

Associations of TLR4 and IL-8 genes polymorphisms with Age-related macular degeneration (AMD): a systematic review and meta-analysis

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Abstract

Background

The results of different studies have indicated the possible associations of TLR4 and IL-8 genes polymorphisms with Age-Related Macular Degeneration (AMD). A meta-analysis study was designed to evaluate the possible associations of TLR4 and IL-8 genes polymorphisms with Age-Related Macular Degeneration (AMD).

Method

A systematic literature search was carried out in PubMed, Embase, Web of Science, and Scopus databases to identify relevant publications. Pooled Odds Ratio (OR) with 95% Confidence Interval (CI) was used to evaluate the power of association.

Results

A total of 12 case-control studies with 4804 AMD patients and 4422 healthy controls were included in this meta-analysis. We found significant associations under the genotypic and allelic models of TLR4/rs4986790 (AA vs. AG+GG: OR=0.73 [0.55-0.97], $P=0.03$; and AA vs. AG: OR=0.71 [0.53-0.95], $P=0.02$) and IL-8/rs2227306 (TT vs. CC: OR=1.63 [1.04-2.56], $P=0.03$; CC vs. TT+TC: OR=0.62 [0.48-0.80], $P<0.001$; CC vs. TC: OR=0.65 [0.47-0.89], $P=0.007$; and C vs. T: OR=0.71 [0.64-0.78], $P<0.001$). However, the data from this meta-analysis declined the associations of TLR4/rs4986791 and IL-8/rs4073 polymorphisms.

Conclusion

The current meta-analysis study suggested that IL-8/rs2227306 and TLR4/rs4986790 polymorphisms are associated with susceptibility to AMD.

Background

Age-related Macular Degeneration (AMD) is the leading cause of irreversible central visual loss in the geriatric population.¹ The primary visible clinical symptom of AMD is called drusen, the yellow sediments in the Bruch's membrane beneath the Retinal Pigment Epithelial (RPE) and photoreceptor cells.² The advanced AMD can be classified clinically into two types: dry (geographic atrophy) and wet (choroidal neovascularization). Although the exact cause of AMD is not entirely known, the results obtained from different studies have demonstrated that the environmental and genetic factors, as well as their impacts, are possible risk factors for AMD. Non-genetic risk factors can be attributed to aging, smoking, gender, race, body mass index, and high-fat diet.³ Based on the pathological features of AMD, the role of dysregulation of inflammatory and immune responses in the etiology of the disease has been demonstrated.⁴

Toll-Like Receptors (TLRs) play a key role in defending the immune system.⁵ TLR4 as a surface receptor for lipopolysaccharides (LPS) is highly expressed in lymphocyte, neutrophil, and monocytes.⁶ Chen *et al.* showed that the TLR4 signaling pathway is a possible mechanism involved in stimulating inflammatory and angiogenic factors in the RPE cells.⁷ Another study indicated that the mRNA level of TLR4 is increased in mice with the retinal degeneration.⁸

Interleukin-8 (IL-8) or C-X-C motif chemokine Ligand 8 (CXCL8) is one of the main inflammatory cytokine and a potent chemoattractant factor, which is linked to the pathogenesis of AMD.⁹ Amyloid-beta as a drusen compound can induce inflammatory cytokine activation (such as IL-1 β and IL-8) in the RPE cells.¹⁰ IL-8 is expressed by cells of the immune system, vascular endothelial, and the RPE cells.¹¹ The investigation has suggested the possible impact of IL-8 level in inflammatory and angiogenic processes, leading to the exudative AMD.¹²

The meta-analysis method is a valuable statistical method that is used by combining multiple studies with the same subject to obtain a general result. To the best of our knowledge, the previous AMD meta-analysis studies have not been included for the TLR4 (rs4986790 and rs4986791) and IL-8 (rs4073 and rs2227306) polymorphisms. Thus, this meta-analysis was

performed based on eligible published literatures to a more accurate description of the potential association between those polymorphisms and AMD.

Method

Search strategy

A comprehensive literature search was conducted to identify all eligible studies regarding TLR4 and IL-8 polymorphisms and AMD risk in PubMed, Web of Science, Scopus databases for articles in English, and SID, Magiran, IranMedex, and IranDoc for articles in Persian up to April 2020 according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist and PICO approach.¹³ The following keywords were used: “Age-related macular degeneration OR AMD”, “interleukin-8 OR IL-8 OR CXCL8 OR C-X-C motif chemokine ligand 8” and “Toll-like Receptor 4 OR TLR4”.

The flowchart of the articles selection process is shown in Figure 1, which included a hierarchical approach based on title, abstract, and full-text reading. Moreover, all listed references of included studies and recent reviews were retrieved for any additional relevant studies.

Inclusion and exclusion criteria

Studies in this meta-analysis met the following inclusion criteria: (1) it was published by April 2020, (2) it was a case-control study or Genome-Wide Association Study (GWAS) that determined the distributions of the TLR4 [rs4986790A/G and rs4986791C/T] and IL-8 [rs2227306C/T and rs4073A/T] polymorphisms, in AMD patients and controls, (3) detailed genotype frequency data could be acquired to calculate the Odds Ratios (ORs) and 95% Confidence Intervals (CIs), and (4) human studies. The exclusion criteria were: (1) abstract, case or series-report review, systematic review, animal studies, and publications in duplicate, (2) unrelated to each of AMD and these polymorphisms, and (3) insufficient data.

Data extraction

Briefly, first author's name, year of publication, ethnicity, mean age of participants, number of cases and controls, method for determination of genotype, studied polymorphisms, and Hardy–Weinberg Equilibrium (HWE) *P*-values were rigorously extracted by two investigators independently from the eligible studies. If there was a disagreement about data, the two investigators rechecked the original data of the included studies and had a discussion to reach consensus; otherwise, the third investigator adjudicated the disagreements. Due to the lack of the mean age and HWE *P*-values for some studies, we were not able to analyze the data based on this information. This study was conducted in the Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences.

Statistical analyses

All statistical manipulations were performed with STATA version-15.0 software (STATA Corporation, College Station, Texas). The strength of the association between TLR4 and IL-8 polymorphisms and AMD was assessed by calculating pooled OR and 95% CI values in all genetic models (allele contrast, homozygous comparison, heterozygous comparison, dominant model, and recessive model). Z-test with *P* < 0.05 was used to authenticate the statistical significance of effect size. Sensitivity analysis was used to investigate the cause of dispersion. Then, outlier studies were removed and analysis was recomputed. In the case of a significant reduction in dispersion (I^2 index), this study was considered as a dispersion factor. With regard to the heterogeneity of the studies, Cochran's Q test (*P*-value [phet] < 0.10 was considered as statistically significant heterogeneity) and I^2 statistics ($75 \leq I^2 < 100$ as extreme heterogeneity, $50 \leq I^2 < 75$ as high heterogeneity, $25 \leq I^2 < 50$ as moderate heterogeneity, and $I^2 < 25$ as no heterogeneity) were used to assess the degree of heterogeneity.¹⁴ The random-effects model was used for this meta-analysis because it accounts for random variability both within and among studies. Publication bias was investigated by the Funnel plot and Egger's test. A *P*-value < 0.05 was considered statistically significant for all analyses.

Result

Literature search and study characteristics

The process of the search was shown in Fig. 1. Relying on the search criteria and manual search of references cited in the relevant articles, 142 and 376 English and Persian language articles related to the TLR4 and IL-8 genes polymorphisms and AMD were initially identified, respectively. After eliminating the duplicated records, and screening the titles, abstracts, and full texts, six studies for TLR4, and six studies for IL-8 were included in this meta-analysis (wherein three studies were eliminated due to: one study due to non-availability of the data, even no responses could be gotten after having sought the relevant data via email contacts, one studies due to the lack of the required number studies for analyzing of the polymorphism, and one study was included only patient's members).

The characteristics of the studies included in the meta-analysis are shown in Table 1. In total, our study included 4804 AMD patients and 4422 healthy controls. Of the 12 articles, studies including different subpopulations were considered as separate studies. Consequently, these groups were independently analyzed.

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

Author(year)	Ethnicity	Sample size	Mean age		Genotyping methods	Studied polymorphism	HWE P _{value} (controls)	ref
			Case/Control	Case				
Sarli A(2017)	Greece	120/103	-	-	PCR-RFLP	TLR4 rs4986790, rs4986791	P > 0.05	19
Güven M (2016)	Turkey	183/200	75 ± 8	73 ± 10	real-time PCR	TLR4 rs4986790, rs4986791	P > 0.05	18
Cho Y (NEI sample) (2009)	USA	130/187	80.1 ± 7.6	65.4 ± 10.3	Taqman SNP Genotyping Assays	TLR4 rs4986790	P > 0.05	17
Cho Y (AREDS sample) (2009)	USA	270/191	68.8 ± 50	66.8 ± 4.2	Taqman SNP Genotyping Assays	TLR4 rs4986790	P > 0.05	17
Cho Y (BMES sample) (2009)	Australia	274/551	75.3 ± 7.6	75.2 ± 7.4	Taqman SNP Genotyping Assays	TLR4 rs4986790	P > 0.05	17
Despriet DD(2008)	USA	368/368	76.31 ± 2.72	75.43 ± 2.07	DHPLC	TLR4 rs4986790	P > 0.05	4
Despriet DD(2008)	The Netherlands	357/173	76.31 ± 2.72	75.43 ± 2.07	DHPLC	TLR4rs4986790, rs4986791	P > 0.05	4
Edwards AO(2008)	USA	387/160	75.01 ± 9.66	67.81 ± 7.94	Taqman SNP Genotyping Assays	TLR4 rs4986790, rs4986791	P > 0.05	16
Zareparsis(2005)	USA	667/438	79.0 ± 7.9	75.1 ± 5.0	PCR-RFLP	TLR4 rs4986790, rs4986791	P > 0.25	15
Cascella R (2018)	Italy	875/993	± 77	± 72	Sanger technique	IL-8 rs2227306	P > 0.05	23
Ambreen (2015)	Pakistan	90/100	70.0 ± 1.07	63.0 ± 0.84	Sanger technique	IL-8 rs2227306, rs4073	P < 0.0001	22
Hautamäki A (2015)	Finland	301/119	> 50 years	> 69 years	PCR-based genotyping	IL-8 rs4073	-	25
Ricci F (2013)	Italy	721/660	75.0 ± 7.6	69.2 ± 11.1	TaqMan assays	IL-8 rs2227306	P > 0.05	21
Tsai YY (2007)	Taiwan	312/180	71.1 ± 7.5	71.5 ± 6.9	PCR-RFLP	IL-8 rs2227306	P > 0.05	20
Goverdhan SV (2008)	UK	474/540	78.8 ± 7.9	69.0 ± 9.7	TaqMan	IL-8 rs4073	P > 0.05	24

Association between TLR4 rs4986790 polymorphism and risk of AMD

In total, nine studies,^{4,15-19} containing 2756 AMD patients and 2371 controls were surveyed at the effect of TLR4 rs4986790 polymorphism in risk of AMD. Our meta-analysis disclosed significant associations under genotypic models in AA vs. AG + GG (OR = 0.733 [0.552–0.973], *P* = 0.032) and AA vs. AG (OR = 0.715 [0.536–0.953], *P* = 0.022). (Table 2 & Fig. 2)

Table 2
Meta-analysis of the associations of TLR4 and IL-8 polymorphisms with AMD risk.

Polymorphism	No. of studies	Comparison	Test of association		Test of heterogeneity		Egger's test (P)	Metareg's test (P)
			OR (95% CI)	P-value	P	I ² (%)		
IL-8 rs4073	3	TT vs. AA + AT	0.880 (0.700-1.106)	0.274	0.811	0.0	0.207	0.727
		AA vs. TT + AT	1.008 (0.566-1.797)	0.978	0.024	73.2	0.455	0.379
		AA vs. AT	0.940 (0.477-1.855)	0.859	0.010	78.1	0.398	0.263
		TT vs. AA	0.883 (0.596-1.309)	0.535	0.229	32.1	0.666	0.576
		A vs. T	1.122 (0.956-1.316)	0.160	0.320	12.2	0.631	0.589
IL-8 rs2227306	4	TT vs. CC + TC	1.419 (0.978-2.060)	0.065	0.059	59.6	0.098	0.294
		CC vs. TT + TC*	0.622 (0.481-0.805)	0.000	0.037	69.7	0.139	0.438
		CC vs. TC*	0.653 (0.479-0.890)	0.007	0.013	76.9	0.200	0.405
		TT vs. CC	1.635 (1.041-2.566)	0.033	0.021	69.0	0.409	0.557
		C vs. T*	0.714 (0.647-0.788)	0.000	0.356	3.3	0.009	0.532
TLR4 rs4986790	9	AA vs. AG + GG	0.733 (0.552-0.973)	0.032	0.050	48.5	0.436	0.361
		GG vs. AG + AA	0.857 (0.403-1.822)	0.688	0.893	0.0	0.342	0.403
		AA vs. AG	0.715 (0.536-0.953)	0.022	0.058	46.8	0.381	0.475
		AA vs. GG	1.146 (0.539-2.439)	0.723	0.889	0.0	0.373	0.197
		A vs. G	0.774 (0.593-1.011)	0.060	0.058	46.8	0.505	0.245
TLR4 rs4986791	5	CC vs. CT + TT	0.688 (0.356-1.330)	0.266	0.013	68.4	0.236	0.672
		TT vs. CC + CT	1.058 (0.227-4.934)	0.943	0.696	0.0	0.989	0.558
		CC vs. CT	0.685 (0.364-1.288)	0.240	0.021	65.4	0.432	0.532
		CC vs. TT	0.927 (0.199-4.324)	0.923	0.680	0.0	0.736	0.388
		C vs. T	0.698 (0.364-1.336)	0.277	0.011	69.3	0.185	0.654

*used sensitivity analysis

Association between TLR4 rs4986791 polymorphism and risk of AMD

In total, five studies,^{4, 15, 16, 18, 19} including 1714 AMD patients and 1074 controls were focused to assess the impacts of TLR4 rs4986791 polymorphism and AMD susceptibility. The analysis of allelic and genotypic models showed no evidence of an association between this polymorphism and AMD. (Table 2 & Fig. 2)

Association between IL-8 rs2227306 polymorphism and risk of AMD

Four studies¹⁹⁻²³ with 1998 AMD patients and 1933 controls were enrolled to examine the association of IL-8 rs2227306 polymorphism in risk of AMD. The present meta-analysis revealed significant associations under genotypic and allelic models in TT vs. CC (OR = 1.635 [1.041–2.566], $P = 0.033$), CC vs. TT + TC (after using sensitivity analysis: OR = 0.622 [0.481–0.805], $P < 0.001$), CC vs. TC (after using sensitivity analysis: OR = 0.653 [0.479–0.890], $P = 0.007$), and C vs. T (after using sensitivity analysis: OR = 0.714 [0.647–0.788], $P < 0.001$). (Table 2 & Fig. 2)

Association between IL-8 rs4073 polymorphism and risk of AMD

Three studies^{22, 24, 25} with 865 AMD patients and 759 controls were enrolled to examine the association of IL-8 rs4073 polymorphism with AMD susceptibility. The analysis of allelic and genotypic models failed to detect any association between this polymorphism and AMD. (Table 2 & Fig. 2)

Tests for publication bias, sensitivity analyses, and heterogeneity

Publication bias was assessed by the Funnel plots, Metareg's test, and Egger test in the overall meta-analysis. A Funnel plot was not carried out for IL-8 rs4073 because it is ineffective when the number of studies is limited. The significant asymmetry was found in C vs. T model of IL-8 rs2227306 by the estimation of the Egger test P -values.

Performing of the sensitivity analysis by omitting the asymmetric study²² (apparent asymmetry in the Funnel plots) and sequentially recalculating the overall effect in CC vs. TT + TC, CC vs. TC, and C vs. T models of IL-8 rs2227306 led to altering the pooled OR and 95% CI (pre-sensitivity analysis data not shown in cases).

In this meta-analysis, the significant statistical heterogeneities were found in most investigations. Thus, random-effect models were elected to synthesize the pooled ORs and 95% CIs for all genetic models. All of the control groups of studies were in HWE, except one study²⁵ which was non-assessed.

Discussion

AMD is an epidemic multi-factorial disorder triggered by a combination of genetic and environmental factors. While the contribution of genetics in susceptibility to the disease is estimated to be 45–70 percent, polymorphisms of genes encoding immune system and inflammation pathways play a major role in this heritability.²⁶ To our knowledge, this is the first systematic review and meta-analysis to determine the possible associations of the TLR4 (rs4986790 (+ 896) and rs4986791 (+ 1196)) and IL-8 (rs2227306 (+ 781) and rs4073 (-251)) genes polymorphisms with AMD risk.

Reducing expression of TLR4 -as a mediator signaling pathway NF- κ B- in AMD models leads to decreasing inflammation and degeneration of the RPE cells.^{4, 27} Two functional missense SNPs (Single Nucleotide Polymorphism) in the coding sequence of TLR4 gene are rs4986790 (A + 896G, Asp299Gly) and rs4986791 (C + 1196T, Thr399Ile), with linkage disequilibrium, modify the extracellular domain of the corresponding protein and lead to a different LPS responsiveness.²⁸ The recent meta-analysis studies have suggested the effects of TLR4 polymorphisms on susceptibility to various diseases such as Open Angle Glaucoma (rs1927911, rs2149356, rs4986791, rs7037117, and rs10759930),²⁹ cancers (rs4986790 and rs4986791),^{30, 31} Pneumonia (rs4986790),³² Asthma (rs4986791),³³ Crohn's disease (rs4986791),³⁴ and Atopic Dermatitis (rs4986790).³⁵

IL-8 as an important chemotactic cytokines has a high level of productions in the nAMD patient's PBMCs and RPE cells.³⁶ A allele of rs4073 (-251) and C allele of rs2227532 (-845) polymorphisms in the promoter region of IL-8 gene cause further

expression of this gene.^{37, 38} Besides, rs2227306 (+ 781) in the first intron has been suggested that C allele contributes to promoting of binding of transcription binding protein within “-251T/+396T/+781C/+1238insA/+1633C/+2767A” haplotype box.³⁹ Most meta-analysis studies have demonstrated that IL-8 (rs4073) is associated with the risk of various cancer diseases such as gastric,⁴⁰ prostate,⁴¹ breast,⁴² lung,⁴³ and oral⁴⁴ cancers, while rs187238 (-137) is associated with head and neck cancer.⁴⁵

In the current study, we utilized meta-analysis, in order to evaluate the association between the TLR4 (rs4986790 and rs4986791) and IL-8 (rs2227306 and rs4073) genes polymorphisms with the susceptibility to AMD. The relatively strong associations were observed in the comparisons of alleles and genotypes of IL-8 rs2227306 polymorphism (CC vs. TT + TC, CC vs. TC, TT vs. CC, and C vs. T). In addition, the results of TLR4 rs4986790 polymorphism provide the evidence of associations with AMD in AA vs. AG + GG and AA vs. AG models. So that, individuals with CC genotype in IL-8 rs2227306 and AA genotype in TLR4 rs4986790 have 38% and 27%, respectively, lower risk of developing AMD than the other genotypes. On the other hand, our meta-analysis disclosed no significant association in the comparisons of allele and genotypes models of TLR4 (rs4986791) and IL-8 (rs4073).

Several limitations in the present meta-analysis study should be brought up. First, heterogeneity and publication bias, for instance, missing articles due to unpublished studies, possibly with negative results, and/or language bias due to writing in other languages (except English and Persian); second, the small number of studies in some polymorphisms may lead to misleading results of the study; third, further studies in other populations are essential for a powerful assessment; fourth, joint effects of SNP-SNP or gene-environment factors, clinical diversities of AMD, as well as gender and age effects, were not investigated in this study due to the inaccessibility or limitations of data which could have significant impacts on the outcomes; lastly, some genotype distributions of control groups do not follow the HWE.

Conclusions

In conclusion, the current meta-analysis study suggested that IL-8 rs2227306 and TLR4 rs4986790 polymorphisms are associated with susceptibility to AMD. Therefore, the presence of genotypes CC of IL-8 rs2227306 and AA of TLR4 rs4986790 polymorphisms in individuals play relatively protective roles against AMD. By understanding the additional genetic and environmental pathogenic mechanisms of AMD, polymorphisms of the immune system have potential implications in clinical trials that would affect monitoring schedules or implementation of preventive treatments. Further Large-scale and well-designed studies are required to validate this conclusion.

Abbreviations

AMD: Age-Related Macular Degeneration; OR:Odds Ratio; CI:Confidence Interval; RPE:Retinal Pigment Epithelial; LPS:lipopolysaccharide; TLR4:Toll-like Receptor; IL-8:Interleukin-8; CXCL8:C-X-C motif chemokine Ligand 8; SNP:Single Nucleotide Polymorphism; GWAS:Genome-Wide Association Study; HWE:Hardy-Weinberg Equilibrium.

Declarations

Authors' contributions

N.R. participated in conception and design, searching and selecting papers, data extraction, writing and approving final paper. **E. S.** participated in design, searching and selecting papers, data extraction, analyzing data, writing and approving final paper. **MG. L.** participated in conception and design, selecting papers, data extraction, analyzing data, writing and approving final paper. **L.V.** participated in the selection of title, keywords, and approving final paper. **SM. G.** participated in data extraction and approving final paper. **A. K.** participated in data extraction and approving final paper. All authors read and approved the final manuscript.

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Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

The meta-analysis is exempt from informed consent because we collected and analyzed data from previous publications in which informed consent had already been obtained.

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Study Highlights

- The polymorphisms of genes encoding immune system and inflammation pathways play a major role in the heritability of AMD.
- Toll-Like Receptors play a key role in defending the immune system.
- TLR4 as a surface receptor for LPS is highly expressed in lymphocyte, neutrophil, and monocytes.
- IL-8 or CXCL8 is one of the main inflammatory cytokine and a potent chemoattractant factor, which is linked to the pathogenesis of AMD.
- The meta-analysis method is a valuable statistical method that is used by combining multiple studies with the same subject to obtain a general result.
- To the best of our knowledge, the previous AMD meta-analysis studies have not been included for the TLR4 (rs4986790 and rs4986791) and IL-8 (rs4073 and rs2227306) polymorphisms.
- The relatively strong associations were observed in the comparisons of alleles and genotypes of IL-8 rs2227306 polymorphism (CC vs. TT+TC, CC vs. TC, TT vs. CC, and C vs. T).
- The results of TLR4 rs4986790 polymorphism provide the evidence of associations with AMD in AA vs. AG+GG and AA vs. AG models.
- Current meta-analysis disclosed no significant association in the comparisons of allele and genotypes models of TLR4 (rs4986791) and IL-8 (rs4073).

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Figures

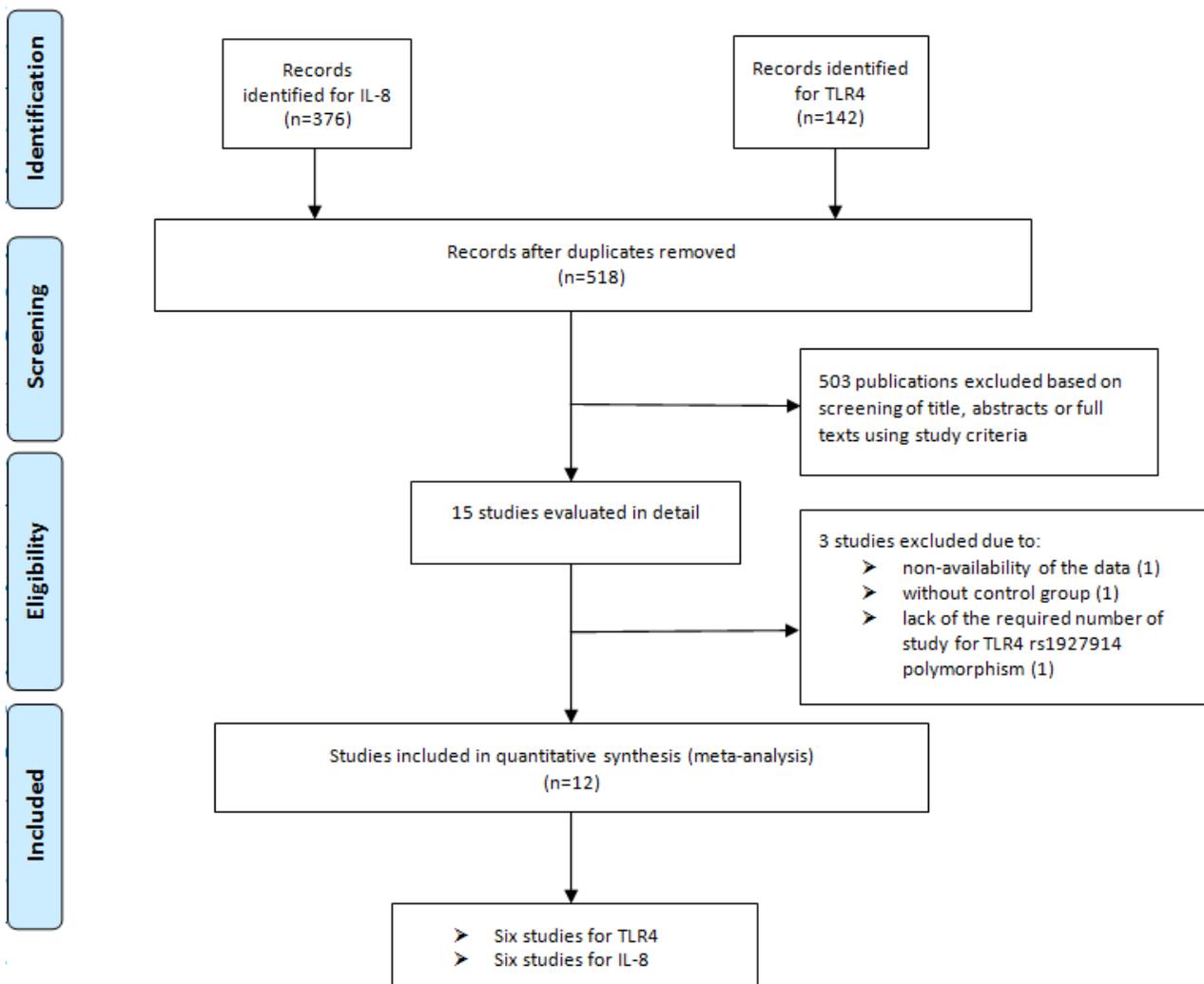


Figure 1

Flow diagram of studies for inclusion in the systematic review and meta-analysis

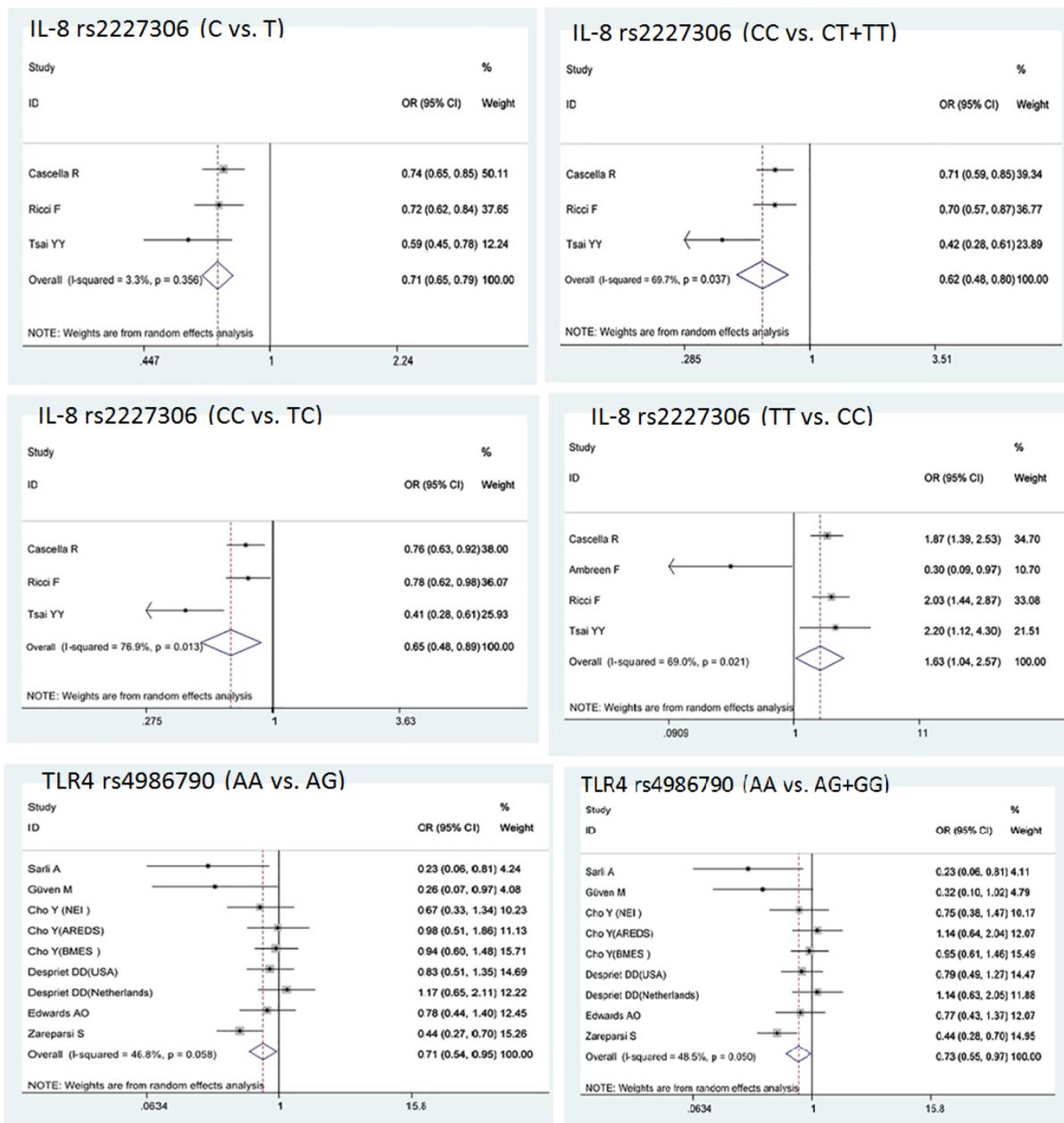


Figure 2

Forest plot of the associations of IL-8 and TLR4 polymorphisms with AMD. Random-effects models were used in all genetic models.