G*Power (http://www.psycho.uni-duesseldorf.de/aap/projects/gpower).

## RESULTS

### Studies included in the meta-analysis

Seventy studies were identified using electronic and manual searches, and 11 of these were selected for full-text review based on title and abstract details (Lin et al., 2003;

Funayama et al., 2004; Tekeli et al., 2008; Khan et al., 2009; Mossböck et al., 2006, 2009; Razeghinejad et al., 2009; Fan et al., 2010; Bozkurt et al., 2012; Wang et al., 2012; Buentello-Volante et al., 2013). One study was excluded because it contained other polymorphism data (Wang et al., 2012). Thus, a total of ten studies met our inclusion criteria (Lin et al., 2003; Funayama et al., 2004; Tekeli et al., 2008; Khan et al., 2009; Mossböck et al., 2006, 2009; Razeghinejad et al., 2009; Fan et al., 2010; Bozkurt et al., 2012; Buentello-Volante et al., 2013) (Figure 1). One of the eight eligible studies contained data from three different glaucoma groups, and was therefore treated independently in the meta-analysis of subtypes of glaucoma (Razeghinejad et al., 2009). Thus, a total of ten studies of TNF-α -308 A/G polymorphisms consisting of 1798 cases and 1683 controls included three European, three Asian, two Turkish, one Latin American, and one Arab population; and three studies of TNF-α -238 A/G polymorphisms consisting of 404 cases and 625 controls included two European and one Turkish population. The ethnicity-specific meta-analysis was restricted to European, Asian, and Turkish populations. Selected details of the individual studies are summarized in Table 1. The statistical power of these studies ranged from 24.7 to 70.8%. None of the studies had a statistical power exceeding 80%.

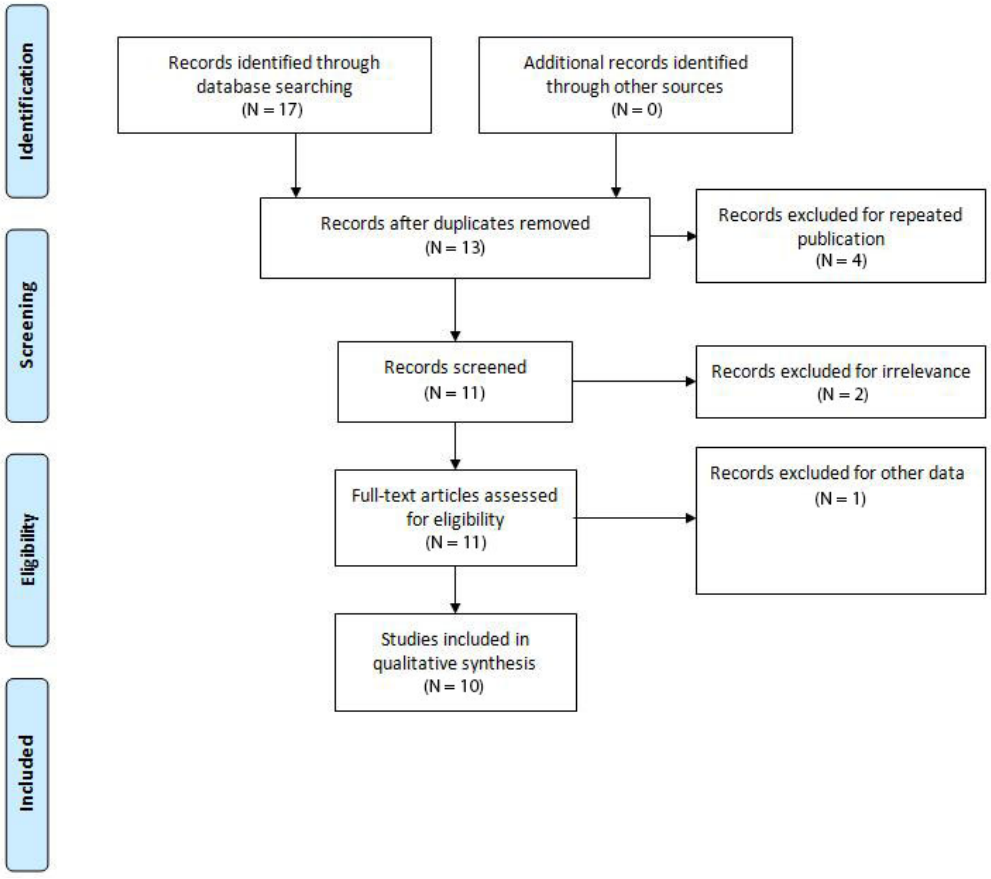


**Figure 1.** Study flow chart.

**Table 1.** Characteristics of the studies included in the meta-analysis.

**A.** TNF-α -308 A/G polymorphism

| Reference | Ethnicity | Numbers | | Case | | | Control | | | HWE P | Association P | Power (%)[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Case | Control | GG | GA | AA | GG | GA | AA | | | |
| Buentello-Volante et al., 2013 | Latin American | 118 | 100 | 109 | 9 | 0 | 93 | 6 | 1 | 0.122 | 0.920 | 31.4 |
| Bozkurt et al., 2012 | Turkish | 86 | 193 | 66 | 19 | 1 | 171 | 21 | 1 | 0.702 | 0.013 | 38.6 |
| Fan et al., 2010 | Asian | 395 | 201 | 350 | 45 | 0 | 167 | 29 | 5 | 0.029 | 0.012 | 68.4 |
| Khan et al., 2009 | European | 122 | 126 | 53 | 39 | 30 | 110 | 13 | 3 | 0.020 | 0.000 | 35.0 |
| Razeghinejad et al., 2009 | Arab | 178 | 200 | 151 | 26 | 1 | 190 | 10 | 0 | 0.612 | 0.001 | 49.3 |
| Mossböck et al., 2009 | European | 204 | 204 | 152 | 48 | 4 | 151 | 51 | 2 | <0.001 | 0.001 | 52.3 |
| Tekeli et al., 2008 | Turkish | 110 | 110 | 103 | 7 | 0 | 92 | 18 | 0 | 0.205 | 0.755 | 31.7 |
| Mossböck et al., 2006 | European | 114 | 228 | 79 | 35 | 0 | 161 | 61 | 6 | 0.938 | 0.919 | 45.6 |
| Funayama et al., 2004 | Asian | 411 | 218 | 403 | 8 | 0 | 212 | 6 | 0 | 0.772 | 0.029 | 70.8 |
| Lin et al., 2003 | Asian | 60 | 103 | 28 | 13 | 19 | 66 | 30 | 7 | 0.192 | 0.824 | 24.7 |

**B.** TNF-α -238 A/G polymorphism

| Reference | Ethnicity | Numbers | | Case | | | Control | | | HWE P | Association P | Power (%)[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Case | Control | GG | GA | AA | GG | GA | AA | | | |
| Bozkurt et al., 2012 | Turkish | 86 | 193 | 79 | 7 | 0 | 180 | 13 | 0 | 0.500 | 0.681 | 38.6 |
| Mossböck et al., 2009 | European | 204 | 204 | 182 | 21 | 1 | 189 | 15 | 0 | 0.449 | 0.187 | 52.3 |
| Mossböck et al., 2006 | European | 114 | 228 | 107 | 7 | 0 | 205 | 23 | 0 | 0.268 | 0.239 | 45.6 |

HWE = Hardy-Weinberg equilibrium; [a]Assuming an odds ratio of 1.5 (small effect size) at a level of significance of 0.05.

## Frequency of the A allele of the TNF-α -308 and TNF-α -238 A/G polymorphisms by ethnicity

The mean frequency of the A allele of the TNF-α -308 A/G polymorphism was 8.8% among all healthy controls. Arabs had a lower A allele prevalence rate than the other ethnic groups (2.5%). Among healthy controls, the frequency of the TNF-α -308 A allele in the Arab, Latin American, Turkish, Asian, and European populations was 2.5, 4.0, 6.8, 8.5, and 13.2, respectively. The mean frequency of the A allele of the TNF-α -238 A/G polymorphism was 4.1% overall among controls, and the Turkish control population had a lower A allele prevalence rate than the European controls (3.4 *vs*. 4.4%) (Table 2).

**Table 2.** Prevalence rates of the A allele of the TNF-α -308 (A) and TNF-α -238 (B) polymorphisms.

| **A.** | | | | | |
|---|---|---|---|---|---|
| Population | No. of studies | Numbers | | A allele (%) | |
| | | Case | Control | Case | Control |
| European | 3 | 440 | 558 | 21.6 | 13.2 |
| Asian | 3 | 856 | 522 | 6.0 | 8.5 |
| Turkish | 2 | 196 | 303 | 7.1 | 6.8 |
| Latin American | 1 | 118 | 100 | 3.8 | 4.0 |
| Arab | 1 | 178 | 200 | 7.9 | 2.5 |
| Overall | 10 | 1798 | 1683 | 10.0 | 8.8 |
| **B.** | | | | | |
| Population | No. of studies | Numbers | | A allele (%) | |
| | | Case | Control | Case | Control |
| European | 2 | 318 | 432 | 4.7 | 4.4 |
| Turkish | 1 | 86 | 193 | 4.1 | 3.4 |
| Overall | 3 | 404 | 625 | 4.6 | 4.1 |

## Meta-analysis of the association between the TNF-α -308 A/G polymorphism and glaucoma

A meta-analysis of all glaucoma patients and of each ethnic group was performed. A summary of the meta-analysis findings regarding the relationship between the TNF-α -308 A/G polymorphism and glaucoma is provided in Table 3. The meta-analysis revealed no association between the TNF-α -308 A allele and glaucoma (OR = 1.403, 95%CI = 0.784-2.513, P = 0.254) (Table 3). Stratification by ethnicity indicated no association between the TNF-α -308 A allele and glaucoma in European, Asian, and Turkish populations (OR = 1.990, 95%CI = 0.557-7.113, P = 0.289; OR = 1.049, 95%CI = 0.329-3.344, P = 0.936; OR = 0.927, 95%CI = 0.162-5.320, P = 0.932, respectively) (Table 3 and Figure 2). Subgroup analysis by disease type revealed no association between the TNF-α -308 A allele and glaucoma in POAG, PEXG, and others. Analysis using the dominant model showed the same pattern for the TNF-α -308 A allele (Table 3).

**Table 3.** Meta-analysis of the association between the TNF-α -308 A/G polymorphism and glaucoma.

| Polymorphism | Population | No. of studies | Test of association | | | Test of heterogeneity | | |
|---|---|---|---|---|---|---|---|---|
| | | | OR | 95%CI | P value | Model | P value | $I^2$ |
| TNF-α -308 | Overall | 10 | 1.403 | 0.784-2.513 | 0.254 | R | 0.000 | 89.9 |
| A *vs* G | HWE | 7 | 1.323 | 0.758-2.309 | 0.325 | R | 0.000 | 78.5 |
| | European | 3 | 1.990 | 0.557-7.113 | 0.289 | R | 0.000 | 95.7 |
| | Asian | 3 | 1.049 | 0.329-3.344 | 0.936 | R | 0.000 | 91.0 |
| | Turkish | 2 | 0.927 | 0.162-5.320 | 0.932 | R | 0.001 | 90.3 |
| | POAG | 7 | 1.365 | 0.771-2.415 | 0.286 | R | 0.000 | 82.9 |
| | PEXG | 3 | 2.445 | 0.398-15.00 | 0.334 | R | 0.000 | 94.2 |
| | Others | 2 | 1.038 | 0.708-1.521 | 0.850 | F | 0.788 | 0 |
| AA + AG *vs* GG | Overall | 10 | 1.413 | 0.807-2.472 | 0.226 | R | 0.000 | 86.4 |
| (Dominant) | HWE | 7 | 1.315 | 0.774-2.233 | 0.312 | R | 0.002 | 71.1 |
| | European | 3 | 2.063 | 0.586-7.268 | 0.260 | R | 0.000 | 94.3 |
| | Asian | 3 | 0.981 | 0.431-2.235 | 0.914 | R | 0.015 | 76.1 |
| | Turkish | 2 | 0.929 | 0.142-6.059 | 0.939 | R | 0.001 | 90.8 |
| | POAG | 7 | 1.392 | 0.836-2.319 | 0.204 | R | 0.001 | 73.5 |
| | PEXG | 3 | 2.427 | 0.359-16.39 | 0.363 | R | 0.000 | 93.9 |
| | Others | 2 | 0.998 | 0.655-1.521 | 0.994 | F | 0.737 | 0 |

OR = odds ratio; CI = confidence interval; HWE = Hardy-Weinberg equilibrium; POAG = primary open-angle glaucoma; PEXG = pseudoexfoliation glaucoma; F = fixed model; R = random model.

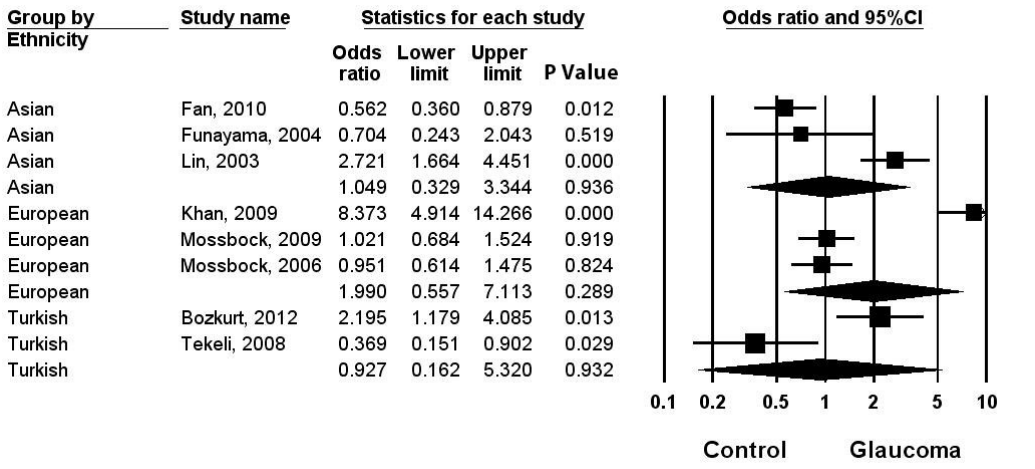


**Figure 2.** Odds ratios (ORs) and 95% confidence intervals (CIs) of individual studies and pooled data for the association between the A allele of the TNF-α -308 A/G polymorphism and glaucoma in each ethnic group.

## Meta-analysis of the association between the TNF-α -238 A/G polymorphism and glaucoma

The meta-analysis revealed no significant association between the TNF-α -238 A allele and glaucoma (OR = 1.120, 95%CI = 0.708-1.773, P = 0.628) (Table 4). Stratification by ethnicity indicated no association between the TNF-α -238 A allele and glaucoma in Europeans or Asians (OR = 1.006, 95%CI = 0.392-2.581, P = 0.990; OR = 1.217, 95%CI = 0.477-3.107,

P = 0.681) (Table 4 and Figure 3). Subgroup analysis by disease type revealed no association between the TNF-α -238 A allele and glaucoma in POAG and PEXG (Table 4). Analysis using the dominant model showed the same pattern for the TNF-α -238 A allele (Table 4).

**Table 4.** Meta-analysis of the association between the TNF-α -238 A/G polymorphism and glaucoma.

| Polymorphism | Population | No. of studies | Test of association | | | Test of heterogeneity | | |
|---|---|---|---|---|---|---|---|---|
| | | | OR | 95%CI | P value | Model | P value | $I^2$ |
| TNF-α -238 | | | | | | | | |
| A *vs* G | Overall | 3 | 1.120 | 0.708-1.773 | 0.628 | F | 0.217 | 34.6 |
| | European | 2 | 1.006 | 0.392-2.581 | 0.990 | R | 0.082 | 66.8 |
| | Turkish | 1 | 1.217 | 0.477-3.107 | 0.681 | NA | NA | NA |
| | POAG | 2 | 0.827 | 0.439-1.559 | 0.557 | F | 0.272 | 17.1 |
| | PEXG | 1 | 1.565 | 0.805-3.045 | 0.187 | NA | NA | NA |
| AA + AG *vs* GG | | | | | | | | |
| (Dominant) | Overall | 3 | 1.096 | 0.685-1.756 | 0.702 | F | 0.232 | 31.5 |
| | European | 2 | 0.981 | 0.384-2.506 | 0.968 | R | 0.091 | 64.9 |
| | Turkish | 1 | 1.227 | 0.472-3.192 | 0.675 | NA | NA | NA |
| | POAG | 2 | 0.819 | 0.429-1.564 | 0.546 | F | 0.261 | 20.7 |
| | PEXG | 1 | 1.523 | 0.766-3.028 | 0.230 | NA | NA | NA |

OR = odds ratio; CI = confidence interval; POAG = primary open-angle glaucoma; PEXG = pseudoexfoliation glaucoma; F = fixed model; R = random model, NA = not available.

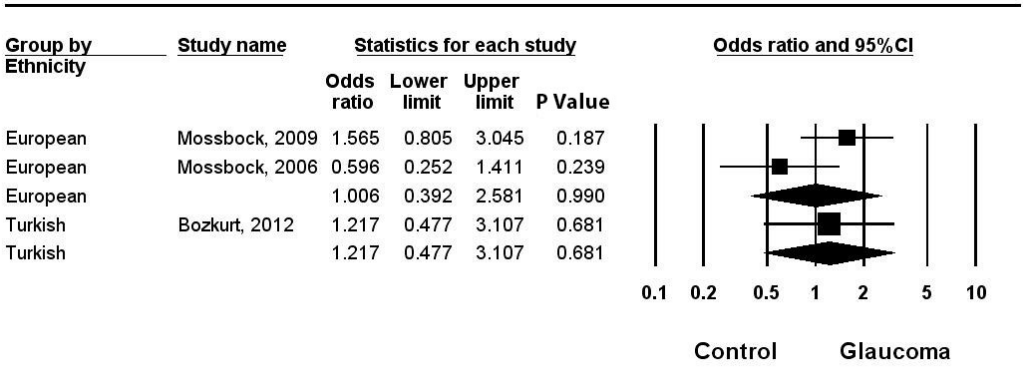


**Figure 3.** Odds ratios (ORs) and 95% confidence intervals (CIs) of individual studies and pooled data for the association between the G allele of the TNF-α -238 A/G polymorphism and glaucoma in each ethnic group.

## Heterogeneity and publication bias

The distribution of genotypes of the TNF-α -308 A/G polymorphism in control groups was consistent with the Hardy-Weinberg equilibrium (HWE), except for three studies, which could imply bias in terms of control selection or genotyping errors (Khan et al., 2009; Mossböck et al., 2009; Fan et al., 2010). When we excluded these studies, the overall result was not substantially affected in the meta-analysis of the TNF-α -308 A/G polymorphism (Table 3). Between-study heterogeneity was found in the meta-analyses of the TNF-α -308 A/G and -238 A/G polymorphisms (Tables 3 and 4). However, no heterogeneity was identified in the meta-analysis of the TNF-α -308 A/G polymorphism in others and the TNF-α -238 A/G polymorphism in the overall group (Tables 3 and 4). Publication bias results in a disproportionate

number of positive studies being published, which poses a problem for meta-analyses. However, no evidence of publication bias was found in the meta-analyses of the A alleles of the TNF-α -308 A/G and -238 A/G polymorphisms across all study subjects (Egger's regression P values = 0.974, 0.580) (Figure 4).

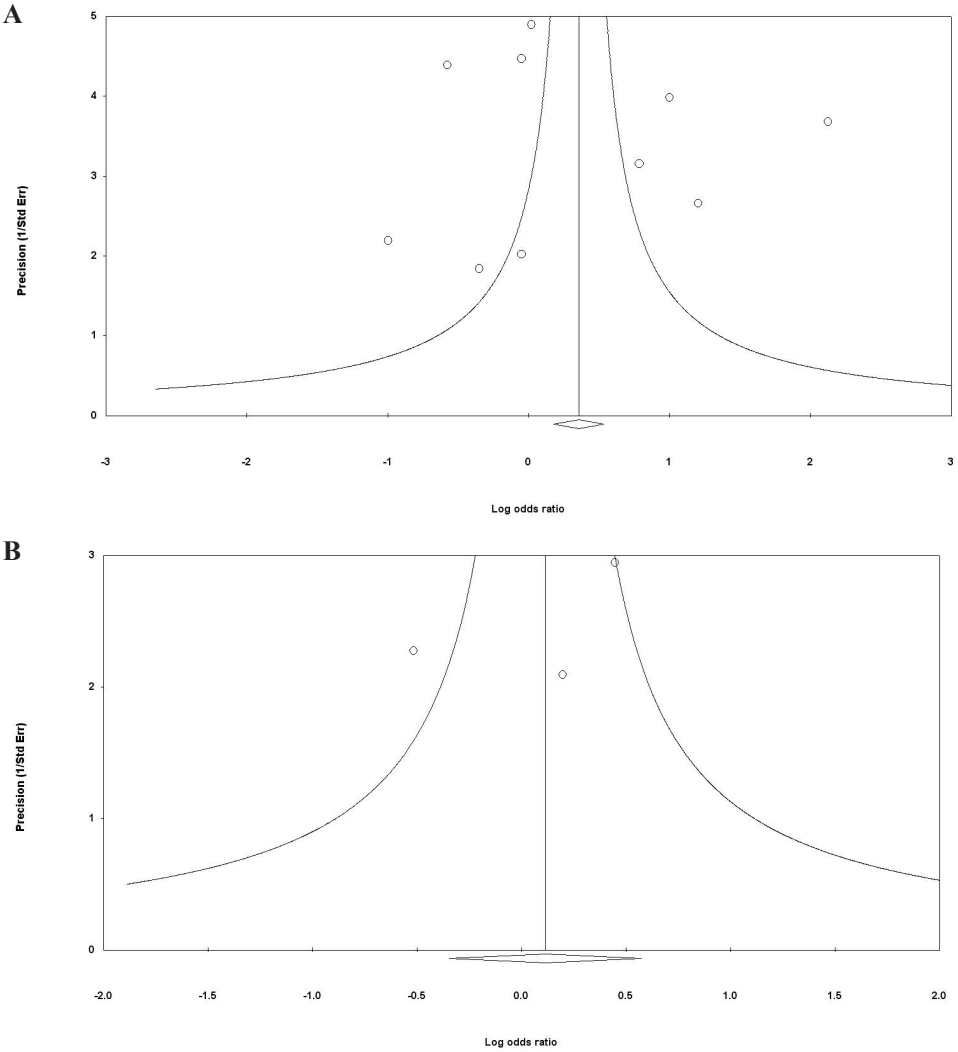


**Figure 4.** Funnel plot of studies for the association between the TNF-α -308A (**A**) and TNF-α -238A (**B**) allele and glaucoma in all subjects (Egger's regression P values = 0.974, 0.580).

## DISCUSSION

The region spanning the TNF cluster has been implicated in susceptibility to numerous immunopathological diseases, including glaucoma. The class III region of the HLA

gene, including the TNF-α gene, lies between the class I and II regions and contains genes that are important for the innate immune system, including the complement components C2 and C4 (DerSimonian and Laird, 1986). Furthermore, the TNF-α -308 A/G and -238 A/G polymorphisms are associated with several autoimmune disorders (Lee et al., 2006a, 2007, 2012). However, studies of the associations between these two polymorphisms and glaucoma have reported conflicting results (Lin et al., 2003; Funayama et al., 2004; Tekeli et al., 2008; Khan et al., 2009; Mossböck et al., 2006, 2009; Razeghinejad et al., 2009; Fan et al., 2010; Bozkurt et al., 2012; Buentello-Volante et al., 2013), which is not altogether surprising. Persistent difficulties regarding robust and replicable results in genetic association studies are almost certainly attributable to small contributions by genetic phenomena. None of the ten individual studies showed a statistical power greater than 80% to detect an association between TNF-α -308 A/G or -238 A/G polymorphism and glaucoma. Thus, many thousands of study subjects are required for adequate statistical power. Meta-analysis can be used as an alternative.

In this meta-analysis, we combined evidence of the associations between the TNF-α -308 A/G and -238 A/G polymorphisms, and susceptibility to glaucoma. Our results revealed no association between TNF-α -308 A/G and -238 A/G polymorphisms and glaucoma in all subjects. Furthermore, subsequent meta-analysis after stratification by ethnicity and disease subtype revealed no significant associations between the two polymorphisms and glaucoma. The prevalence of the A allele was found to vary among ethnic controls from 2.5 to 13.2% for the TNF-α -308 polymorphism and from 3.4 to 4.4% for the TNF-α -238 A/G polymorphism. The frequency of the TNF-α -308A allele was lowest among Arabs and highest among Europeans. However, meta-analysis failed to reveal any association between the TNF-α -308 A/G and -238 A/G polymorphisms and glaucoma in each ethnic group.

Our meta-analysis results are not consistent with functional studies of TNF-α that have suggested TNF-α involvement in glaucoma pathogenesis. TNF-α is upregulated in optic nerve microglia and astrocytes of glaucoma patients (Tezel et al., 2001). TNF-α plays a critical role in optic neuropathy through inflammatory pathways and leads to the apoptotic death of retinal ganglion cells in glaucoma (Nakazawa et al., 2006). However, because glaucoma is a complex disease, epidemiologic results sometimes do not coincide with the results of functional studies. Multiple genes, genetic backgrounds, and environmental factors contribute to glaucoma development. The lack of any association between the TNF-α -308 A/G and -238 A/G polymorphisms and glaucoma can be explained in four ways. First, genetic heterogeneity for glaucoma may exist in different populations. In fact, genetic association studies on glaucoma have demonstrated genetic heterogeneity (Lin et al., 2003; Funayama et al., 2004; Tekeli et al., 2008; Khan et al., 2009; Mossböck et al., 2006, 2009; Razeghinejad et al., 2009; Fan et al., 2010; Bozkurt et al., 2012; Buentello-Volante et al., 2013). Second, clinical heterogeneities and differences between patient populations may be responsible. Third, discrepancies may be caused by different linkage disequilibrium (LD) patterns. For example, these polymorphisms may be in LD with a nearby causal variant in one ethnic group but not in another. Fourth, our meta-analysis results might be simply due to type II error.

This meta-analysis differs from a previous meta-analysis on the relationship between the TNF-α -308 A/G polymorphism and glaucoma risk performed by Yu and Yao (2012). Our study included three more studies on the TNF-α -308 A/G polymorphism, 599 more glaucoma patients, 494 more controls (Fan et al., 2010; Bozkurt et al., 2012; Buentello-Volante et al., 2013), a subgroup analysis by excluding studies not consistent with HWE (Khan et al., 2009;

Mossböck et al., 2009; Fan et al., 2010), and an additional meta-analysis including the TNF-α -238 A/G polymorphism. Nonetheless, the results of our meta-analysis of the association between the TNF-α -308 A/G polymorphism and the development of glaucoma were in agreement with the previous study.

The present study has some limitations that should be considered. First, heterogeneity and confounding factors may have distorted the analysis. In particular, significant heterogeneity has been found among most meta-analyses, and this heterogeneity may prevent conclusions on the absence of an association between the TNF polymorphisms and glaucoma. Second, there are varying levels of severity for each disease, and the activity level of glaucoma was unclear. Further research is required to examine whether an association exists between TNF-α -308 A/G and -238 A/G polymorphisms and the activity or clinical features of glaucoma (Yamaguchi et al., 2001). Third, we only included data from European, Asian, and Turkish patients in our ethnicity-specific meta-analysis. Our ethnicity-associated results are only applicable to these ethnic groups.

This meta-analysis demonstrated no association between the TNF-α -308 A/G and -238 A/G polymorphisms and glaucoma, and thus, our findings do not support the notion that the TNF-α -308 A/G and -238 A/G polymorphisms play important roles in glaucoma. However, this study does show that the prevalence of the TNF-α -308 A allele is ethnicity dependent. Larger-scale studies in populations with different ethnicities are required to further explore the relationship between polymorphisms in the TNF-α gene and the pathogenesis of glaucoma.

## Conflicts of interest

The authors declare no conflict of interest.

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