Association of Tumor Necrosis Factor-Alpha-308 G/A and -238 G/A Polymorphism with Diabetic Retinopathy: A Systematic Review and Updated Meta-Analysis

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Keywords

Diabetic retinopathy · Tumor necrosis factor-alpha · Polymorphism · Meta-analysis

Abstract

Background: Mounting evidence has suggested that tumor necrosis factor-alpha (TNF- α) can promote the development of diabetic retinopathy (DR), and TNF- α gene variants may influence DR risk. However, the results are quite different. **Objectives:** To comprehensively address this issue, we performed the meta-analysis to evaluate the association of TNF- α -308 G/A and -238 G/A polymorphism with DR. **Method:** Data were retrieved in a systematic manner and analyzed using STATA Statistical Software. Crude odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the strength of associations. Allelic and genotypic comparisons between cases and controls were evaluated. **Results:** For the TNF- α -308 G/A polymorphism, overall analysis suggested a marginal association with DR (the OR [95% CI] of [GA vs. GG], [GA + AA] vs. GG, and [A vs. G] are 1.21 [1.04, 1.41], 1.20 [1.03, 1.39], and 1.14 [1.01, 1.30], respectively). And the subgroup analysis indicated an enhanced association among the European population. For the TNF- α -238 G/A polymorphism,

there was a mild correlation in the entire group (the OR [95% CI] of [GA vs. GG] is 1.55 [1.14, 2.11]), which was strengthened among the Asian population. **Conclusion:** The meta-analysis suggested that -308 A and -238 A allele in TNF- α gene potentially increased DR risk and showed a discrepancy in different ethnicities.

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Introduction

Vascular complications are related to the hazards of diabetes mellitus (DM), which is an increasingly common metabolic disease and one of the significant resources of crippling diseases. It is widely accepted that diabetic retinopathy (DR), a progressive microvascular complication of DM, occurs in about 18.5%–34.6% individuals with diabetes, especially among patients from 60 to 69 years old, and the risk grew sharply with the duration of DM [1, 2].

In the presence of sustained hyperglycemia, pathological changes to retinal microvasculature are caused by a cascade of events involving the formation of advanced glycation end products [3, 4]. Secondary to the binding of glycation end products to their corresponding receptors

anchored on the surface of immune cells and endothelial cells, various proinflammatory cytokines, including tumor necrosis factor- α (TNF- α), are synthesized and secreted [3]. It was reported that TNF- α was mainly involved in the destruction of the blood retinal barrier and the formation of new blood vessels [5]. The research conducted by Yao et al. [6] suggested that the expression of TNF- α in DR patients was significantly distinct from that in healthy controls, and inhibition of TNF has shown promising outcomes in preventing the progression of DR in the mice diabetic model [7]. Therefore, TNF- α serves as a critical element in the pathogenesis of DR.

TNF gene is located on the 6 chromosome in the human leukocyte antigen class III region. Genetic variations in its promoter region may regulate the production of TNF. Contrasting with the limitation of studies concerning other mutants, such as rs1799724 and rs11574936, rs1800629 and rs361525, where the guanine (G) is substituted by the adenine (A) at positions -308 and -238, respectively, are 2 TNF- α promoter variants being the most extensively studied. Individuals homozygous for the less common TNF- α -308 A allele have also been shown to have higher circulating TNF- α levels than those homozygous for the G allele and have worse outcomes in response to infectious diseases [8–10]. As a result, numerous studies have attempted to investigate whether there is a certain association between TNF- α -308 G/A and/or -238 G/A polymorphism and DR risk. However, those results remain conflicting. Although related meta-analyses have been published [11–13], more researches have been carried out in recent years. To address this issue and derive a more precise estimation, we conduct this systematic review and updated meta-analysis to discuss if the association has ethnic disparity or is biased by the classification of DM.

Methods

Search Strategy

In this systematic review, a comprehensive literature research was conducted in the database of PubMed, Embase, Ovid Medline, ISI Web of Knowledge, and the China National Knowledge Internet (to May 11, 2020) using research terms including “diabetic retinopathy,” “diabetic retinopathies,” “DR,” “tumor necrosis factor- α ,” “tumor necrosis factor alpha,” “tumor necrosis factor,” “tumor necrosis factor- α ,” “TNF- α ,” “TNF- α ,” “TNF,” “polymorphism, genetic,” “polymorphism,” “genotype,” “single nucleotide polymorphism,” “variant,” “variation,” “allele,” “mutation,” and the combined phrases to acquire all genetic studies on the relationship between TNF- α -308 G/A and/or -238 G/A polymorphism and the risk of DR. There was no language limitation. We also searched references of original

studies or reviews on this topic to identify additional studies. Moreover, we have registered this study on the Prospero Website with the registration number of CRD42020189022.

Inclusion and Exclusion Criteria

Two authors screened trials independently and discussed with a third author when inconsistencies occurred. Studies were included in accordance with the following criteria: only case-control studies referring to the association between TNF- α -308 G/A and/or -238 G/A polymorphism and DR were included, and for the meta-analysis, studies included must also contain the detailed and correct numbers of different genotypes for estimating an odds ratio (OR) with a 95% confidence interval (CI). When several publications reported on the same population data, the largest or the most complete study was selected.

Quality Assessment

The methodological quality of included case-control studies was evaluated based on the Newcastle-Ottawa Scale (NOS). Two authors assessed the quality independently, and divergence was arbitrated by a third author. According to the scores of 1–3, 4–6, and 7–9, eligible literatures were categorized as low-, intermediate-, and high-quality researches, respectively.

Data Extraction

Two investigators assessed articles for inclusion or exclusion independently, resolved disagreements, and attained consistency. For each eligible study, the following information was recorded: the first author's name, the year of publication, ethnicity, type of DM, genotyping methods, the number of patients with DR or without DR (DWR), sex ratio, average age, mean DM duration, and the distribution of each TNF- α genotype. Both proliferative DR (PDR) and nonproliferative DR (NPDR) were classified as DR. Different ethnicities were divided into the European and the Asian, and DM type was sorted into type 1 DM (T1DM) and type 2 DM (T2DM). We contacted corresponding authors for missing data.

Statistical Analysis

The strength of the relationship between TNF- α -308 G/A and/or -238 G/A polymorphism and DR risk was estimated by calculating pooled ORs with 95% CIs. We evaluated the risk using the dominant model (AA vs. GG; GA vs. GG), the dominant model ([GA + AA] vs. GG), the recessive model (AA vs. [GG + GA]), and the allelic model (A allele vs. G allele). Hardy-Weinberg equilibrium (HWE) was tested by the χ^2 test. If genotype distributions in the controls of all studies are in agreement with HWE, the sensitivity analysis will not be undertaken. If not, the sensitivity analysis will be performed by omitting 1 study at a time to assess the stability of the meta-analysis results. Between-study heterogeneity was appraised by the inconsistency index I^2 statistic (ranging from 0 to 100%). Heterogeneity was considered significant if $I^2 > 50\%$, in which case the random-effects model (the Dersimonian-Laird method) was performed to pool the data. Otherwise, the fixed-effects model (the Mantel-Haenszel method) was used. Studies were categorized into subgroups based on ethnicity and the type of DM. Begg's funnel plot and Egger's test were undertaken to assess publication bias ($p < 0.05$ was considered as a representative of statistical significance). All statistical analyses were performed using the STATA Statistical Software (v.12.0; StataCorp, LP, College Station, TX, USA).

Fig. 1. Flowchart of included studies. TNF- α , tumor necrosis factor- α ; CNKI, China National Knowledge Internet.

Results

Eligible Studies

A flowchart of the literature search, according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines, is shown in Figure 1. In total,

11 case-control studies were selected in our systematic review [14–24], all of which were referred to TNF- α -308 G/A polymorphism and 4 of which were related to TNF- α -238 G/A polymorphism [16, 17, 20, 21]. Owing to the insufficient information about the distribution of genotypes in several studies [14–16], there were only 8

case-control studies including 1,698 cases (DR) and 2,064 controls (DWR) [17–24] and 3 case-control studies including 659 cases and 576 controls [17, 20, 21], which correlate with TNF- α -308 G/A and TNF- α -238 G/A polymorphism, respectively, were included in the meta-analysis.

Characteristics of the Studies and Data Analysis

The main characteristics of 11 studies included in the systematic review were shown in Table 1. The quality evaluation of included studies by Newcastle-Ottawa Scale suggested that all of studies had high quality, except the study conducted by Bućan et al. [21] in 2009 which had intermediate quality. On the TNF- α -308 G/A polymorphism side, there were 6 studies of the Asians and 5 studies of the Europeans among these eligible publications. Ten studies were related to T2DM compared with 4 studies for T1DM, since 3 of them referred to both T1DM and T2DM. Ten studies showed no association between TNF- α -308 G/A polymorphism and DR in spite of the ethnicity or DM type, while a case-control study in Brazil concerning the European T2DM subjects proved TNF- α -308 A allele was in connection with an increased risk of PDR [17]. In the matter of TNF- α -238 G/A polymorphism, 1 study of the Asians and 3 studies of the Europeans were included. Three studies were related to T2DM, while 2 studies were in association with T1DM, since 1 of them referred to both T1DM and T2DM. The findings of 3 studies did not indicate any association between TNF- α -238 G/A polymorphism and DR, while that of 1 study connected with the Asian T2DM patients stated that TNF- α -238 A was a potent hazard factor for the occurrence of PDR.

For the further quantitative analysis, the related data of these studies were shown in Table 2. Among studies investigating the TNF- α -308 G/A polymorphism, the methods for genotyping included real-time polymerase chain reaction (PCR), PCR-sequence-specific primers, amplification refractory mutation system-polymerase chain reaction, PCR-restriction fragment length polymorphism method, allele-specific PCR, and Taq-PCR. Besides, only the study conducted by Bućan et al. [21] in 2009 having a deviation from HWE in the controls of those studies. And for the TNF- α -238 G/A polymorphism, the methods for genotyping involve real-time PCR, amplification refractory mutation system-PCR, and PCR-restriction fragment length polymorphism method. The control of the study is conducted by Sesti et al. [17] in 2015 deviating from HWE.

Meta-Analysis

The main results of the meta-analysis and the heterogeneity test are shown in Table 3. For the TNF- α -308 G/A polymorphism, the results showed that no significant heterogeneity was detected in each model. Overall, we found a marginally statistical significant association between the allele A and DR risk in overall population (for GA vs. GG: OR 1.21, 95% CI 1.04–1.41; for [GA + AA] vs. GG: OR 1.20, 95% CI 1.03–1.39; for A vs. G: OR 1.14, 95% CI 1.01–1.30). And in reference to the TNF- α -238 G/A polymorphism, significant heterogeneity existed among all the models, apart from the codominant model (GA vs. GG). Besides, a mild correlation was found in the entire group (for GA vs. GG: OR 1.55, 95% CI 1.14–2.11).

Subgroup Analysis

In the case of TNF- α -308 G/A polymorphism, significantly increased risks were observed among the European population in the subgroup analysis by ethnicity (for GA vs. GG: OR 1.27, 95% CI 1.06–1.51; for [GA + AA] vs. GG: OR 1.25, 95% CI 1.05–1.49; for A vs. G: OR 1.17, 95% CI 1.01–1.36; shown in Table 3). In the stratified analysis by the type of DM, the TNF- α variation was not found associated with elevated DR risk in either T1DM patients or T2DM patients in any model (shown in Table 3). And when it comes to the TNF- α -238 G/A polymorphism, every model's heterogeneity was eliminated when the subgroup analysis was made according to ethnicity (for each model, $I^2 = 0.0$), but remained no change by DM type. Therefore, it was the factor, ethnicity, that accounted for the heterogeneity of included studies (shown in Table 3). What's more, the susceptibility of DR enhanced among the Asian population (for AA vs. GG: OR 5.79, 95% CI 2.47–13.57; for GA vs. GG: OR 1.91, 95% CI 1.29–2.83; for [GA + AA] vs. GG: OR 2.30, 95% CI 1.58–3.33; for AA vs. [GA + GG]: OR 4.65, 95% CI 2.01–10.77; for A vs. G: OR 2.29, 95% CI 1.68–3.12; shown in Table 3). What's more, in the subgroup analysis by DM type, except for the model GA versus GG which indicated low coherence between TNF- α -238 G/A and T2DM (OR 1.55, 95% CI 1.13–2.11), no significant relationship was examined.

Sensitivity Analysis

Given the study not conforming to HWE, we performed the sensitivity analysis. With regard to the TNF- α -308 G/A polymorphism, when the study conducted by Bućan et al. [21] in 2009 was removed, the pooled ORs did not change substantially in all comparison models, and it represented the good stability and reliability of outcomes of the meta-analysis (shown in

Table 1. Main characteristics of case-control studies included in the systematic review

First author	Ethnicity	DM type	Sample sizes	Age, years	DM duration, years	Association with DR	NOS score
<i>TNF-α-308 G/A</i> Fan et al. [14]	Asian	T2	314 255	– –	13.0 $\pm$ 8.0 14.7 $\pm$ 6.0	No	8
Wang et al. [15]	Asian	T1	120 100	58.6 $\pm$ 15.2	15.2 $\pm$ 6.6	No	7
		T2	120 100	61.2 $\pm$ 16.6	18.4 $\pm$ 7.4	No	7
Kaidonis et al. [16]	European	T1	340* 260	– –	– –	No	8
		T2	993* 901	– –	– –	No	8
Sesti et al. [17]	European	T2	404 322	61.6 $\pm$ 9.3 60.0 $\pm$ 9.6	14.8 $\pm$ 8.9 11.8 $\pm$ 6.9	Yes	7
Rodrigues et al. [18]	European	T2	66 36	56.6 $\pm$ 8.5 52.8 $\pm$ 9.2	17.3 $\pm$ 9.2 8.8 $\pm$ 7.7	No	7
Sikka et al. [19]	Asian	T2	162 300	58.9 $\pm$ 7.5 59.4 $\pm$ 9.7*	13.3 $\pm$ 7.2 10.1 $\pm$ 7.8	No	7
Paine et al. [20]	Asian	T2	253 240	52.0 $\pm$ 15.0 54.0 $\pm$ 12.0	16.0 $\pm$ 6.0 17.0 $\pm$ 7.0	No	8
Bućan et al. [21]	European	T1	19 28	30.8 $\pm$ 13.8*	13.9 $\pm$ 9.1*	No	6
Lindholm et al. [22]	European	T1	315 307	38.2 $\pm$ 13.6*	18.1 $\pm$ 15.6*	No	7
		T2	295 583	61.2 $\pm$ 11.7*	2.58 $\pm$ 7.0*	No	7
Wang et al. [23]	Asian	T2	109 72	64.5 $\pm$ 9.9* 63.8 $\pm$ 10.0*	14.9 $\pm$ 3.8* 13.9 $\pm$ 3.3*	No	7
Yoshioka et al. [24]	Asian	T2	75 176	62.3 $\pm$ 9.0 61.9 $\pm$ 10.5	14.3 $\pm$ 6.4 12.3 $\pm$ 6.3	No	8
<i>TNF-α-238 G/A</i> Kaidonis et al. [16]	European	T1	340* 260	– –	– –	No	8
		T2	993* 901	– –	– –	No	8
Sesti et al. [17]	European	T2	387 308	61.6 $\pm$ 9.3* 59.9 $\pm$ 9.6*	14.8 $\pm$ 8.8* 11.8 $\pm$ 7.0*	No	7
Paine et al. [20]	Asian	T2	253 240	52.0 $\pm$ 15.0 54.0 $\pm$ 12.0	16.0 $\pm$ 6.0 17.0 $\pm$ 7.0	Yes	8
Bućan et al. [21]	European	T1	19 28	30.8 $\pm$ 13.8*	13.9 $\pm$ 9.1*	No	6

Data are displayed as mean $\pm$ standard deviation. In each item, the upper line represents the DR group, while the lower line represents the DWR group. DM, diabetes mellitus; NOS, Newcastle-Ottawa Scale; –, not available; DR, patients with diabetic retinopathy; DWR, patients without diabetic retinopathy. * Estimated values.

Table 2. Description of key data for studies included in the meta-analysis

First author	Genotyping methods	Sex ratio (M/F)	Genotypes					HWE
			GG	GA	AA	G	A	
<i>TNF-α-308 G/A</i>								
Sesti et al. [17]	Real-time PCR	(223, 181) (137, 185)	284 251	108 68	12 3	676 570	132 74	0.49
Rodrigues et al. [18]	PCR-SSP	(15, 51) (4, 32)	49 29	17 6	0 1	115 64	17 8	0.35
Sikka et al. [19]	ARMS-PCR	(−, −) (−, −)	142 263	19 36	1 1	303 562	21 38	0.84
Paine et al. [20]	ARMS-PCR	(133, 120) (128, 112)	165 161	77 70	11 9	407 392	99 88	0.69
Bućan et al. [21]	PCR-RFLP	(−, −) (−, −)	11 20	7 5	1 3	29 45	9 11	0.02
Lindholm et al. [22]	Real-time PCR	(−, −)	145	154	16	444	186	0.15
		(−, −)	154	134	19	442	172	
		(−, −)	200	85	10	485	105	
		(−, −)	379	179	25	937	229	
Wang et al. [23]	Taq-PCR	(−, −) (−, −)	94 63	15 8	0 1	203 134	15 10	0.23
Yoshioka et al. [24]	AS-PCR	(39, 36) (115, 61)	74 172	1 4	0 0	149 348	1 4	0.88
<i>TNF-α-238 G/A</i>								
Sesti et al. [17]	Real-time PCR	(212, 175) (133, 175)	347 277	38 28	2 3	732 582	42 34	0.02
Paine et al. [20]	ARMS-PCR	(133, 120) (128, 112)	130 170	92 63	31 7	352 403	154 77	0.69
Bućan et al. [21]	PCR-RFLP	(−, −) (−, −)	17 26	2 2	0 0	36 54	2 2	0.84
In each item, the upper line represents the DR group, while the lower line represents the DWR group. M, male; F, female; HWE, Hardy-Weinberg equilibrium; PCR, polymerase chain reaction; PCR-SSP, PCR-sequence-specific primers; ARMS-PCR, amplification refractory mutation system PCR; PCR-RFLP, PCR restriction fragment length polymorphism method; ASPCR, allele-specific PCR; DR, patients with diabetic retinopathy; DWR, patients without diabetic retinopathy.								

Fig. 2a–e). Similarly, as for the TNF- α -238 G/A polymorphism, when the study of Sesti et al. [17] was omitted, the results also did not have any qualitative alteration, which indicated that the results of this meta-analysis were basically stable and robust (shown in Fig. 2f–j).

Publication Bias

On the part of TNF- α -308 G/A polymorphism, the Begg's funnel plot appeared symmetric (shown in Fig. 3a–e), and the Egger's test supported that there was no significant statistical evidence of publication bias for genetic

models AA versus GG, GA versus GG (GA + AA) versus GG, AA versus (GG + GA), and A versus G model, with the *p* values of 0.293, 0.805, 1.000, 0.293, and 0.621, respectively. In terms of TNF- α -238 G/A polymorphism, from the Begg's funnel plot of models GA versus GG and (GA + AA) versus GG, studies included were within the range of CIs, while those of models AA versus GG, AA versus (GG + GA), and A versus G were not (shown in Fig. 3f–j). However, the Egger's test showed no publication bias for any of 5 genetic models, with the *p* values of 0.317, 0.602, 0.602, 0.317, and 0.602, respectively.

Table 3. Summary of odds ratios and 95% confidence intervals of TNF- α -308 G/A and -238 G/A polymorphism and diabetic retinopathy risk

Comparisons	Studies, <i>n</i>	OR (95% CI)	<i>I</i> ² (%)	Alleles/genotypes (DR/DWR)
<i>TNF-α-308 G/A</i>				
AA versus GG				
Total	8	1.08 (0.73, 1.58)	2.1	2,769 (1,215/1,554)
Ethnicity				
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)
Type of DM				
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)
GA versus GG				
Total	8	1.21 (1.04, 1.41)	0.0	3,649 (1,647/2,002)
Ethnicity				
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)
Type of DM				
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)
(GA + AA) versus GG				
Total	8	1.20 (1.03, 1.39)	0.0	3,762 (1,698/2,064)
Ethnicity				
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)
Type of DM				
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)
AA versus (GA + GG)				
Total	8	1.00 (0.69, 1.47)	5.7	3,762 (1,698/2,064)
Ethnicity				
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)
Type of DM				
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 (1,364/1,729)
A versus G				
Total	8	1.14 (1.01, 1.30)	0.0	7,524 (3,396/4,128)
Ethnicity				
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)
Type of DM				
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)
<i>TNF-α-238 G/A</i>				
AA versus GG				
Total	3	2.01 (0.20, 20.56)	82.0	1,010 (527/483)
Ethnicity				
European	2	0.53 (0.09, 3.21)	0.0	672 (366/306)
Asian	1	5.79 (2.47, 13.57)	0.0	338 (161/177)
Type of DM				
T1	1	Excluded	–	43 (17/26)
T2	2	2.01 (0.20, 20.56)	82.0	967 (510/457)
GA versus GG				
Total	3	1.55 (1.14, 2.11)	32.3	1,192 (626/566)

Table 3 (continued)

Comparisons	Studies, <i>n</i>	OR (95% CI)	<i>I</i> ² (%)	Alleles/genotypes (DR/DWR)
Ethnicity				
European	2	1.11 (0.67, 1.82)	0.0	737 (404/333)
Asian	1	1.91 (1.29, 2.83)	0.0	455 (222/233)
Type of DM				
T1	1	1.53 (0.20, 11.92)	5.4	47 (19/28)
T2	2	1.55 (1.13, 2.11)	66.1	1,145 (607/538)
(GA + AA) versus GG				
Total	3	1.57 (0.80, 3.07)	69.1	1,235 (659/576)
Ethnicity				
European	2	1.05 (0.65, 1.70)	0.0	742 (406/336)
Asian	1	2.30 (1.58, 3.33)	0.0	493 (253/240)
Type of DM				
T1	1	1.53 (0.20, 11.92)	0.0	47 (19/28)
T2	2	1.57 (0.71, 3.43)	84.5	1,188 (640/548)
AA versus (GA + GG)				
Total	3	1.82 (0.22, 15.04)	78.4	1,235 (659/576)
Ethnicity				
European	2	0.53 (0.09, 3.18)	0.0	742 (406/336)
Asian	1	4.65 (2.01, 10.77)	0.0	493 (253/240)
Type of DM				
T1	1	Excluded	–	47 (19/28)
T2	2	1.82 (0.22, 15.04)	78.4	1,188 (640/548)
A versus G				
Total	3	1.53 (0.74, 3.15)	77.4	2470 (1,318/1,152)
Ethnicity				
European	2	1.00 (0.64, 1.58)	0.0	1,484 (812/672)
Asian	1	2.29 (1.68, 3.12)	0.0	986 (506/480)
Type of DM				
T1	1	1.50 (0.20, 11.14)	0.0	94 (38/56)
T2	2	1.53 (0.67, 3.50)	88.7	2,376 (1,280/1,096)

OR, odds ratio; TNF- α , tumor necrosis factor-alpha; DM, diabetes mellitus; DR, patients with diabetic retinopathy; DWR, patients without diabetic retinopathy; CI, confidence interval.

Discussion

Unraveling genes that contribute to the pathogenic risk of DR has been one of the major foci of basic researches in DR over the past few decades. A growing number of putative genes and genetic variants have been discussed to detect the genetic risk loci associated with DR [25, 26]. However, the relationship between TNF- α polymorphism and the presence and progression of DR remained unclear due to controversial findings generated by several relatively small studies. Consequently, we are intended to discuss this issue in depth.

Relevance of TNF- α -308 G/A Polymorphism and DR

The earliest investigation covering the theme of TNF- α -308 G/A polymorphism and DR risk was report-

ed by Wang et al. [27]. However, owing to the failure of reporting the genotype distribution of TNF- α -308 G/A polymorphism in DR and DWR patients, it is impossible to determine whether there is a correlation between them. Our systematic review covered 11 available relevant researches and revealed that compared with 10 studies arguing no linkage between TNF- α -308 G/A polymorphism and DR whatever the ethnicity or DM type is, 1 study recruiting the European T2DM subjects suggested that -308 A allele might raise the morbidity of PDR. Next, the meta-analysis included 8 studies involving 1,698 DR cases and 2,064 controls. Under the codominant model (GA vs. GG), the dominant model ([GA + AA] vs. GG), and the allelic model (A allele vs. G allele), all summary ORs indicated that TNF- α -308 G/A polymorphism was marginally significantly associated with an increased risk

Fig. 2. Sensitivity analyses to explore the influence of the individual dataset to the pooled ORs. **a–e** The models of AA versus GG, GA versus GG (GA + AA) versus GG, AA versus (GG + GA) and A versus G about the TNF- α -308 G/A polymorphism, respectively. **f–j** Those of the TNF- α -238 G/A polymorphism, respectively. OR, odds ratio; TNF- α , tumor necrosis factor- α ; CI, confidence interval.

of DR. Because differences in ethnic groups, which bring out different genetic backgrounds, might affect genetic predispositions to human diseases [28], the subgroups were based on ethnicity. In the stratified analysis, significant differences were observed with the European ethnicity, while not among the Asian population. In addition, there was evidence suggesting that rs1800629 was found a consistent association with the risk of T1DM [29], but not with T2DM [30]. As a result, we further explored the difference in the separate analyses of T1DM and T2DM.

However, the finding implied the lack of a relationship between this polymorphism and the type of DM. What's more, it should be noted that the genetic distributions of the controls in the study conducted by Buc'an et al. [21] in 2009 deviated from HWE, indicating the possibility of bias. Thus, we conducted the sensitivity analyses to omit the study. Results from the pooled ORs before and after omitting the study deviating from HWE were similar, suggesting that the result was little affected by the study. Furthermore, no significant publication bias was ob-

Fig. 3. Funnel plot of the meta-analysis of TNF- α polymorphism and risk for diabetic retinopathy. **a–e** The models of AA versus GG, GA versus GG, (GA + AA) versus GG, AA versus (GG + GA), and A versus G about the TNF- α -308 G/A polymorphism, respectively. **f–j** Those of the TNF- α -238 G/A polymorphism, respectively. TNF- α , tumor necrosis factor- α ; OR, odds ratio.

served in the pooled results in any genetic model, demonstrating the robustness of our meta-analysis.

Previously, meta-analyses conducted by Liu et al. [11] and Meng et al. [13] showed that there was no obviously statistical relevancy between DR and TNF- α -308 G/A polymorphism in global and/or subgroup analyses by DM type and ethnicity. In our meta-analysis, 2 extra studies were included, and that might explain why marginal significance was detected among the European population. Besides, some studies also focused on the issue that whether there is a relationship between TNF- α polymorphism and different stages of DR, such as NPDR and PDR. For example, Sesti et al. [17] found that although having no difference between the NPDR subjects and DWR subjects, the genetic frequency of -308 A polymorphism in PDR subjects was more than that of DWR subjects. Then, this was upheld by the achievements of Fan and his colleagues [12]. However, there were also studies deeming no significant association between this promoter polymorphism and different DR phenotypes [15]. Further, on the basis of the divergence about the microaneurysms turnover and the central retinal thickness, Simões et al. [31] stratified 307 NPDR patients of T2DM into 3 different phenotypes, and by massively genotyping single nucleotide polymorphisms of several candidate genes where TNF- α -308 G/A was comprised, they still did not discover any definite correlation between the variant and NPDR.

Unexpectedly, while the findings of our research suggested an enhanced association between rs1800629 and DR in the European population, Liu et al. [11] observed a stronger connection between rs1800629 and diabetic nephropathy (DN), another major complication of DM, in the Asian population. The ethnic difference in the susceptibility to DR and DN of rs1800629 may need a further in-depth study.

Relevance of TNF- α -238 G/A Polymorphism and DR

Four researches were retrieved for the qualitative analysis. Among them, 3 articles suggested that TNF- α -238 G/A was irrelevant with DR in both T1DM and T2DM, and 1 study found certain association between -238 A allele and PDR. Further, 3 studies involving 659 DR cases and 576 controls were included for the meta-analysis. Under the codominant model (GA vs. GG), the summary OR demonstrated a mild correlation between TNF- α -238 G/A variant and DR. Similar to rs1800629, there was also ethnic disparity about the pathogenicity of rs361525 in some diseases [32]. Thus, the subgroup analysis based on ethnicity was performed. On one hand, heterogeneity among studies included descended sharply, which proved

that ethnic differences could lead to the deviation of the results and may account for the sources of heterogeneity. On the other hand, statistical correlations were observed among the Asian population in all analysis models, suggesting that -238 A carriers might have a high risk of DR in Asian subjects. Besides, there were evidences that functions of the variant might vary depending on the type of DM [33, 34]. Therefore, we next discussed the effect of T1DM and T2DM. Neither heterogeneity among included studies nor the general results appeared any marked change. So we concluded that DM type had no notable effect on the findings. Furthermore, though the control's genotype distribution in the study of Sesti et al. [17] did not comply with HWE, the sensitivity analysis proved that it would not alter the ultimate result strikingly. Meanwhile, no remarkable publication bias was detected in the pooled results for any analysis model, which strengthened the reliability of our meta-analysis.

Similarly, there were also a few of studies exploring the relationship between TNF- α -238 G/A polymorphism and various degrees of DR. Sesti et al. [17] reported that the -238 G and A allele frequencies in NPDR patients were not significantly distinct with those of DWR patients. Unlike the variant of rs1800629, the meta-analysis published by Fan et al. [12] did not find any correlation between rs361525 and DR in total or in the PDR subgroup. The reason for such a difference might be due to the difference of analysis models adopted. The meta-analysis of Fan et al. only used 3 analysis models, namely the dominant model ([GA + AA] vs. GG), the recessive model (AA versus [GG + GA]), and the allelic model (A allele vs. G allele). By contrast, not only those models but also the codominant models (AA vs. GG, GA vs. GG) were undertaken in our meta-analysis. And it was just the codominant model (GA vs. GG) that demonstrated the association between TNF- α -238 G/A polymorphism and DR. Consequently, in case of missing some potential findings, it is necessary to perform comprehensive analyses. What is more, 1 extra study was also included in our meta-analysis.

However, reports about the relationship between TNF- α -238 G/A polymorphism and DN are still limited. Therefore, there may be a need to carry out the relevant research.

Issues Remaining

Several inadequacies exist in our meta-analysis, and there is still much left to be improved. To begin with, in our meta-analysis, the accurate disease durations, ages, and classifications of DR for individual patients, which can matter much in the final conclusions, are unavailable. For example, relevant researches have showed that the predisposi-

tion to immunity stimuli among the elderly often has to do with altered gene frequencies of some inflammatory factors, such as TNF- α [35], and sex can also play a vital role in the association between TNF and certain diseases [36]. As a result, it is essential to investigate the relationship of TNF- α polymorphism and DR risk in different age and/or gender groups. Next, it merits attention that several studies are not in line with HWE. This may heighten the selection bias in the controls, although sensitivity analyses have suggested the high stability of our meta-analysis's conclusions. Afterward, this meta-analysis was limited by the small sample size – especially for the TNF- α -238 G/A polymorphism – though Egger's tests testify no publication bias. Finally, we have not carried out in-depth explorations on some potential gene-gene interactions. For example, Kumar et al. [33] ascertained that among Indian patients, it is the linkage disequilibrium between TNF- α -308 G/A and -238 G/A and particular HLA-DR3-DQ2 haplotypes that results in the associations of them with T1DM. Those discrepancies indicate the need to implement rigorous quality control procedures in future studies.

Conclusions

Our systematic review suggests that TNF- α polymorphism may be associated with elevated DR risk in DM patients, especially TNF- α -308 G/A polymorphism in the European population and TNF- α -238 G/A polymorphism in the Asian population. Because of the small sample size and the limitations of our study, future larger scale epidemiological investigation of this topic should be conducted to validate our findings.

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Statement of Ethics

Our paper only makes a secondary analysis of published studies and does not include the original overview of clinical trials, and ethical approval is not required. Thus, in our paper, the ethical statement is not included. In addition, we have registered this study on the Prospero Website with the registration ID of CRD42020189022.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Yang Liu contributed to the idea and design of this study and revised the manuscript. Both Wenna Gao and RuiLin Zhu carried out the screening procedure and data extraction. Besides, Wenna Gao participated in the design of the study, performed the statistical analysis, and drafted the manuscript. Ruilin Zhu revised the manuscript, helped to improve the English language, and gave some suggestions to the manuscript. All authors read and approved the final manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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