Research Article

The association of TNF-α −308G/A and −238G/A polymorphisms with type 2 diabetes mellitus: a meta-analysis

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Introduction

Diabetes is a global epidemic, with an estimated worldwide prevalence of 1 in 11 adults (approximately 425 million people in 2017), and is projected to increase to 629 million people by 2045 (http://www.diabetesatlas.org/). Individuals with type 2 diabetes mellitus (T2DM) accounted for 90% of this total [1]. T2DM is a complex metabolic disorder and usually involves pancreatic islet dysfunction and insulin-secreting β cell failure in the endocrine pancreas (Islets of Langerhans), allowing for the secretion of more insulin to counteract insulin resistance in peripheral tissues (adipose, skeletal muscle and liver). Ultimately, T2DM shows an uncontrolled increase in blood glucose levels [2], therefore the pathogenesis of T2DM is insulin resistance [3].

Some in vivo and in vitro studies have shown that tumor necrosis factor-α (TNF-α) induces insulin resistance to some extent, through the inhibition of intracellular signaling from the insulin receptor [4,5]. The disease has a strong genetic component, however few genes have been identified [1]. Several genome-wide association scans (GWAS) have been performed for T2DM and several candidate genes...
have been proposed [6–10]. Of multiple candidate genes, the TNF-α promoter polymorphisms −308G/A and −238G/A have been studied in T2DM etiology [11].

Currently, it is inconclusive whether these polymorphisms (−308G/A and −238G/A) in the TNF-α promoter lead to T2DM susceptibility. Two large-scale British association analyses found these polymorphisms were not robustly associated with T2DM [11,12] and similar results have been observed in China [13,14] and India [15]. However, studies have also suggested that −308G/A and −238G/A are risk factors for T2DM in Egypt [16] and Iran [17]. Studies from different racial backgrounds may produce conflicting results and these independent studies are confusing and controversial. Therefore, we performed a large-scale meta-analysis to investigate associations between these polymorphisms and T2DM.

Materials and methods

Literature search

This meta-analysis was conducted according to the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis 2009 (PRISMA2009). All published studies up to October 2018 were searched using the PubMed, Embase, EBSCO, OVID, and Web of science database. We used the following terms: 'TNF-α', 'TNF-alpha', 'tumor necrosis factor-alpha', 'T2DM', 'type 2 diabetes mellitus', 'type 2 diabetes', 'type II diabetes', 'non-insulin dependent diabetes', 'NIDDM', 'polymorphism', 'variation', '−308G/A', 'rs1800629', '−238G/A' and 'rs361525'. Relevant references in selected articles were also included.

All articles were independently reviewed by two investigators. Studies were assessed against the following inclusion criteria: (1) the associated study of TNF-α polymorphisms (−308G/A and −238G/A) with the risk of T2DM, (2) the study was case–control designed, (3) sufficient information on genotype frequencies (GG, AA and GA) in both cases and controls to estimate an odds ratio (OR) with a 95% confidence interval (95% CI), (4) all data were original. Exclusion criteria were as follows: (1) other DM (diabetes) types were excluded, (2) non-human studies, (3) reviews, meta-analysis and non-case–control studies and (4) studies not published in English.

Quality score assessment

Study quality was assessed to guarantee the strength of results and conclusions. Quality assessment was performed according to the Newcastle–Ottawa Quality Assessment Scale (NOS), which is a validated scale for nonrandomized studies in meta-analyses [18]. This NOS uses a star system to assess the quality of a study in three domains: selection, comparability and exposure/outcome. The NOS assigns a maximum of 5 stars for selection (in the case of cross-sectional studies), 2 stars for comparability, and 3 stars for outcome/exposure. Studies achieving a score of at least 8 stars were classified as being at low risk of bias (i.e., thus reflecting the highest quality). A maximum of 9 scores, including selection, comparability and exposure items were awarded. Any score disagreements were decided by a third researcher.

Data extraction

Data were independently extracted by two investigators using a standardized form. For each study, the following information was extracted: (1) name of first author; (2) year of publication; (3) ethnicity of population; (4) sample sizes and genotype distributions; (5) allele frequency of the major variant. Ethnicity was categorized as Caucasian, Asian and African.

Statistical analysis

The Hardy–Weinberg equilibrium (HWE) test was calculated using the Chi-squared test. The distribution of allele frequencies in controls was considered to deviate from HWE when $P < 0.05$. STATA (15.0; Stata Corporation, College Station, TX, U.S.A.) software was used to calculate meta-analysis results. Individual study heterogeneity was assessed by Cochran’s Q test and the $I^2$ statistic ($P < 0.10$ and $I^2 > 50\%$ indicates evidence of heterogeneity) [19]. The fixed-effects model (Mantel–Haenszel method) was used to estimate the pooled OR [20], when there was no evidence of heterogeneity, otherwise the random-effects model (DerSimonian and Laird method) was used [20,21]. ORs with corresponding 95% CIs were calculated to assess associations between TNF-α promoter polymorphisms (−308G/A and −238G/A) and T2DM risks. Five genetic models were used in this meta-analysis: (1) the allele model (A allele vs. G allele); (2) the dominant model (GA+AA vs. GG); (3) the recessive model (AA vs. GA+GG); (4) the codominant model (GA vs. GG; AA vs.GG) and (5) the overdominant model (GG+AA vs. GA). A $P$-value $<0.05$ was accepted as the significant threshold for each genetic model. Three subgroups, including Caucasian, Asian and African, based on ethnicity, were analyzed to reduce influences from genetic backgrounds. A meta-regression was
Figure 1. Study flow diagram

**Identification**

977 citations searched in PubMed, Embase, EBSCO, OVID and Web of science databases and through search of reference signs in relevant articles

766 were excluded for not relevant to TNF-α -308G/A, -238G/A and type 2 diabetes

**Screening**

211 publications for further assignment

124 were excluded due to no fulfilling inclusion criteria (review, meta-analysis and not case-control studies)

**Eligibility**

87 publications left for data extraction

37 publications were excluded due to not provide sufficient genotype information

**Included**

49 publications were included in the meta-analysis

14 studies for TNF-α -238G/A

49 studies for TNF-α -308G/A
used to search the source of heterogeneity [22], which contained publication year, sample size, ethnicity, HWE and number of studies. The 10000 times Monte Carlo permutation test approach was used for assessing the statistical significance of meta-regression [23,24]. \( I^2 \) res explained the proportion of residual variation due to heterogeneity, and \( \text{adj } R^2 \) explained the proportion of between-study variation due to heterogeneity [25,26]. An \( I^2 \) res close to 100% and \( \text{adj } R^2 \) close to 0% further indicated no effects on heterogeneity. Pooled estimates were performed to sensitivity analysis which involved omitting one study at a time followed by recalculation to test for robustness of the summary effects [26]. To increase transparency, risk of bias ratings and meta-analyses were displayed together. Funnel plots were used to investigate the risk of publication bias [23]. Egger’s and Begg’s regression tests evaluated publication bias with quantitative analysis [27]. A \( P \)-value <0.05 was accepted as statistically significant.

### Figure 2. Forest plot of the association of TNF-\( \alpha \) −308G/A and type 2 diabetes (A vs. G) in random-effects model

Each square is proportional to the study-specific weight.
Figure 3. Forest plot of the association of TNF-α −238G/A and type 2 diabetes (A vs. G) in fixed-effects model
Each square is proportional to the study-specific weight.

Results
Study characteristics
Based on the above search strategy, 977 publications were identified in the initial search. Approximately 766 articles were excluded after scanning titles and abstracts as being non-relevant to T2DM and TNF-α −308G/A and −238G/A. Through in-depth full-text analysis of the remaining 211 publications, 49 publications were used for the final meta-analysis (Figure 1). These 49 publications contained 16246 patients and 13973 controls and were included in the −308G/A analysis, of which 14 publications, with 4935 patients and 5260 controls, were included in the −238G/A analysis. According to NOS classifications, three points or lower indicated low quality, however no publications were of low quality. The main characteristics of selected publications are shown in Table 1.

Overall population
The meta-analysis showed a significant association between TNF-α −308G/A and T2DM risk in the allele model (OR = 1.239, 95% CI = 1.108–1.385, P = 0.000); the dominant model (OR = 1.280, 95% CI = 1.116–1.469, P = 0.000); the recessive model (OR = 1.57, 95% CI = 1.065–2.29, P = 0.001); the overdominant model (OR = 1.49, 95% CI = 1.02–2.21, P = 0.049); and the codominant model (OR = 1.691, 95% CI = 1.310–2.184, P = 0.000). TNF-α −238G/A was not associated (P > 0.05) with T2DM in all genetic models (Table 2). After Bonferroni correction, our results were also significantly associated. The forest plot of the −308G/A polymorphism is shown in Figure 2 and −238G/A is shown in Figure 3.

Subgroup by ethnicity
To derive heterogeneity and assess the genetic background, we carried out a subgroup analysis, where the overall population was divided into three subgroups, namely Caucasian, Asian and African. The subgroup analysis showed significant associations between −308G/A and T2DM risk in the Caucasian population in the allele model (OR = 1.224, 95% CI = 1.060–1.413, P = 0.006); the dominant model (OR = 1.282, 95% CI = 1.085–1.514, P = 0.004); the overdominant model (OR = 1.225, 95% CI = 1.050–1.423, P = 0.005), and also in Asian populations in the allele model (OR = 1.324, 95% CI = 1.078–1.626, P = 0.007); the dominant model (OR = 1.367, 95% CI = 1.065–1.754, P = 0.014); the recessive model (OR = 1.789, 95% CI = 1.357–2.357, P = 0.000); the codominant model (OR = 2.368,
| Author          | Year | Country | Ethnicity | Genotype in case | Genotype in control | P of HWE | NOS |
|-----------------|------|---------|-----------|------------------|---------------------|----------|-----|
| Patel et al.    | 2018 | India   | Asian     | 388 (90.5%)      | 351 (91.1%)         | 0.348    | 6   |
| Umapathy et al. | 2018 | India   | Asian     | 538 (56.1%)      | 402 (75.4%)         | 0.001    | 4   |
| Hemmed et al.   | 2018 | India   | Asian     | 862 (61.3%)      | 673 (75.4%)         | 0.080    | 5   |
| Fathy et al.    | 2018 | Kuwaiti | Caucasian | 117 (73.5%)      | 62 (90.7%)          | 0.001    | 6   |
| Rodrigues et al.| 2018 | Brazil  | Caucasian | 102 (76.5%)      | 67 (85.7%)          | 0.279    | 6   |
| Mortazavi et al.| 2018 | India   | Asian     | 214 (13.8%)      | 91 (12.1%)          | 0.080    | 5Fathy et al. | 2018 | Kuwaiti | Caucasian | 117 (73.5%) | 62 (90.7%) | 0.001 | 6 |
| Rodriquez et al.| 2017 | Brazil  | Caucasian | 102 (76.5%)      | 67 (85.7%)          | 0.279    | 6   |
| Mortazavi et al.| 2018 | India   | Asian     | 214 (13.8%)      | 91 (12.1%)          | 0.080    | 5   |
| Jamil et al.    | 2017 | India   | Asian     | 198 (178.9%)     | 138 (87.0%)         | 0.004    | 7   |
| Churnosov et al.| 2017 | Russia  | Caucasian | 236 (74.6%)      | 156 (92.6%)         | 0.180    | 5   |
| Mortazavi et al.| 2017 | Iran    | Caucasian | 214 (13.8%)      | 91 (12.1%)          | 0.080    | 5   |
| Rodrigues et al.| 2017 | Brazil  | Caucasian | 102 (76.5%)      | 67 (85.7%)          | 0.279    | 6   |
| Mortazavi et al.| 2017 | Iran    | Caucasian | 214 (13.8%)      | 91 (12.1%)          | 0.080    | 5   |
| Jamil et al.    | 2017 | India   | Asian     | 198 (178.9%)     | 138 (87.0%)         | 0.004    | 7   |
| Churnosov et al.| 2017 | Russia  | Caucasian | 236 (74.6%)      | 156 (92.6%)         | 0.180    | 5   |
| Mortazavi et al.| 2017 | Iran    | Caucasian | 214 (13.8%)      | 91 (12.1%)          | 0.080    | 5   |
| Rodrigues et al.| 2017 | Brazil  | Caucasian | 102 (76.5%)      | 67 (85.7%)          | 0.279    | 6   |
| Mortazavi et al.| 2017 | Iran    | Caucasian | 214 (13.8%)      | 91 (12.1%)          | 0.080    | 5   |
| Jamil et al.    | 2017 | India   | Asian     | 198 (178.9%)     | 138 (87.0%)         | 0.004    | 7   |
| Churnosov et al.| 2017 | Russia  | Caucasian | 236 (74.6%)      | 156 (92.6%)         | 0.180    | 5   |

Continued over
| Author                  | Year | Country | Ethnicity | TNF-α -308G/A | Genotype in case | Genotype in control | P of HWE | NOS |
|------------------------|------|---------|-----------|---------------|------------------|---------------------|----------|-----|
|                        |      |         |           | Total GG (%)  | GA (%)           | AA (%)              |          |     |
| Wang et al. [66]       | 2008 | China   | Asian     | 181 (86.7%)   | 23 (12.7%)       | 1 (0.6%)            | 0.362    | 5   |
| Kim et al. [34]        | 2006 | Korea   | Asian     | 198 (87.9%)   | 24 (12.1%)       | 0 (0.0%)            | 0.240    | 4   |
| Willer et al. [67]     | 2006 | Finland | Caucasian | 761 (74.6%)   | 184 (24.1%)      | 9 (1.2%)            | 0.235    | 6   |
| Santos et al. [68]     | 2006 | Chile   | Caucasian | 30 (90.6%)    | 3 (10.0%)        | 0 (0.0%)            | 0.552    | 4   |
| Zeggini et al. [12]    | 2005 | Britain | Caucasian | 761 (76.8%)   | 260 (33.5%)      | 32 (4.1%)           | 0.480    | 6   |
| Tsaiou et al. [69]     | 2004 | Greece  | Caucasian | 30 (90.6%)    | 3 (9.4%)         | 0 (0.0%)            | 0.538    | 4   |
| Zouari et al. [70]     | 2004 | Tunisia | African   | 280 (70.0%)   | 64 (22.9%)       | 20 (7.1%)           | 0.698    | 4   |
| Shiau et al. [14]      | 2003 | China   | Asian     | 257 (84.8%)   | 35 (13.6%)       | 4 (1.6%)            | 0.002    | 5   |
| Li et al. [71]         | 2003 | Sweden  | Caucasian | 488 (68.2%)   | 141 (28.9%)      | 14 (2.9%)           | 0.456    | 6   |
| Heijmans et al. [72]   | 2002 | Netherlands | Caucasian | 79 (83.8%)   | 22 (27.8%)       | 6 (7.6%)            | 0.012    | 5   |
| Furuta et al. [73]     | 2002 | Japan   | Asian     | 132 (89.3%)   | 3 (2.3%)         | 0 (0.0%)            | 0.899    | 5   |
| Rasmussen et al. [74]  | 2000 | Denmark | Caucasian | 243 (74.5%)   | 98 (29.9%)       | 5 (1.5%)            | 0.896    | 4   |
| Kamizono et al. [75]   | 2000 | Japan   | Asian     | 213 (98.1%)   | 4 (1.9%)         | 0 (0.0%)            | 0.751    | 4   |
| Pandey et al. [76]     | 1999 | Belgium | Caucasian | 214 (67.3%)   | 61 (28.5%)       | 9 (4.2%)            | 0.233    | 4   |
| Hamann et al. [77]     | 1995 | America | Caucasian | 138 (78.3%)   | 27 (19.6%)       | 3 (2.2%)            | 0.604    | 5   |
| Kung et al. [78]       | 2010 | China   | Asian     | 23 (100.0%)   | 0 (0.0%)         | 0 (0.0%)            | 0.001    | 6   |
| Ko et al. [79]         | 2003 | China   | Asian     | 339 (83.8%)   | 50 (14.7%)       | 5 (1.5%)            | 0.238    | 4   |
| Morris et al. [80]     | 2003 | Australia | Caucasian | 91 (58.2%)   | 32 (32.2%)       | 6 (6.6%)            | 0.427    | 4   |
| Sobic et al. [81]      | 2012 | India   | Asian     | 113 (5.4%)    | 100 (88.5%)      | 7 (8.1%)            | 0.000    | 5   |
|                        |      |         |           | Total GG (%)  | GA (%)           | AA (%)              |          |     |
| Rasmussen et al. [82]  | 2000 | Denmark | Caucasian | 236 (86.9%)   | 31 (13.1%)       | 0 (0.0%)            | 0.459    | 4   |
| Kim et al. [34]        | 2007 | Korea   | Asian     | 198 (77.7%)   | 21 (10.6%)       | 0 (0.0%)            | 0.491    | 4   |
| Sesti et al. [50]      | 2015 | Britain | Caucasian | 695 (89.4%)   | 66 (9.5%)        | 5 (0.7%)            | 0.365    | 7   |
| Santos et al. [68]     | 2006 | Chile   | Caucasian | 30 (90.3%)    | 6 (6.7%)         | 0 (0.0%)            | 0.604    | 4   |
| Li et al. [71]         | 2003 | Sweden  | Caucasian | 488 (94.3%)   | 27 (9.5%)        | 1 (0.2%)            | 0.581    | 6   |
| Dhamodharan et al. [53]| 2015 | India   | Asian     | 133 (67.2%)   | 29 (19.8%)       | 4 (2.9%)            | 0.805    | 5   |
| Patel et al. [15]      | 2018 | India   | Asian     | 320 (91.3%)   | 27 (8.4%)        | 1 (0.3%)            | 0.785    | 7   |
| Fathy et al. [44]      | 2018 | Kuwait  | Asian     | 172 (98.3%)   | 2 (1.7%)         | 0 (0.0%)            | 0.938    | 6   |
| Boraska et al. [63]    | 2010 | Britain | Caucasian | 133 (88.5%)   | 170 (11.3%)      | 3 (0.2%)            | 0.296    | 6   |
| Zeggini et al. [12]    | 2005 | Britain | Caucasian | 560 (83.9%)   | 87 (15.5%)       | 3 (0.5%)            | 0.908    | 6   |
| Jamil et al. [47]      | 2017 | India   | Asian     | 88 (86.7%)    | 12 (12.2%)       | 1 (1.0%)            | 0.094    | 7   |
| Shiau et al. [14]      | 2003 | China   | Asian     | 257 (84.8%)   | 35 (13.6%)       | 4 (1.6%)            | 0.002    | 5   |
| Guzman-Flores et al. [61] | 2011 | Mexico  | Caucasian | 259 (84.9%)   | 31 (12.0%)       | 8 (3.1%)            | 0.622    | 5   |
| Mukhopadhyaya et al. [83] | 2010 | India   | Asian     | 40 (85.7%)    | 3 (7.5%)         | 2 (5.0%)            | 0.805    | 4   |

1 Deviated from HWE.
## Table 2 Association between TNF-α -308G/A and -238G/A and type 2 diabetes

| Genetic model       | Ethnicity | $I^2$ (%) | $P$ (heterogeneity) | OR (95% CI)      | $P$-value | $P$ for publication bias | Effects model |
|---------------------|-----------|-----------|---------------------|-------------------| ---------|--------------------------|---------------|
|                     |           |           |                     |                   |          |                         |               |
| **TNF-α -308G/A A vs G** | Overall  | 73.7      | 0.000              | 1.239 (1.108–1.385) | 0.000    | 0.268                    | 0.000         | Random  |
|                     | Caucasian | 74.6      | 0.000              | 1.224 (1.060–1.413) | 0.006    | 0.135                    | 0.363         | Random  |
|                     | Asian     | 69.2      | 0.000              | 1.324 (1.078–1.626) | 0.007    | 0.809                    | 0.249         | Random  |
|                     | African   | 56.2      | 0.077              | 0.960 (0.679–1.356) | 0.815    | 0.174                    | 0.015         | Random  |
| **GA+AA vs GG**     | Overall  | 74.6      | 0.000              | 1.280 (1.116–1.469) | 0.000    | 0.096                    | 0.275         | Random  |
|                     | Caucasian | 74.6      | 0.000              | 1.282 (1.085–1.514) | 0.004    | 0.069                    | 0.376         | Random  |
|                     | Asian     | 71.7      | 0.000              | 1.367 (1.065–1.754) | 0.014    | 0.174                    | 0.532         | Random  |
|                     | African   | 57.6      | 0.070              | 0.844 (0.522–1.363) | 0.487    | 0.487                    | 0.234         | Random  |
| **AA vs GG+GA**     | Overall  | 38.3      | 0.008              | 1.446 (1.154–1.813) | 0.001    | 0.207                    | 0.125         | Random  |
|                     | Caucasian | 51.3      | 0.005              | 1.240 (0.908–1.692) | 0.176    | 0.469                    | 0.276         | Random  |
|                     | Asian     | 0.0       | 0.497              | 1.789 (1.357–2.357) | 0.000    | 0.284                    | 0.363         | Random  |
|                     | African   | 9.4       | 0.346              | 1.809 (0.890–3.677) | 0.102    | 0.497                    | 0.561         | Random  |
| **GA vs GG+AA**     | Overall  | 67.8      | 0.000              | 1.181 (1.041–1.341) | 0.008    | 0.364                    | 0.604         | Random  |
|                     | Caucasian | 66.3      | 0.000              | 1.225 (1.050–1.423) | 0.005    | 0.243                    | 0.594         | Random  |
|                     | Asian     | 63.7      | 0.000              | 1.230 (0.977–1.548) | 0.079    | 0.846                    | 0.619         | Random  |
|                     | African   | 50.5      | 0.109              | 0.707 (0.455–1.098) | 0.123    | 0.174                    | 0.452         | Random  |
| **AA vs GG**        | Overall  | 47.4      | 0.001              | 1.691 (1.310–2.184) | 0.000    | 0.285                    | 0.068         | Random  |
|                     | Caucasian | 62.8      | 0.000              | 1.399 (0.969–2.018) | 0.073    | 0.506                    | 0.244         | Random  |
|                     | Asian     | 0.0       | 0.842              | 2.368 (1.779–3.153) | 0.000    | 0.365                    | 0.157         | Random  |
|                     | African   | 11.6      | 0.335              | 1.605 (0.765–3.369) | 0.211    | 1.000                    | 0.942         | Random  |
| **AA vs GA**        | Overall  | 31.8      | 0.029              | 1.150 (0.918–1.441) | 0.224    | 0.285                    | 0.068         | Random  |
|                     | Caucasian | 46.8      | 0.013              | 1.031 (0.756–1.405) | 0.847    | 0.506                    | 0.244         | Random  |
|                     | Asian     | 0.0       | 0.533              | 1.138 (0.834–1.553) | 0.414    | 0.365                    | 0.157         | Random  |
|                     | African   | 0.0       | 0.414              | 2.230 (1.160–4.287) | 0.016    | 1.000                    | 0.942         | Random  |
| **TNF-α -238G/A A vs G** | Overall  | 23.0      | 0.205              | 1.064 (0.944–1.200) | 0.309    | 0.524                    | 0.821         | Fixed   |
|                     | Caucasian | 32.3      | 0.170              | 1.076 (0.938–1.234) | 0.295    | 0.453                    | 0.860         | Fixed   |
|                     | Asian     | 22.0      | 0.288              | 1.027 (0.802–1.316) | 0.832    | 0.881                    | 0.639         | Fixed   |
| **GA+AA vs GG**     | Overall  | 8.3       | 0.382              | 1.045 (0.921–1.187) | 0.936    | 0.396                    | 0.947         | Fixed   |
|                     | Caucasian | 15.8      | 0.306              | 1.056 (0.914–1.220) | 0.459    | 0.293                    | 0.801         | Fixed   |
|                     | Asian     | 13.5      | 0.328              | 1.011 (0.774–1.320) | 0.492    | 0.881                    | 0.719         | Fixed   |
| **AA vs GG+GA**     | Overall  | 0.0       | 0.497              | 1.554 (0.896–2.692) | 0.085    | 0.881                    | 0.754         | Fixed   |
|                     | Caucasian | 31.2      | 0.202              | 1.795 (0.888–4.533) | 3.628    | 0.573                    | 0.350         | Fixed   |
|                     | Asian     | 0.0       | 0.810              | 1.243 (0.516–2.977) | 0.619    | 0.327                    | 0.680         | Fixed   |
| **GA vs GG+AA**     | Overall  | 0.0       | 0.462              | 1.021 (0.897–1.162) | 0.758    | 0.396                    | 0.908         | Fixed   |
|                     | Caucasian | 4.1       | 0.398              | 1.029 (0.889–1.192) | 0.698    | 0.453                    | 0.689         | Fixed   |
|                     | Asian     | 8.4       | 0.383              | 0.990 (0.751–1.304) | 0.943    | 0.662                    | 0.813         | Fixed   |
| **AA vs GG**        | Overall  | 0.0       | 0.496              | 1.569 (0.905–2.721) | 0.078    | 0.881                    | 0.748         | Fixed   |
|                     | Caucasian | 31.6      | 0.198              | 1.807 (0.894–3.654) | 0.064    | 0.348                    | 0.414         | Fixed   |
|                     | Asian     | 0.0       | 0.811              | 1.262 (0.523–3.048) | 0.596    | 0.142                    | 0.356         | Fixed   |

Continued over
Table 2 Association between TNF-α -308G/A and -238G/A and type 2 diabetes (Continued)

| Genetic model | Ethnicity | I² (%) | P (heterogeneity) | OR (95% CI) | P-value | P for publication bias | Effects model |
|---------------|-----------|--------|-------------------|-------------|---------|----------------------|--------------|
| AA vs GA      | Overall   | 0.0    | 0.533             | 1.429 (0.808–2.526) | 0.178   | 0.881 | 0.748 | Fixed |
|               | Caucasian | 24.3   | 0.252             | 1.688 (0.822–3.466) | 0.117   | 0.348 | 0.414 | Fixed |
|               | Asian     | 0.0    | 0.778             | 1.079 (0.424–2.748) | 0.852   | 0.142 | 0.356 | Fixed |

† P<0.05.
‡ P<0.01.
§ P<0.001.

95% CI = 1.779–3.153, P=0.000) and no associations between −308G/A and T2DM risk in African populations (P>0.05). For −238G/A, it was not associated (P>0.05) with T2DM in the subgroup population (Table 2).

Meta-regression and sensitivity analysis
The following covariates were considered for meta-regression: publication year, sample size, ethnicity and HWE in controls. The −308G/A results revealed no influence on the publication year (I² res = 91.89%, adj R² = 5.37%, P=0.084), sample size (I² res = 94.31%, adj R² = 1.11%, P=0.215), HWE (I² res = 92.83%, adj R² = 2.97%, P=0.882) and ethnicity, including Caucasian (P=0.106), Asian (P=0.127), using the 10000 times Monte Carlo permutation test. The −238G/A results revealed no influence from publication year (P=0.573), sample size (P=0.498) and ethnicity, including Caucasian (P=0.864) and Asian (P=0.735), using the 10000 times Monte Carlo permutation test. Sensitivity analysis revealed that some studies [17,28–32] have observed bias (Figure 4). But no significant changes in heterogeneity were observed after excluding these studies except study by Golshani et al. [17]. After its removal, the heterogeneity was greatly reduced in the Caucasian subgroup (from 74.6 to 47.4), but there was still a significant association between −308G/A and T2DM (OR = 1.148, 95% CI = 1.033–1.277, P=0.011).

Publication bias
Publication bias data for TNF-α −308G/A and −238G/A, in all genetic models are shown in Table 2. The continuity corrected results showed no existing publication bias (P>0.05). The Begg’s and Egger’s tests showed no existing publication bias in the overall population for all genetic models (Table 2). There are no bias and asymmetry found in Begg’s and Egger’s funnel plots (Figures 5 and 6).

Discussion
T2DM is a complex disease where environmental and genetic factors interact. Family-based studies have found that T2DM has a strong genetic component [33] with several candidate genes identified [1]. Among these candidate genes, the TNF-α −308G/A and −238G/A polymorphisms have been widely studied. Although numerous studies have focused on these associations, their conclusions have been controversial [13,17,34,35]. A previous meta-analysis by Feng et al. [36], did not find any significant associations between the TNF-α −308 G/A polymorphism and T2DM risk in Caucasian and Asian populations. In contrast, a more recent meta-analysis by Zhao et al. [37], suggested that the TNF-α −308A variant increased by approximately 21% in T2DM incidence. Similarly, the results of two meta-analyses, of small sample sizes, showed that TNF-α −238G/A was not associated with T2DM [38,39]. Moreover, some meta-analyses were limited to specific countries and regions [40–42]. Therefore, we performed a comprehensive large-scale meta-analysis to investigate these associations.

For this meta-analysis, in order to derive reliable results, we added 12 new studies, performed quality score assessments and added multiple genetic models. Compared with previous meta-analyses [36,37], we demonstrate that TNF-α −308G/A is a risk factor for T2DM, not only in Asian but also in Caucasian populations. Additionally, we found that TNF-α −238G/A is not associated with T2DM in overall and subgroup populations. These observations illustrate the necessity for more comprehensive analyses and multiple genetic models. To prevent possible interference from heterogeneity to our results, we sought to explain the source of heterogeneity and eliminate it. First, subgroup analysis of ethnicity and genetic models reduced between-study heterogeneity. We found that heterogeneity was reduced, but there was still high heterogeneity. Next, our meta-regression analysis attempted to reveal these heterogeneous sources. These results showed that publication year, sample size, ethnicity...
Figure 4. Sensitive analysis in TNF-α $-308$G/A study (A) and $-238$G/A study (B).

There is a bias and asymmetry in TNF-α $-308$G/A study.

(Caucasian, Asian, African) and HWE were not the sources of between-study heterogeneity ($P>0.05$). Finally, we performed sensitivity analysis to explore the impact of a single study; our results revealed that the study by Golshani et al. [17] may have been the major contributor to this heterogeneity.
Begg’s funnel plot shows centered at the fixed-effect summary OR, visual inspection of the funnel plot is roughly symmetrical and indicates that there is no bias ($P > 0.05$). Egger’s funnel plot with fitted regression line, intercept represents the degree of asymmetry, close to zero, the smaller the bias. The Egger’s test indicates that there are no small-study effects (intercept = 0.514, 95% CI = $-1.504–1.532$) and bias ($P > 0.05$).
Figure 6. Publication bias of Begg’s test (A) and Egger’s test (B) in TNF-α -238G/A study

Begg’s funnel plot shows centered at the fixed-effect summary OR, visual inspection of the funnel plot is roughly symmetrical and indicates that there is no bias ($P>0.05$). Egger’s funnel plot with fitted regression line, intercept represents the degree of asymmetry, close to zero, the smaller the bias. The Egger’s test indicates that there are no small-study effects (intercept = $-0.048$, 95% CI = $-1.405$–$1.309$) and bias ($P>0.05$).
The advantages of this meta-analysis are that it expands to large-scale studies. While strictly complying with the inclusion criteria, we updated 12 studies not included in previous meta-analysis, our results are more comprehensive. To guarantee the quality of the meta-analysis, NOS and HWE analyses were conducted to assess the quality of included studies to avoid potential influences and increase the strength of the results. A strict search strategy of literature inclusion and data extraction was performed by two investigators according to inclusion and exclusion criteria. Furthermore, sensitivity analysis and meta-regression were also performed to increase the robustness of our conclusions. Subgroup analysis by ethnicity and the source of the control population were used to explain the effect of genetic background and study design.

There were some limitations to this meta-analysis. First, only studies in English were included, studies published in other languages were excluded. Second, because we excluded literature without original data, some studies were excluded. Third, other potential interactions including environmental factors, environment–gene interactions and gene–gene interactions. Additionally, some potential covariates (e.g. age, sex) were not included due to insufficient information from selected publications.

In conclusion, our meta-analysis identified that TNF-α –308G/A were associated with T2DM susceptibility. Additionally, we found that TNF-α –238G/A is not associated with T2DM in overall and subgroup populations. In the future, the influences of genetic loci, combined with environmental factors, may provide important treatment therapies for T2DM, therefore, well-conceived studies are warranted to confirm the important data presented here.

Author Contribution

All authors have contributed to the paper. Lidan Xu and Songbin Fu participated in the design of the study. Xiaoliang Guo and Chenxi Li drafted the article and wrote the manuscript. Jiawei Wu, Chang Liu, Qingbu Mei and Wenjing Sun assisted with analysis and interpretation of data. All authors read and approved the final manuscript.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

GWAS, genome-wide association scans; HWE, Hardy–Weinberg equilibrium; NOS, Newcastle–Ottawa Quality Assessment Scale; OR, odds ratio; TNF-α, tumor necrosis factor-α; T2DM, type 2 diabetes mellitus; 95% CI, 95% confidence interval.

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