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TNF-α G-308A polymorphism is associated with insulin resistance: a meta-analysis

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ABSTRACT. Tumor necrosis factor-α (*TNF-α*) promoter polymorphisms has been reported to be associated with obesity and insulin resistance and gained widespread attention. However, results obtained so far are quite conflicting. We therefore performed a meta-analysis to address this issue, basing on 17 studies from electronic databases (MEDLINE and EMBASE). No evidence of significant effect of *TNF-α* G-308A polymorphism on body mass index (BMI) or obesity risk was detected (BMI: WMD_{RE} = 0.05, 95%CI: -0.62 to 0.73; risk of obesity: OR_{FE} = 1.09, 95%CI: 0.87 to 1.35). G-308A variant was significantly associated with increased insulin levels in the overall (SMD_{FE} = 0.12, 95%CI: 0.03 to 0.20) and obese subgroup analysis (SMD_{FE} = 0.16, 95%CI: 0.03 to 0.29). In total, no significant result was observed for the association between *TNF-α* G-308A variant and HOMA-IR index. Nevertheless, subgroup analysis showed G-308A polymorphism was significantly associated with increased HOMA-IR in Caucasians (WMD_{FE} = 0.49, 95%CI: 0.03 to 0.94). Our results

indicate that *TNF-α* G-308A polymorphism has a significant effect on insulin resistance. However, it is unlikely that G-308A variant contributes to obesity.

Key words: TNF-α; Insulin resistance; Obesity; Polymorphism; Meta-analysis

INTRODUCTION

The worldwide prevalence of obesity and insulin resistance soared over the last decade. Both are major risk factors for type 2 diabetes, cardiovascular disease and related devastating complications (Kahn et al., 2006; Schenk et al., 2008; Graves, 2010). There is growing evidence that inflammation plays an important role in the development of obesity and insulin resistance (Dandona et al., 2004; Sun et al., 2011). Tumor necrosis factor-α (TNF-α), one of the most typical pro-inflammatory cytokines, has been shown to be correlated with adiposity and insulin resistance (Hotamisligil et al., 1995; Matsushita et al., 2006; Belkina and Denis, 2010). However, the exact effects of TNF-α on these conditions are not well defined.

Genetic susceptibility of disease has been a research focus in recent years. Four common variants in the promoter region of *TNF-α* gene, G-308A (rs1800629), G-238A (rs361525), C-863A (rs1800630) and C-857A (rs1799724), were considered to alter *TNF-α* gene transcriptional activity (Wilson et al., 1997; Skoog et al., 1999; Bayley et al., 2001), and have been viewed as candidate genetic risk factors for obesity and insulin resistance.

To date, several genetic association studies have investigated the effect of G-308A, the most extensive researched *TNF-α* polymorphism, on obesity and insulin resistance. However, the data obtained so far are quite conflicting. Here, we specifically reevaluate the influence of *TNF-α* G-308A variant on obesity and insulin resistance by performing a comprehensive meta-analysis, to address discrepancy in the genetic association studies.

MATERIAL AND METHODS

Search strategy and selection criteria

We carried out a systematic literature search for articles published prior to December 20, 2013 on the association between *TNF-α* G-308A variant and obesity and/or insulin resistance. Different combinations of key words: tumor necrosis factor or TNF; obesity, overweight, body mass index, BMI, insulin resistance, homeostasis model assessment, or HOMA; and gene, polymorphism or variant were used to identify potentially relevant publications in MEDLINE (using PubMed) and EMBASE databases. Reference lists of relevant articles were also screened to identify additional publications.

Cross-sectional or case-control studies were eligible for inclusion in the meta-analysis if they satisfied the following inclusion criteria: i) adult subjects with age ≥18 years, without any evidence of apparent metabolic diseases other than obesity; ii) evaluation on *TNF-α* G-308A polymorphism; iii) data reported on BMI, risk of obesity, fasting plasma insulin levels, or HOMA of insulin resistance (HOMA-IR) index; iv) published in English language journals. Studies involved subjects with diabetes, hyperlipidemia, hypertension, hyperuricemia, coronary artery disease, liver or renal disease were excluded. For studies in which the relevant

information was not reported, we contacted original authors to ensure sufficient data were obtained. When overlapping studies were provided by the same investigators, only the recent ones with the largest number of participants were selected. Animal studies, case reports, reviews and studies with incomplete data were excluded.

Data extraction

Measurements related to obesity and insulin resistance (i.e. BMI, fasting plasma insulin levels and HOMA-IR) were retrieved using a data extraction form. General characteristics of studies included were also extracted: first author, year of publication, gender, age, ethnicity, sample size, BMI condition, genotype distribution and genotyping methods. Two of the authors extracted the data separately. Discrepancies were resolved by discussion. When information was reported for more than one subpopulation (i.e., male or female subjects, participants from different age groups) in one study, each subgroup was considered as a separate study.

Statistical analysis

All the continuous variables are reported as means ± SD. Weighted mean difference (WMD) or standardized mean difference (SMD) with 95%CIs was used to pool effect estimates, depending on whether the units of measurements were consistent across studies or not (Welton et al., 2009). For dichotomous variables, OR with the corresponding 95%CIs was calculated in the meta-analysis. Because of the low frequency of the minor allele in population, a dominant model was used in this meta-analysis, in which carriers of the minor allele were compared with those homozygous for wild-type allele. In addition to main (overall) analysis, subgroup analysis was performed by ethnicity (Caucasians or East Asians) and BMI status (lean subgroup: BMI<25 kg/m^2 or obese subgroup: BMI≥30 kg/m^2).

Between-study heterogeneity was assessed by Q-statistic test and I^2 statistics. Heterogeneity was considered significant if $P < 0.10$. I^2 represents the percentage of total variation across studies, with higher values indicating greater degrees of heterogeneity (Zintzaras and Ioannidis, 2005). When heterogeneity was detected, the pooled effect estimates were calculated according to the random-effects (RE) model; otherwise, the fixed-effects (FE) model was used.

Cumulative meta-analysis was performed to evaluate the trend of pooled effect estimates over time as more data accumulated (Zintzaras and Lau, 2008). Hardy-Weinberg equilibrium (HWE) was tested for all studies by Pearson's χ^2 test. We performed the meta-analysis via excluding the studies not in HWE to test the stability of the results. Furthermore, a full sensitivity analysis was performed by deleting each study in turn to assess the influence of individual study on the overall effect. Publication bias was estimated by Egger and Begg-Mazumdar tests.

All statistical analyses were conducted using the Review Manager 5.1 software (The Cochrane Collaboration, Oxford, UK) and the Stata software (version 11.0; Stata Corporation, College Station, Texas, USA). A two-sided P value of less than 0.05 was considered to be statistically significant.

RESULTS

Study characteristics

The initial search identified 152 articles. After title and abstract examination, 106 ir-

relevant articles were excluded. The remained articles were further evaluated for appropriateness and 32 articles were excluded (Figure 1). Finally, 15 articles met the inclusion criteria (Fernandez-Real et al., 1997; Walston et al., 1999; Hayakawa et al., 2000; Hoffstedt et al., 2000; Ishii et al., 2000; Rasmussen et al., 2000; Brand et al., 2001; Romeo et al., 2001; Dalziel et al., 2002; Um et al., 2003; Wybranska et al., 2003; De Luis et al., 2011; Vikram et al., 2011; Hedayati et al., 2012; Wingeyer et al., 2012). One article (Ishii et al., 2000) provided separate data on young (aged 21 to 29) and older (aged 45 to 65) subjects, and one article (Hoffstedt et al., 2000) on male and female subjects. Each subpopulation was treated as a separate study. Therefore, 17 studies were included in the meta-analysis.

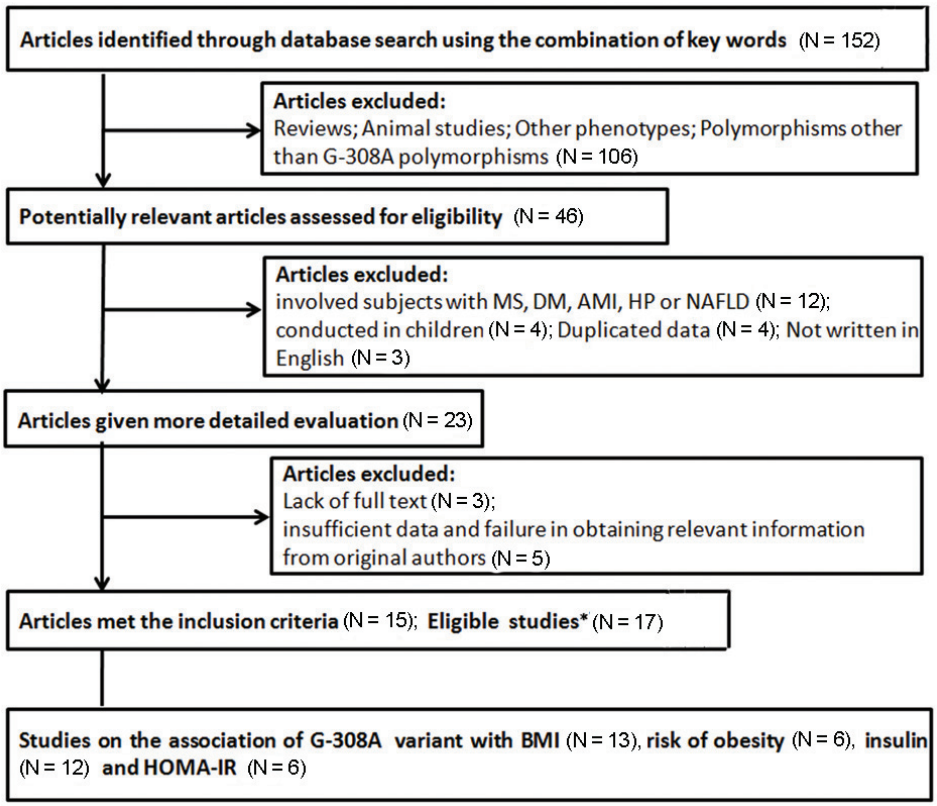

Figure 1. Process of studies inclusion/exclusion in the meta-analysis. MS = metabolic syndrome; DM = diabetes mellitus; AMI = acute myocardial infarction; HP = hypertension; NAFLD = non-alcoholic fatty liver disease. *Two articles provided information on two subpopulation, and each subpopulation was treated as a separate study.

The studies identified were undertaken in a wide range of ethnicities: 9 providing data on Caucasians, 4 on East Asians (Japanese and Korean), and 4 on other ethnic origins. Five studies involved lean subjects with normal weight (BMI < 25 kg/m^2), 3 involved obese subjects (BMI ≥ 30 kg/m^2), and 9 involved both obese and lean subjects. The genotype distributions in two studies were deviated from HWE (Wybranska et al., 2003; Vikram et al., 2011). The studies were published between 1997 and 2012. Among the included studies, 13 studies involved BMI, 12 studies involved insulin and 6 studies involved HOMA-IR. Six studies

dealt with the relationship between G-308A variant and risk of obesity. The characteristics of included studies were summarized in **Table S1**.

Effect of G-308A polymorphism on BMI and risk of obesity

Overall, the result did not show significant association between *TNF-α* G-308A polymorphism and BMI (WMD_{RE} = 0.05, 95%CI: -0.62 to 0.73; Figure 2, Table 1). Similarly, no significant effect estimates were observed in the subgroup analyses stratified by ethnicity. Significant heterogeneity between studies was detected ($P_{Q\text{-}Test}$ = 0.03, I^2 = 49%). After stratifying by ethnicity, heterogeneity was eliminated in East Asians ($P_{Q\text{-}Test}$ = 0.47, I^2 = 0%).

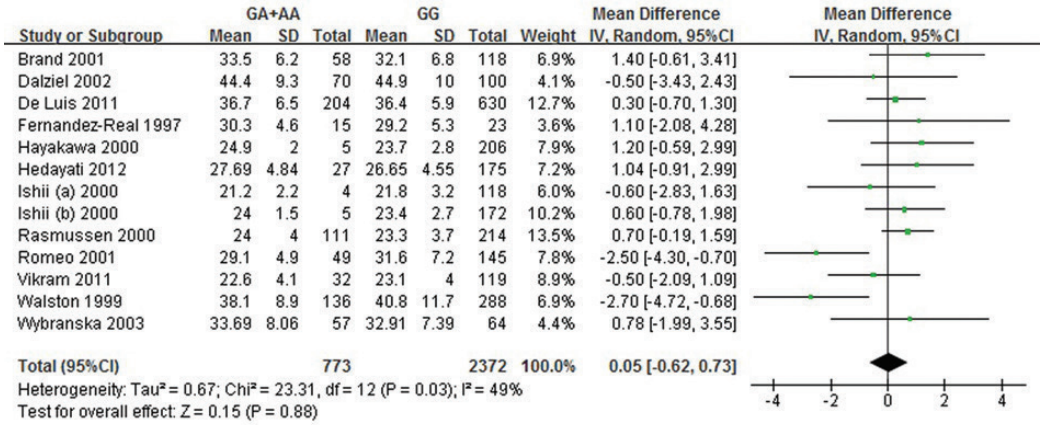

Figure 2. Meta-analysis for the association between *TNF-α* G-308A polymorphism and BMI. Pooled effect estimate is shown under the random-effects (RE) model.

Table 1. Meta-analysis results of *TNF-α* G-308A polymorphism for BMI, insulin levels and HOMA-IR.

Overall and subgroup analysis	Studies (N)	$P_{Q\text{-}Test}$	I^2 (%)	Fixed-effects		Random-effects	
				WMD/SMD (95%CI)	P	WMD/SMD (95%CI)	P
BMI							
All	13	0.03	49	0.17 (-0.27, 0.62)	0.44	0.05 (-0.62, 0.73)	0.88
All with HWE	11	0.01	55	0.22 (-0.25, 0.68)	0.37	0.06 (-0.71, 0.83)	0.88
Caucasians	7	0.07	49	0.29 (-0.28, 0.85)	0.32	0.16 (-0.76, 1.09)	0.73
East Asians	3	0.47	0	0.55 (-0.43, 1.53)	0.27	0.55 (-0.43, 1.53)	0.27
Insulin							
All	12	0.28	17	0.12 (0.03, 0.20)	0.01	0.12 (0.02, 0.23)	0.02
All in HWE	10	0.43	0	0.13 (0.03, 0.22)	0.01	0.13 (0.03, 0.22)	0.01
Caucasians	8	0.27	20	0.13 (0.04, 0.22)	0.00	0.14 (0.04, 0.25)	0.01
East Asians	3	0.65	0	0.21 (-0.32, 0.74)	0.43	0.21 (-0.32, 0.74)	0.43
Lean	5	0.47	0	0.03 (-0.16, 0.21)	0.77	0.03 (-0.16, 0.21)	0.77
Obese	3	0.29	19	0.16 (0.03, 0.29)	0.02	0.18 (0.02, 0.34)	0.03
HOMA-IR							
All*	6	0.18	34	0.20 (-0.05, 0.45)	0.12	0.26 (-0.06, 0.58)	0.11
Caucasians	3	0.35	5	0.49 (0.03, 0.94)	0.04	0.49 (0.02, 0.96)	0.04
East Asians	3	0.21	37	0.07 (-0.23, 0.37)	0.66	0.11 (-0.28, 0.50)	0.59
Lean	3	0.21	37	0.07 (-0.23, 0.37)	0.66	0.11 (-0.28, 0.50)	0.59
Obese	2	0.15	52	0.51 (-0.08, 1.10)	0.09	0.65 (-0.32, 1.63)	0.19

All, all of the studies eligible for meta-analyses; All in HWE, the studies with genotype distribution in accordance with HWE. *The genotype distribution of all the studies for HOMA-IR analysis is in accordance with HWE.

Regarding the effect of *TNF-α* G-308A polymorphism on the risk of obesity, no evidence of heterogeneity was found ($P_{Q\text{-}Test}$ = 0.74, I^2 = 0%). There was no statistically significant association between G-308A polymorphism and obesity risk under dominant model (OR_{FE} = 1.09, 95%CI: 0.87 to 1.35).

Effect of G-308A polymorphism on insulin levels and HOMA-IR index

The results of aggregated effect estimates and heterogeneity test were summarized in Table 1. There is no significant heterogeneity for insulin or HOMA-IR comparisons ($P_{Q\text{-}Test}$ = 0.28, I^2 = 17%; $P_{Q\text{-}Test}$ = 0.18, I^2 = 34%; respectively).

In total, *TNF-α* G-308A variant was significantly associated with increased insulin levels (SMD_{FE} = 0.12, 95%CI: 0.03 to 0.20; Figure 3). In the analysis stratified by ethnicity, significant association remained in Caucasians (SMD_{FE} = 0.13, 95%CI: 0.04 to 0.22), but not in East Asians (SMD_{FE} = 0.21, 95%CI: -0.32 to 0.74). Interestingly, the results of subgroup analyses showed an increased trend of pooled SMD from lean to obese individuals (lean subgroup: SMD_{FE} = 0.03, 95%CI: -0.16 to 0.21; obese subgroup: SMD_{FE} = 0.16, 95%CI: 0.03 to 0.29), with statistically significant result only observed in obese subpopulation.

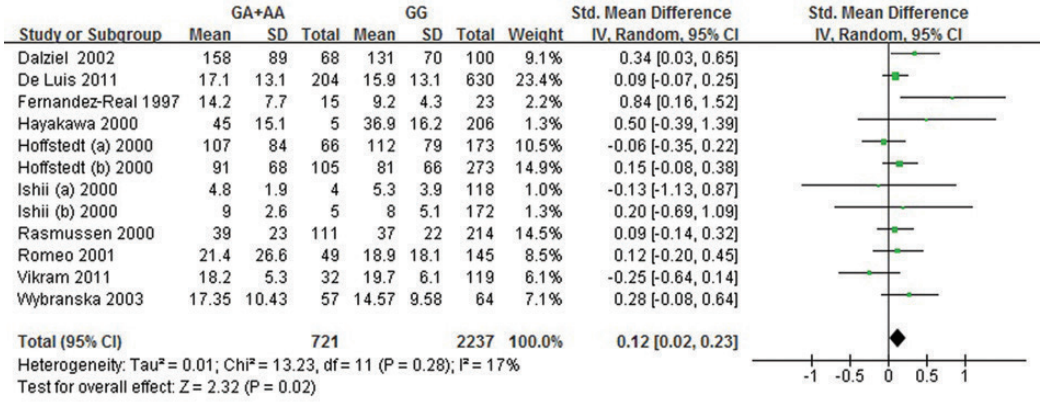

Figure 3. Meta-analysis for the association between *TNF-α* G-308A polymorphism and insulin levels. Pooled effect estimate is shown under the fixed-effects (FE) model.

The cumulative meta-analysis for the dominant model showed a trend of positive association between *TNF-α* G-308A polymorphism and elevated insulin levels as evidence accumulated, with the 95%CIs of pooled effect estimates being narrower (Figure 4).

No significant result was observed for the association between G-308A variant and HOMA-IR index (WMD_{FE} = 0.20, 95%CI: -0.05 to 0.45; **Figure S1**). Analysis stratified by ethnicity showed *TNF-α* G-308A polymorphism was significantly associated with increased HOMA-IR in Caucasians (WMD_{FE} = 0.49, 95%CI: 0.03 to 0.94), but not in East Asians (WMD_{FE} = 0.07, 95%CI: -0.23 to 0.37). No evidence of significant association was detected in the subgroup analysis stratified by BMI categories.

Sensitivity analysis

Exclusion of studies deviating from HWE did not alter the pattern of results (Table 1).

Additionally, the effect estimations for BMI, risk of obesity, insulin, and HOMA-IR did not substantially change after dropping each study every time in the sensitivity analysis, suggesting high stability of the current comparisons.

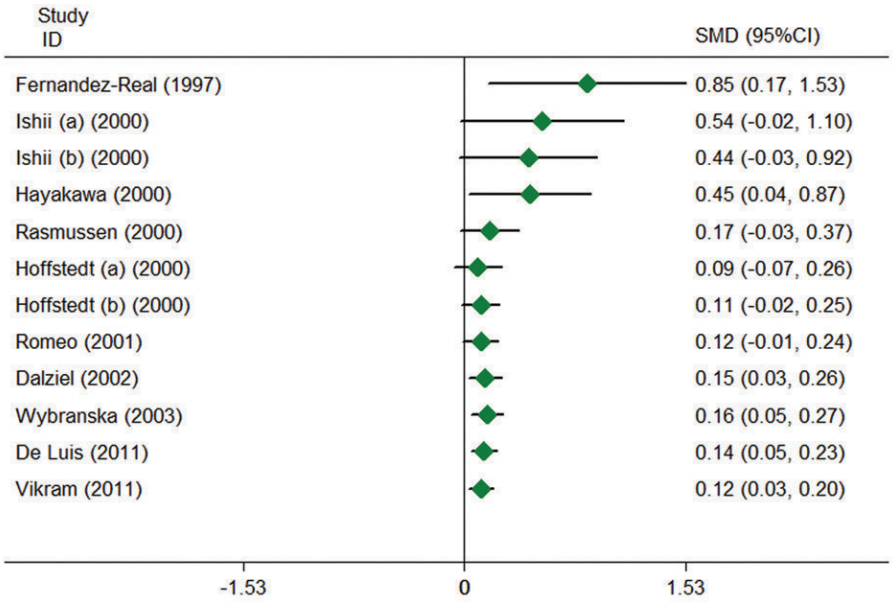

Figure 4. Cumulative meta-analysis for the association between *TNF-α* G-308A polymorphism and insulin levels. The pooled effect estimate with the 95%CI is shown, as each study is added. Studies are displayed by descending order of publication year.

Publication bias

Formal statistical tests did not show significant publication bias for *TNF-α* G-308A polymorphism comparisons (BMI: $P_{Egger} = 0.58$, $P_{Begg} = 0.86$; risk of obesity: $P_{Egger} = 0.84$, $P_{Begg} = 1.0$; insulin: $P_{Egger} = 0.40$, $P_{Begg} = 0.84$; HOMA-IR: $P_{Egger} = 0.45$, $P_{Begg} = 0.85$).

DISCUSSION

The present meta-analysis is conducted to investigate the association of *TNF-α* G-308A polymorphism with obesity and insulin resistance. We excluded publications involving participants with frank metabolic diseases during study selection, since they would introduce bias to the magnitude of genetic effects. Accordingly, our inclusion/exclusion criteria led to selecting studies with a clear definition of disease, which was different from the meta-analysis by Sookoian et al. (2005). It could therefore be argued that the discrepancy in the pooled effect estimates between Sookoian's and our studies is partly attributed to different selection criteria.

We fail to observe that *TNF-α* G-308A polymorphism is associated with the mean BMI or risk of obesity. Additional analysis on this polymorphism and waist-to-hip ratio (WHR) relation also yields no significant results (data not shown). These findings are in line with the

evidence from biological functions of TNF-α in cell cultures and animal studies. Although there are grounds for considering TNF-α cytokine is overexpressed in adipose tissue during obesity (Cawthorn and Sethi, 2008), the specific effect of TNF-α on fat tissue is unclear so far. It is of note that TNF-α has been shown to inhibit adipocyte differentiation and lipogenesis, promote lipolysis, and induce adipose cell apoptosis in *in vitro* studies (Prins et al., 1997; Zhang et al., 2002; Cawthorn and Sethi, 2008). All these actions suggest that TNF-α account for reduced adiposity. Considering the results in our meta-analysis, it is unlikely that *TNF-α* G-308A polymorphism contributes to obesity development.

Insulin resistance is generally viewed as a condition in which insulin levels are higher than expected relative to glucose levels because of the reduced responsiveness of target organs to insulin. It should be recognized that insulin resistance is tethered to hyperinsulinemia by definition (Shanik et al., 2008). Importantly, our results provide statistical evidence for the effect of *TNF-α* G-308A polymorphism on elevated insulin levels, indicating that such polymorphism may play a role in the pathogenesis of insulin resistance.

We demonstrate for the first time that the role of *TNF-α* G-308A polymorphism in causing elevated insulin levels is potentially modified by high BMI in the current study. In fact, insulin resistance is considered to be multifactorial and its development may be accounted for by genetic variants interacting with environmental factors, especially obesity (McKillop and Flatt, 2011). It should be noticed that the genetic effect of *TNF-α* G-308A variant may be modest, which is insufficient to significantly affect *per se* insulin resistance development. Rather, it is likely to play a role in obese subjects who already have an elevated amount of TNF-α. Further investigations are necessary to provide more precise estimation of the joint effects of *TNF-α* G-308A variant and high BMI in modulating insulin levels.

Subgroup analyses stratified by ethnicity indicate that *TNF-α* G-308A variant has significant effect on insulin levels in Caucasians but not East Asians. Such findings could also be explained by considering obesity as a covariate modifying the genetic effect of G-308A variant on insulin levels. Generally, both the percentage of obese individuals and the degree of obesity were higher among Caucasians than Asians. More than 21.9% of adults in United States and Europe are obese, whereas the rates for Asian population lag behind largely (Kelly et al., 2008). Hence, further studies should take obesity into consideration when investigating the effect of *TNF-α* G-308A polymorphism on insulin resistance.

The HOMA-IR index, which is derived from a mathematical assessment of fasting glucose and insulin levels, is regarded as a simple and reliable surrogate measure of insulin resistance (Wallace et al., 2004). No significant association is observed for *TNF-α* G-308A variant and HOMA-IR. One possible explanation for the negative result on HOMA-IR index may be that most original studies selected subjects with low BMI, leading to an underestimation of the gene effect. After stratifying by ethnicity, we find significant association between this polymorphism and HOMA-IR in Caucasians in which the percentage and the degree of obesity were higher than in Asians (Kelly et al., 2008). This result further confirms and strengthens the hypothesis that *TNF-α* G-308A variant may play a role in insulin resistance with obesity acting as an effect modifier.

A limitation of our study is the use of aggregated data (AD) rather than individual patient data (IPD) for continuous variables. Compared with AD approach, IPD meta-analysis has many advantages which synthesizes the raw data from each study directly, including adjusting patient-level covariates (e.g., age, gender) (Riley et al., 2008). A more precise estimation of the association between *TNF-α* G-308A polymorphism and obesity and insulin resistance

could be conducted if detailed individual data are available. Lack of information on TNF-α protein levels is another limitation. Since *TNF-α* G-308A polymorphism is assumed to be the "gain of function" variation leading to increased expression activity, TNF-α protein levels in plasma or adipose tissue may directly and substantially confirm the functionality of the genetic variants in humans.

Nonetheless, our study has several strengths. First, in contrast to novel genetic findings from genome-wide association (GWA) studies, many data have existed on TNF-α biological functions, thereby allowing an easy interpretation of these genetic association results. Second, the current study clearly suggests that *TNF-α* G-308A polymorphism-insulin resistance relation be modified by high BMI levels. Findings from our study have great significance in the early identification and therapy monitoring of subject subgroups at high risk for insulin resistance, as well as a better understanding of the complex pathogenesis of such disease.

In summary, our results indicate that *TNF-α* G-308A polymorphism has a significant effect on insulin resistance, which is potentially modified by high BMI. However, it is unlikely that *TNF-α* G-308A variant contributes to obesity.

Conflicts of interest

The authors declare no conflict of interest.

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Supplementary material

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