

# Tumor necrosis factor- $\alpha$ 308 G>A polymorphism and retinopathy risk in diabetes mellitus: an updated meta-analysis

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## Research article

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## Abstract

Background Growing evidence has indicated that tumor necrosis factor (TNF)- $\alpha$  is a candidate for increasing risk of diabetic retinopathy. Lots of researches have suggested that the variation of the TNF- $\alpha$  gene promoter may play a vital role in the risk of this disease. To solve this issue more clearly, we performed an updated meta-analysis to evaluate the relationship of TNF- $\alpha$  -308 G>A polymorphism with diabetic retinopathy in diabetes mellitus. Methods Literatures were retrieved in a systematic manner and analyzed using STATA Statistical Software. Crude odds ratios (ORs) with 95% confidence intervals (CIs) were adopted to estimate the strength of association. Results Eight studies with 1,698 cases and 2,064 controls were included. Genotypic and allelic comparisons between cases and controls were evaluated. Integral analysis shows a marginal association of the TNF- $\alpha$  -308 A allele polymorphism with diabetic retinopathy. Additionally, in stratified analysis by ethnicity, an association among the European population was found. Conclusions Our meta-analysis proved that -308 A allele polymorphism in the TNF- $\alpha$  gene potentially raised the risk of diabetic retinopathy and presented a differential frequency in distinct ethnicities.

## Background

Vascular complications are related with the hazard of diabetes mellitus (DM), which is a more and more common metabolic illness and is regarded as one of the significant resources of lethal and/or crippling diseases. It has been widely acknowledged that diabetic retinopathy (DR), which is a progressive microvascular complication of DM, occurs in almost all of the type 1 DM (T1DM) and about 75% of the type 2 DM (T2DM) subjects within 15 years onset of DM [1,2], and is the primary reason of blindness among the population aged from 20 to 75 years old [3].

The condition of persistent hyperglycaemia contributes to a cascade of events, and then the abnormal changes of retinal microvasculature occur [4,5]. During the pathological process, advanced glycation end products bind to the corresponding receptors anchored on the surface of endothelial cells and immune cells, thus various pro-inflammatory cytokines are synthesized and secreted, where tumor necrosis factor (TNF)- $\alpha$  are included [4]. It is reported that TNF- $\alpha$  is primarily involved in the destruction of blood retinal barrier and the formation of neovascularization. As a result, it is considered as one of the major effective elements of DR [6].

The gene of TNF is located on the 6 chromosome in the human leukocyte antigen class III region. Genetic variations in its promoter region may regulate the expression of TNF. rs1800629, where the guanine (G) at positions -308 is substituted by the adenine (A), is a TNF- $\alpha$  promoter variant being the most extensively studied. There is a higher circulating levels of TNF in the serum of T1DM subjects with proliferative DR (PDR) compared with those without retinopathy[7]. Both mRNA level of TNF and the expression of soluble TNF receptors are elevated in the vitreous humor of patients with PDR[8]. Furthermore, the result of inhibiting TNF with angiopoietin-1 has also suggested a promising prospect in preventing early DR in the animal diabetic model[9]. Several vitro studies have shown that rs1800629 may bring about a rising level of transcription and expression for TNF gene than that of wild-type[10,11]. Bayley and his colleagues [12] have proved that the circulating TNF levels are higher among subjects homozygous of -308 A allele compared with those homozygous of -308 G allele, and the former also present worse prognosis in response to some infectious illnesses.

As a result, an enormous amount of studies have tried to investigate if there is a certain connection between TNF -308 G>A polymorphism and DR[13-24], but the results remain conflicting. A related meta-analysis has been published in 2014[25], but there are still a few of new researches carried out in recent years. To address this issue and derive a more precise estimation, we conduct this updated meta-analysis and try to discuss whether the association has ethnic divergences or is biased by the classification of DM.

## Methods

### Search strategy

An extensive and systematic document search for the Embase database, the Medline database (by the Ovid database) and the Web of Science database (to May 29, 2019) was executed by two investigators separately making use of those search

terms including 'diabetic retinopathy', 'tumor necrosis factor', 'polymorphism', 'genotype', 'allele', 'single nucleotide polymorphism', 'variant', 'variation', 'mutation' and the combined phrases to get access to all related genetic researches on the association between TNF- $\alpha$  -308 G>A polymorphism and the risk of DR. All relevant studies before May 29, 2019 were taken into account. There was no language limitation. For example, the full electronic search strategy for the Embase database is ('genetic variation'/exp OR 'genetic variation' OR 'heredity'/exp OR 'heredity') AND ('diabetic retinopathy'/exp OR 'diabetic retinopathy') AND ('tumor necrosis factor'/exp OR 'tumor necrosis factor').

Besides, the references of reviews and original studies on this theme were also hand-searched to obtain additional studies. When it was necessary, we would contact the corresponding authors to get the essential information, for example, for those abstracts and unpublished studies, and half of them replied. Qualified studies were screened according to the following criteria: a case-control study on the relationship between TNF-a -308 G>A polymorphism and DR; containing right and definite numbers of different genotypes to evaluate odd ratios (ORs) and the corresponding 95% confidence intervals (CIs). When the same research data was reported in some different publications, the most complete or the largest research was selected. At the end, eight eligible studies were included and further analyzed in this meta-analysis.

#### Data extraction

Two investigators evaluated the studies for inclusion or exclusion independently, discussed differences, and reached agreements in the end. Those following information were extracted from each qualified research: first author's name, publication year, nationality, ethnicity, definition and type of DM, case definition, genotyping methods, total number of subjects with or without DR (DWR), along with the corresponding sex ratio, average age, mean DM duration and the distribution of each TNF-a genotype. Both PDR and nonproliferative DR (NPDR) are considered as DR. Different ethnicities were classified as European and Asian. DM was divided into T1DM and T2DM.

#### Statistical analysis

Pooled ORs with 95% CIs were calculated to evaluate the strength of the association between TNF-a -308 G>A polymorphism and the risk of DR. We assessed the risk by making use of the codominant model (AA versus GG; GA versus GG), the dominant model [(GA + AA) versus GG], the recessive model [AA versus (GG + GA)] and the allelic model (A allele versus G allele). The inconsistency index  $I^2$  statistic (documented for percentage of the observed between study variability due to heterogeneity instead of chance), which ranges from 0 to 100%, was adopted to assess between-study heterogeneity. If  $I^2 > 50\%$ , heterogeneity was considered significant, in which case the random-effects model (the DerSimonian-Laird method) was used to analyze the data. If  $I^2 < 50\%$ , the fixed-effects model (the Mantel-Haenszel method) was adopted. When heterogeneity between studies was relatively low, the two models showed similar outcomes. Studies were classified into subgroups by ethnicity and the

type of DM. Hardy-Weinberg equilibrium was assessed using the  $c^2$  test. If total genotype distributions in the controls of all studies included are in agreement with Hardy-Weinberg equilibrium, the sensitivity analysis will not be undertaken. If not, the sensitivity analysis will be performed by omitting one study at a time to assess the stability of the meta-analysis results. Begg's funnel plot and Egger's test were undertaken to assess publication bias. ( $P < 0.05$  was considered representative of statistical significance). All statistical analyses were performed in STATA Statistical Software (v.12.0; StataCorp, LP, College Station, TX). When the two sided  $P$ -value  $< 0.05$ , it was regarded to be significant statistically.

## Results

#### Qualified studies

Overall, eight case-control studies were included in this meta-analysis, referring to 1,698 cases (DR) and 2,064 controls (DWR). According to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines, Figure 1 shows a flow chart of the literature retrieval [26]

These studies' primary characteristics are presented in Table 1. DM was defined according to the American Diabetes Association diagnostic criteria (ADA) or the World Health Organization's criteria (WHO). Among these qualified publications, studies for Europeans and Asians were both four. Since one study was related to both T1DM and T2DM, studies concerning T1DM and T2DM were two and seven, respectively [18]. The methods for genotyping includes polymerase chain reaction (PCR), PCR-sequence specific Primers(PCR-SSP), amplification refractory mutation system-polymerase chain reaction (ARMS-PCR), allelic discrimination method (AMD), PCR-restriction fragment length polymorphism method (PCR-RFLP) fluorescent allele-specific DNA primer assay system and GeneAmp PCR. Among all the included studies, there was one study conducted by Bućan et al. in 2009, having deviation from HWE in the control for the TNF-a -308 G>A (rs1800629) variant among all of studies[17].

## Meta-analysis

The major results of the meta-analysis, as well as the heterogeneity test, are presented in Table 2. The results showed that no significant heterogeneity was detected among all the models. As a whole, there was a marginally statistical significance correlation between the allele A and DR risk among overall population being detected (for GA versus GG: OR 1.21, 95% CI 1.04 to 1.41, Figure 2A; for (GA + AA) versus GG: OR 1.20, 95% CI 1.03 to 1.39, Figure 2B; for A versus G: OR 1.14, 95% CI 1.01 to 1.30, Figure 2C).

## Subgroup analysis

Significantly raised risks were found in the subgroup of European population ((for GA versus GG: OR 1.27, 95% CI 1.06 to 1.51, Figure 3A; for (GA + AA) versus GG: OR 1.25, 95% CI 1.05 to 1.49, Figure 3B; for A versus G: OR 1.17, 95% CI 1.01 to 1.36, Figure 3C). However, after stratification with the type of DM, the TNF-a variation was still not found related with increased DR risk among either T1DM or T2DM subjects (for AA versus GG: OR 1.10, 95% CI 0.69 to 1.77, Figure 4).

## Sensitivity analysis

Sensitivity analyses were performed after the sequential removal of each included studies to evaluate the influence of each individual study on the pooled ORs. There was no single study qualitatively changing the pooled ORs in all genetic model, which indicated that the outcomes of our meta-analysis were basically robust and stable (Figure 5).

## Publication bias

Begg's funnel plot, along with Egger's test, was conducted to quantitatively evaluate the potential existence of publication bias. The appearance of Begg's funnel plot is symmetrical. And the Egger's test further proved that there was no definitively statistical testimony for publication bias among any of the five genetic models (for AA versus GG:  $P = 0.815$ ; for GA versus GG:  $P = 0.704$ ; for (GA+AA) versus GG:  $P = 0.976$ ; for AA versus (GG+GA):  $P = 0.790$ ; for A versus G:  $P = 0.890$ ). Figure 6 indicates the appearance of the funnel plot of AA versus GG integrally.

## Discussion

It has become one of the main foci of fundamental researches in DR to unravel those genes that accelerate the pathogenic risk of DR among the diabetic patients over the past few decades. A growing number of putative genes and genetic variants have been discussed to investigate the genetic risk loci having a relationship with DR[27]. Functional studies have indicated a vital role for TNF as a biomarker in DR. Experimental studies in animal diabetic models have suggested that TNF-a is responsible for capillary degeneration, pericyte loss and so on, which all are features of DR[28]. Nevertheless, the association between TNF-a polymorphism and the occurrence and progression of DR still remained conflicting as a result of controversial findings generated by some relatively small researches. The first meta-analysis conducted by Meng et al.[25], showed that there was no obviously statistical difference between T2DM with or without retinopathy and TNF-308 G>A polymorphism.

Available data concerning the association between TNF-308 G>A polymorphism and DR risk in 8 studies, which involve 1,698 DR cases and 2,064 controls, were calculated in the present meta-analysis. Insignificant heterogeneity was detected among all those five models. Under the codominant model (GA versus GG), the dominant model [(GA + AA) versus GG] and the allelic model (A allele versus G allele), all summary ORs indicated that TNF-308 G>A polymorphism was marginally significantly related with an increased risk of DR. As ethnic differences may lead to different genetic backgrounds, and then may have an influence on genetic predispositions in some pathological conditions [29,30], the subgroup analysis was also further performed divided by ethnicity. In the stratified analysis, significantly statistical differences were observed with European ethnicity. Furthermore, there is evidence showing that genetic predisposition is more probable in type 1 DM in some genes. For example, Zhou et al.[31], have proved that a genetic relationship between aldose reductase C(-106)T polymorphism and DR risk of T1DM but not T2DM. As a result, we further explored the difference in the separate analysis of T1DM and T2DM. However, the results

implied a lack of association between TNF-a -308 G>A polymorphism and the type of DM. What's more, it is worth noting that the controls' genetic distributions in the research conducted by Bućan et al.[17] in 2009 deviated from HWE, supporting the probability of bias. Therefore, we undertook the sensitivity analysis afterwards to omit it. And the findings from the overall ORs before and after omitting the research were consistent, indicating that there was little effect to the result of this meta-analysis by the study. Furthermore, no significant publication bias was found in the pooled results in any genetic model, which further demonstrated the robustness of our meta-analysis.

The earliest investigation into TNF-a polymorphism and DR risk, reported by Wang et al.[19], revealed a negative relationship between the -308 A allele of TNF-a and DR risk in Chinese patients. However, owing to the essential information is not enough, we excluded this study in our meta-analysis. In the subsequent studies, it was suggested that there was a trend of lacking association [20,19,17,16,15,14]. But in a larger case-control study in Scandinavia that contained a total of 622 patients with T1DM and 878 patients with T2DM, Lindholm et al.[18] reported a significantly higher frequency of A allele (OR: 1.53, 95%CI 1.04 to 2.25) in T2DM with DR but not in T1DM, indicating -308 A allele in TNF-a locus as a risky factor for DR. And the research manifested by Sesti et al.[13] is in consistent with the research above. Our meta-analysis confirmed that the -308 A allele of the TNF-a carried more risk in European but not Asian participants, even if the overall effect was positive. What's more, all the studies in Asian populations showed no relationship between TNF-a polymorphism and DR risk. The divergences in ethnic backgrounds, living environment, nutrition and lifestyle may partly account for the discrepancy[32].

Further, several studies had also focused on the question that whether there is a certain connection between TNF-a polymorphism and different stages of DR, such as NPDR and PDR[23,22,24]. However, there was no significant association to be found between the promoter polymorphism and different DR phenotypes.

There are several major differences between the previous meta-analysis, performed by Meng et al.[25] and our study. First, the authors made a conclusion that there was no association between TNF-a -308 G>A (rs1800629) polymorphism and DR in T2DM, while we found TNF-a polymorphism may be associated with increased DR risk among DM patients, especially among the European population. Second, they did not include the study deviating from HWE, and this may result in the loss of some data and thus lead to the incomprehensiveness of the meta-analysis. We included the study conducted by Bućan et al.[17] in 2009. At the same time, a sensitivity analysis was performed to detect whether the study affected the pooled result. Third, the sample being made use of in this research was larger than that in the previous meta-analysis. We included 3 case-control studies which are published later, and thereby it could strengthen the statistical efficiency and elevate the credibility of the outcomes.

A few of potential limitations existed in our study and the results should be interpreted carefully. First, our meta-analysis is based on unadjusted estimates due to a lack of original data. For example, the accurate disease's time-course of each patient was not available. Therefore, our classification criterion could not be performed according to the duration of the diabetes, which may affect the results. Second, it should be noticed that there was one study deviating from HWE. This possibly have enhanced the possibility of selective bias in controls. However, when the research was eliminated, the pooled OR of the rest studies was invariant, which indicated the fairly good stability of the findings in our meta-analysis. Third, the sample size of this meta-analysis was limited, although the Egger's test indicated no publication bias[33]. Fourth, genotyping methods were various among those included studies, which might influence the outcomes. This discrepancy suggests the necessity to carry out strict quality control procedures in future researches.

## Conclusions

Our meta-analysis indicates that TNF-a polymorphism may have a connection with elevated DR risk in DM patients, especially in the European population. Because of the small sample size and other limitations in our study, a larger scale of epidemiological investigations on this theme should be performed to validate our findings in the future.

## Abbreviations

TNF tumor necrosis factor

ORs odds ratios

CIs confidence intervals

DM diabetes mellitus

DR diabetic retinopathy

T1DM type 1 DM

T2DM	type 2 DM
PDR	proliferative DR
DWR	patients without DR
NPDR	nonproliferative DR
ADA	American Diabetes Association diagnostic criteria
WHO	World Health Organization
PCR	polymerase chain reaction
PCR-SSP	PCR-sequence specific Primers
ARMS-PCR	amplification refractory mutation system-polymerase chain reaction
AMD	allelic discrimination method
PCR-RFLP	PCR-restriction fragment length polymorphism method

## Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Some of the datasets analyzed during the current study are included in those published articles and their supplementary information files, and the other are available from the corresponding authors on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LY contributed to the idea and design of this study and revised the manuscript. Both WN and RL carried out the screening procedure. Besides, WN participated in the design of the study, performed the statistical analysis and drafted the manuscript. RL and JT participated in the study design, revised the manuscript and gave some suggestions to this manuscript. All authors read and approved the final manuscript.

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## Tables

**Table 1. Main characteristics of case-control studies included in the meta-analysis.**

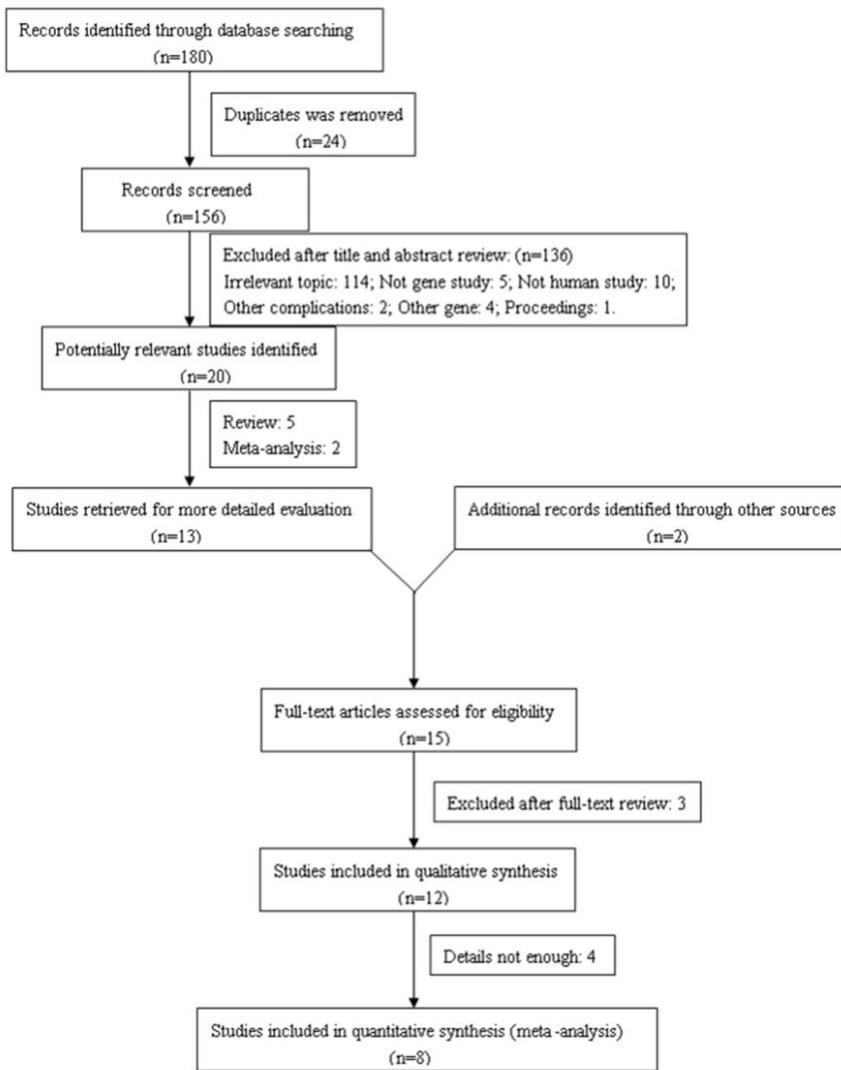
First author (year)	Country	Ethnicity	Definition	Type	Genotyping	Sample size (M/F)	Average age (years)	Mean DM duration (years)	Distribution of TNF-a genotypes				HWE	
									of DM	of DM	Methods	GG	A	
Sesti LF (2015)	Brazil	European	ADA	T2	PCR	404 (223,181)	61.6 ± 9.3	14.8 ± 8.9	284	108	12	676	132	0.49
						322 (137,185)	60.0 ± 9.6	11.8 ± 6.9	251	68	3	570	74	
Rodrigues KF (2015)	Brazil	European	ADA	T2	PCR-SSP	66 (15,51)	56.6 ± 8.5	17.3 ± 9.2	49	17	0	115	17	0.35
						36 (4,32)	52.8 ± 9.2	8.8 ± 7.7	29	6	1	64	8	
Sikka R (2014)	India	Asian	ADA	T2	ARMS-PCR	162 (290,172)	58.9 ± 7.5	13.3 ± 7.2	142	19	1	303	21	0.84
							59.4 ± 9.7*	10.1 ± 7.8	263	36	1	562	38	
Paine SK (2012)	India	Asian	WHO	T2	ARMS-PCR	253 (133,120)	52.0 ± 15.0	16.0 ± 6.0	165	77	11	407	99	0.69
						240 (128,112)		54.0 ± 12.0	161	70	9	392	88	
Bućan K (2009)	Croatia	European	--	T1	PCR-RFLP	19(--,-)	13.9±9.1*	19.2 ± 7.4*	11	7	1	29	9	0.02
						28(--,-)		7.4 ± 5.6*	20	5	3	45	11	
Lindholm E (2008)	Scandinavia	European	WHO	T1	AMD	315(--,--)	38.2±13.6*	18.1 ± 15.6*	145	154	16	444	186	0.15
						307(--,--)		200	154	134	19	442	172	
Wang N (2008)	China	Asian	ADA, WHO	T2	GeneAmp PCR	295(--,--)	61.2 ± 11.7*	2.5 8± 7.0*	200	85	10	485	105	0.51
						583(--,--)		379	179	25	937	229		
Yoshioka K (2006)	Japan	Asian	WHO	T2	FASDPAS	109((--,--))	64.5 ± 9.9*	14.9 ± 3.8*	94	15	0	203	15	0.23
						72((--,--))	63.8 ± 10.0*	63	8	1	134	10		
						75(39,36)	62.3 ± 8.95	14.3 ± 6.4	74	1	0	149	1	0.88
						176(115,61)	61.9 ± 10.5	12.3 ± 6.3	172	4	0	348	4	

DM: diabetes mellitus; HWE: Hardy-Weinberg equilibrium; ADA: American Diabetes Association criteria; WHO: World Health Organization's criteria; DR: diabetic retinopathy; PCR: polymerase chain reaction; PCR-SSP: PCR sequence specific primers; ARMS-PCR: amplification refractory mutation system-polymerase chain reaction; AMD: allelic discrimination method; FASDPAS: fluorescent allele-specific DNA primer assay system; RFLP: restriction fragment length polymorphism method; \*: estimated values; --: not available

Table 2. Summary of  $I^2$  values and p values of TNF-a -308 G>A polymorphism and diabetic retinopathy risk.

Comparisons	Number of studies	OR (95% CI)	$I^2$ (%)	Alleles/genotypes
				(DR/DWR)
<b>AA versus GG</b>				
<b>Ethnicity</b>				
Total	8	1.08 (0.73, 1.58)	2.1	2,769 (1,215/1,554)
European	4	1.07 (0.69, 1.66)	40.2	1,612 (728/ 884)
Asian	4	1.08 (0.48, 2.45)	0.0	1,157 (487/670)
<b>Type of DM</b>				
Total	9	1.02 (0.69, 1.50)	0.0	2,769 (1,215/1,554)
T1	2	0.87 (0.44, 1.69)	0.0	369 (173/196)
T2	7	1.10 (0.69, 1.77)	21.1	2,400 (1,042/1,358)
<b>GA versus GG</b>				
<b>Ethnicity</b>				
Total	8	1.21 (1.04, 1.41)	0.0	3,649 (1,647/2,002)
European	4	1.27 (1.06, 1.51)	0.0	2,285 (1,060/1,225)
Asian	4	1.05(0.78, 1.42)	0.0	1,364 (587/777)
<b>Type of DM</b>				
Total	9	1.14 (0.97, 1.33)	0.0	3,649 (1,647/2,002)
T1	2	1.27 (0.93, 1.74)	5.4	630 (317/313)
T2	7	1.10 (0.92, 1.31)	0.0	3,019 (1,330/1,689)
<b>(GA+AA) versus GG</b>				
<b>Ethnicity</b>				
Total	8	1.20 (1.03, 1.39)	0.0	3,762 (1,698/2,064)
European	4	1.25 (1.05, 1.49)	0.0	2,375 (1,099/1,276)
Asian	4	1.05 (0.79, 1.41)	0.0	1,387 (599/788)
<b>Type of DM</b>				
Total	9	1.13 (0.97, 1.31)	0.0	3,762 (1,698/2,064)
T1	2	1.21 (0.89, 1.64)	0.0	669 (334/335)
T2	7	1.10 (0.92, 1.31)	0.0	3,093 (1,364/1,729)
<b>AA versus (GA+GG)</b>				
<b>Ethnicity</b>				
Total	8	1.00 (0.69, 1.47)	5.7	3,762 (1,698/2,064)
European	4	0.99 (0.64, 1.52)	42.0	2,375 (1,099/1,276)
Asian	4	1.06 (0.47, 2.39)	0.0	1,387 (599/788)
<b>Type of DM</b>				
Total	9	0.97 (0.66, 1.42)	0.0	3,762 (1,698/2,064)
T1	2	0.77 (0.40, 1.49)	0.0	669 (334/335)
T2	7	1.09 (0.68, 1.75)	15.6	3,093 (1,364/1729)
<b>A versus G</b>				
<b>Ethnicity</b>				
Total	8	1.14 (1.01, 1.30)	0.0	7,524 (3,396/4,128)
European	4	1.17 (1.01, 1.36)	14.4	4,750 (2,198/2,552)
Asian	4	1.05 (0.81, 1.36)	0.0	2,774 (1,198/1,576)
<b>Type of DM</b>				
Total	9	1.09 (0.96, 1.24)	0.0	7,524 (3,396/4,128)
T1	2	1.09 (0.86, 1.38)	0.0	1,338 (668/670)
T2	7	1.09 (0.93, 1.27)	17.0	6,186 (2,728/3,458)

## Figures



**Figure 1**

Flow chart of included.

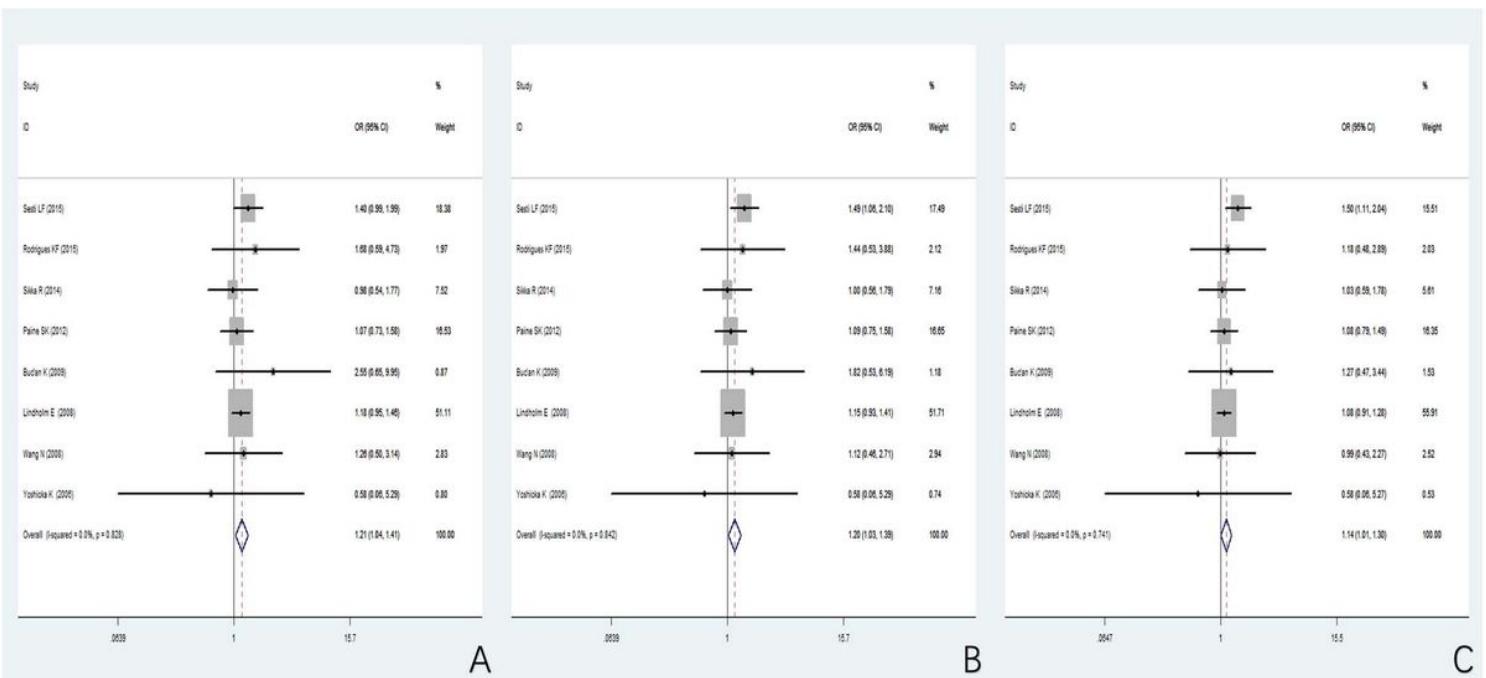


Figure 2

Odds ratios of diabetic retinopathy associated with TNF- $\alpha$  polymorphism in overall studies. A: the codominant model (GA versus GG); B: the dominant model [(GA + AA) versus GG]; C: the allelic model (A versus G).

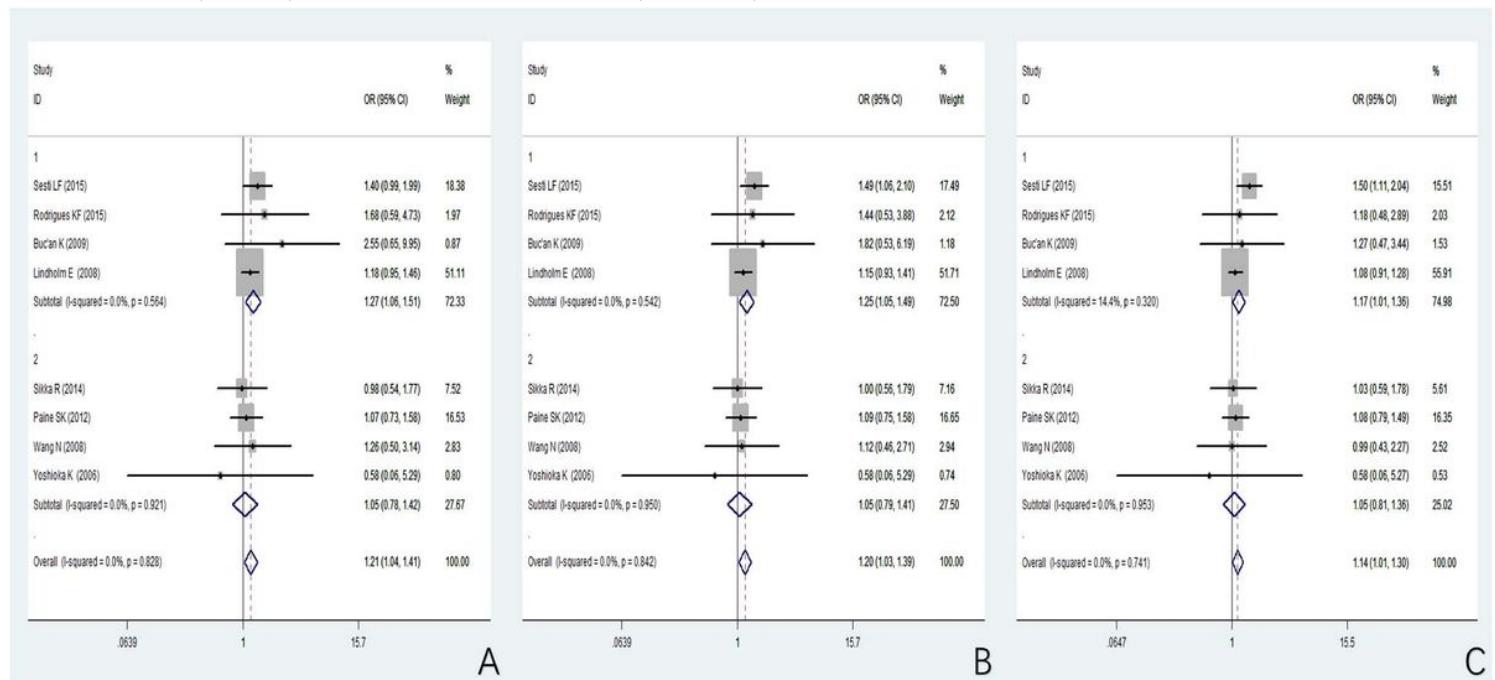
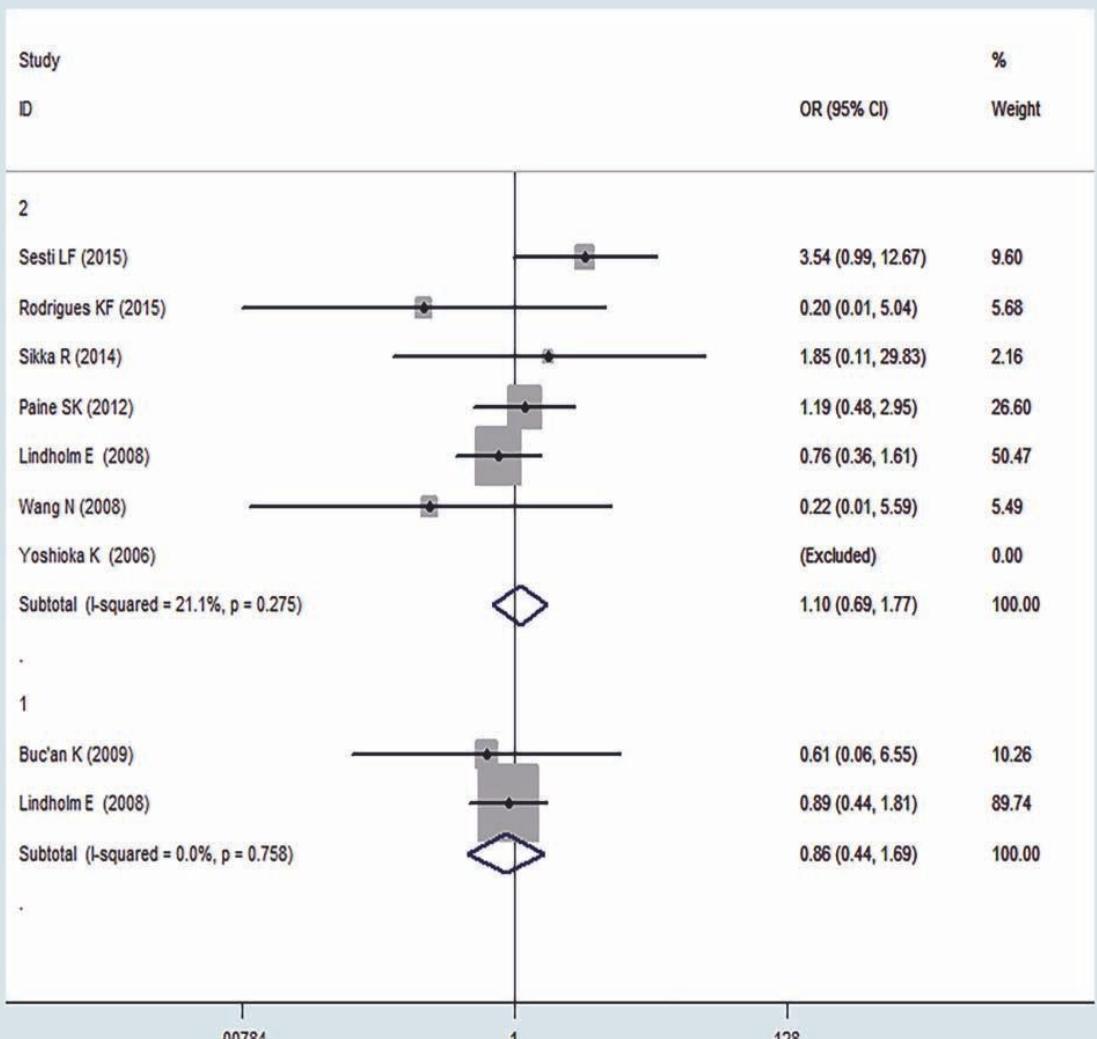


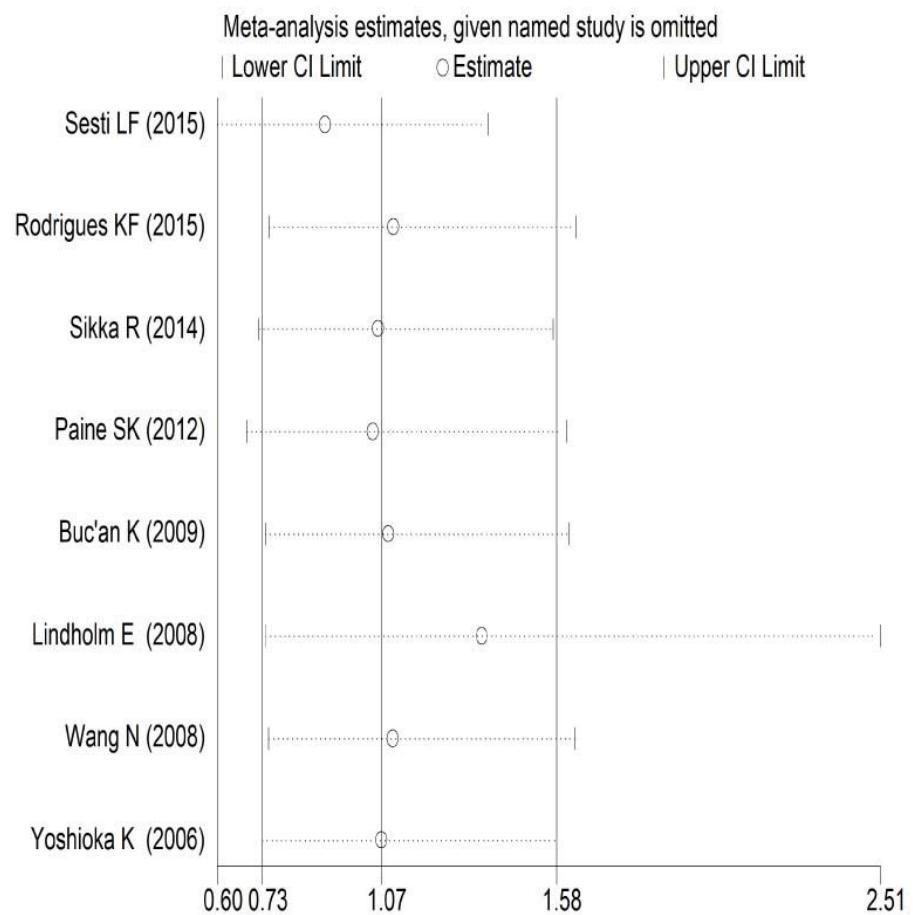
Figure 3

Odds ratios of diabetic retinopathy associated with TNF- $\alpha$  polymorphism in the subgroup analysis by ethnicity. A: the codominant model (GA versus GG); B: the dominant model [(GA + AA) versus GG]; C: the allelic model (A versus G).



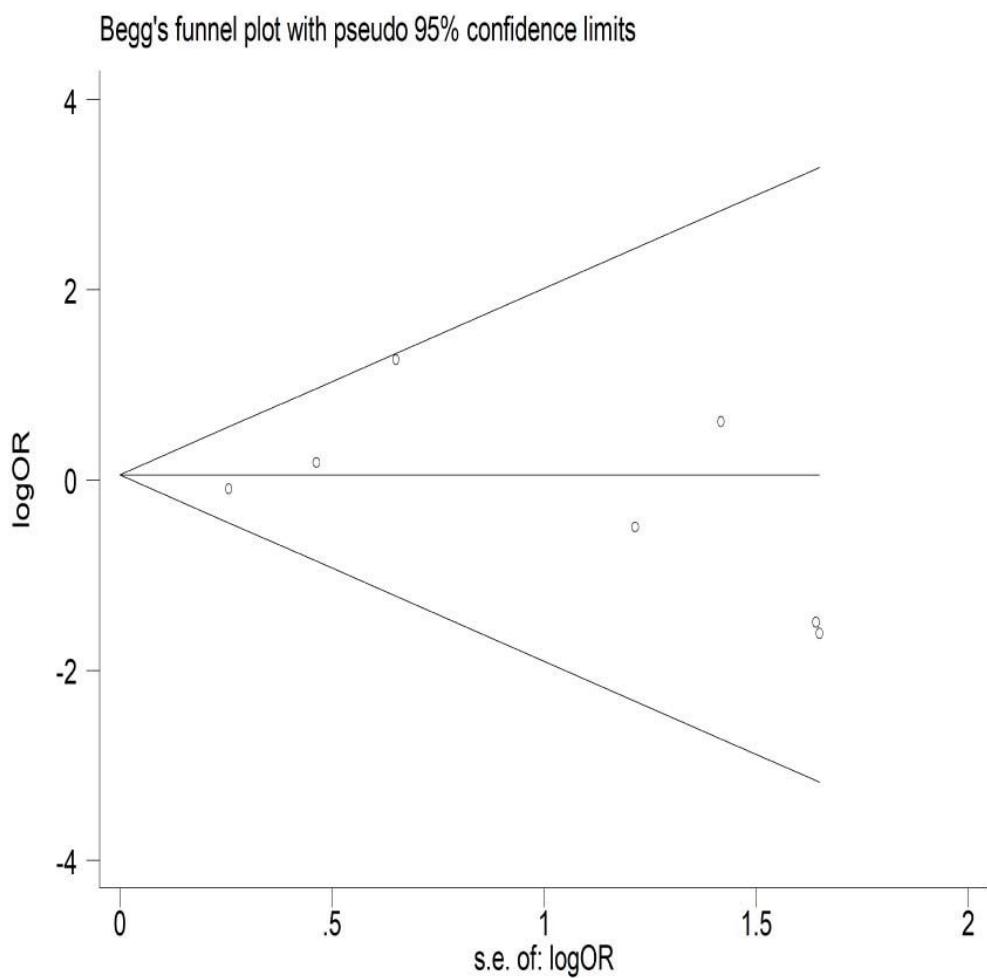
**Figure 4**

Odds ratios of diabetic retinopathy associated with TNF- $\alpha$  polymorphism (AA versus GG) in the subgroup analysis by the type of DM.



**Figure 5**

Sensitivity analyses through deletion of one study at a time to reflect the influence of the individual dataset to the pooled ORs (AA versus GG).



**Figure 6**

Funnel plot of the meta-analysis of TNF- $\alpha$  polymorphism and risk for diabetic retinopathy (AA versus GG).

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [BOPHD1900398PRISMAchecklist.doc](#)