Meta-analysis of the correlation between the *TNF-α* 308G/A polymorphism and polycystic ovary syndrome

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ABSTRACT. Previous studies have suggested that the tumor necrosis factor alpha (*TNF-α*) gene 308G/A polymorphism may be associated with polycystic ovary syndrome (PCOS) risk. However, this relationship is controversial. The present meta-analysis aimed to evaluate the correlation between the *TNF-α* 308G/A polymorphism and susceptibility to PCOS. A systematic electronic search of PubMed and Embase databases was conducted using specific inclusion criteria. Summary odds ratios (ORs) and 95% confidence intervals (95%CIs) were calculated, and all statistical analyses were performed using STATA 12.0. The results of our meta-analysis showed no significant association between the *TNF-α* 308G/A polymorphism and PCOS risk (AA *vs* GG: OR = 0.80, 95%CI = 0.31-2.08; AG *vs* GG: OR = 1.03, 95%CI = 0.59-1.81; dominant model: OR = 1.02, 95%CI = 0.60-1.71; recessive model: OR = 0.87, 95%CI = 0.35-2.16). Based on the statistical data, our meta-analysis indicates that the *TNF-α* 308G/A

sequence variation may be not related to PCOS susceptibility. Further large and well-designed studies are needed to confirm this conclusion.

Key words: Tumor necrosis factor alpha; Meta-analysis; Polymorphism; Polycystic ovary syndrome

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine-metabolic disorder among women, affecting approximately 5-10% of the adult female population, and is frequently diagnosed during adolescence (Goodarzi et al., 2011). PCOS is primarily characterized by hyperandrogenism, insulin resistance, chronic anovulation, infertility, and irregular menses (Motta, 2012). Although there is evidence supporting the involvement of genetic and environmental factors in PCOS development, the exact mechanism responsible remains unknown. Previous data have demonstrated that insulin resistance and hyperandrogenism play a key role in the pathophysiology of PCOS (Stepto et al., 2013). To date, a large number of candidate genes have been studied concerning their potential roles in the etiology of PCOS, such as insulin secretion and action, energy metabolism, steroid hormone biosynthesis, and interleukin activity (Prapas et al., 2009).

Tumor necrosis factor alpha (TNF-α) is a polyfunctional pro-inflammatory cytokine involved in many physiologically and pathologically important processes in a variety of tissues and organs, including inflammation, immunoregulation, proliferation, and apoptosis (Esposito and Cuzzocrea, 2009). In addition, TNF-α has been shown to affect ovarian function, ovulation, fertilization, and implantation in women suffering from PCOS (Vural et al., 2010). Furthermore, PCOS patients have been found to have higher serum and follicular fluid concentrations of TNF-α than women without this condition (Amato et al., 2003). The *TNF-α* gene is located on chromosome 6p21.3, spans approximately 3 kb, and contains four exons. Genetic variations in its promoter can alter the function of this gene and the metabolism of TNF-α. Of these, the 308G/A (rs1800629) polymorphism is the most commonly studied (Fargion et al., 2001). This variant consists of a G to A transition at nucleotide position 308 in the promoter region of the *TNF-α* gene. A previous study has demonstrated that this polymorphism is associated with increased release of TNF-α (Wilson et al., 1997).

Numerous studies have tested the connection between PCOS risk and the *TNF-α* 308G/A polymorphism. However, the results remain controversial. A single case-control study may not have sufficient statistical power to comprehensively demonstrate this complicated interrelationship because of the relatively small sample size involved. Meta-analysis is a useful tool for detecting associations that may otherwise remain masked in studies of limited sample size, particularly in those evaluating low-frequency, rare-allele polymorphisms (Ammar et al., 2012). In the present study, we investigated whether the *TNF-α* 308G/A polymorphism is associated with PCOS risk by performing a meta-analysis.

MATERIAL AND METHODS

Literature search

We searched PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and Embase databases

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(http://www.embase.com) for all articles containing the following terms: "tumor necrosis factor alpha/TNF-α", "308G/A", "polycystic ovary syndrome/PCOS", "polymorphism", "single nucleotide polymorphism", and "genetic polymorphism" (the most recent search was conducted on September 1, 2015). References included in retrieved articles were also manually searched for additional relevant and eligible studies. When investigations had overlapping study subjects, we selected the study with the largest sample size.

Eligibility of relevant studies

Articles were included if they met the following criteria: 1) they evaluated the relationship between the *TNF-α* 308G/A polymorphism and PCOS risk; 2) they used a case-control study design; and 3) sufficient data were available to estimate an odds ratio (OR) with its 95% confidence interval (95%CI). The following were excluded: 1) studies that did not use a case-control design; 2) case reports, letters, reviews, meta-analyses, and editorial articles.

Data extraction

Two authors independently extracted all data based on the inclusion criteria listed above. Any disagreement was subsequently resolved by consensus with a third author. The following data were abstracted directly from the included studies: first author, year of publication, location, number of patients and controls, genotype and allele distributions, and evidence of Hardy-Weinberg equilibrium (HWE; Table 1).

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

Study	Year	Location	Ethnicity	Cases/controls	Genotypes (cases)			Genotypes (controls)			HWE test
					AA	AG	GG	AA	AG	GG	
Milner et al.	1999	Australia	Caucasian	84/108	2	23	59	3	42	63	0.19
Vural et al.	2010	Turkey	Caucasian	97/95	3	16	78	3	15	77	0.05
Deepika et al.	2013	India	Asian	283/306	3	270	10	3	293	10	0.00
Grech et al.	2014	Greece	Caucasian	39/140	0	31	154	1	15	149	0.37

HWE = Hardy-Weinberg equilibrium.

Statistical analysis

We first assessed the departure of *TNF-α* 308G/A genotype frequencies in the control group of each study from their expected values under HWE using the goodness-of-fit test (chi-square or Fisher exact test). Homozygote (AA *vs* GG) and heterozygote comparisons (AG *vs* GG), and dominant (AA + AG *vs* GG) and recessive models (AA *vs* AG + GG) were used to compare study groups. We quantified the effect of heterogeneity using the I^2 test, for which values of 25, 50, and 75% were defined as low, moderate, and high estimates, respectively. When an I^2 value > 50% indicated heterogeneity across studies, the random-effects model (the DerSimonian-Laird method) was used, while in all other cases the fixed-effects model (the Mantel-Haenszel method) was utilized (Lu et al., 2012). Sensitivity analysis was performed by omission of any study containing data not conforming to HWE, to test the robustness of the results. Potential publication bias was assessed statistically with the Begg's test and Egger's test (P < 0.05 represented statistical significance). All calculations were performed using Stata version 12.0 (StataCorp, College Station, TX, USA).

RESULTS

Characteristics of the included studies

Articles relating to the association between PCOS susceptibility and the *TNF-α* 308G/A polymorphism were retrieved based on the search criteria above. The study selection process is shown in Figure 1. A total of 83 citations were identified during the initial search. After the primary screening of titles and abstracts, six papers remained. Following detailed evaluation, two studies were excluded owing to their lack of genotype frequency data. Finally, four case-control studies assessing the association between the *TNF-α* 308G/A polymorphism and PCOS risk published between 1999 and 2014 (Milner et al., 1999; Vural et al., 2010; Deepika et al., 2013; Grech et al., 2014), and comprising 503 PCOS cases and 649 controls were included in the meta-analysis (baseline data and other details are shown in Table 1). The distribution of genotypes in the control groups was consistent with HWE in all but one of the studies (Deepika et al., 2013).
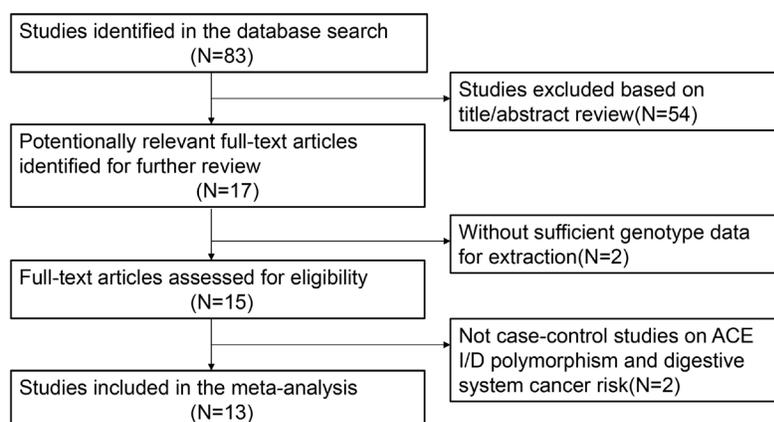
Figure 1. Flow chart representing the process by which studies were selected. PCOS = polycystic ovary syndrome.

Overall analyses

A summary of the results of our meta-analysis testing the association between the *TNF-α* 308G/A polymorphism and PCOS risk is provided in Figures 2-5 and Table 2. Under two of the genetic models, substantial between-study heterogeneity was observed, thus both fixed- and random-effects models were used to calculate pooled estimates. The meta-analysis failed to identify a significant relationship between the polymorphism of interest and susceptibility to PCOS (AA *vs* GG: OR = 0.80, 95%CI = 0.31-2.08; AG *vs* GG: OR = 1.03, 95%CI = 0.59-1.81; dominant model: OR = 1.02, 95%CI = 0.60-1.71; recessive model: OR = 0.87, 95%CI = 0.35-2.16).

Publication bias and sensitivity analysis

Sensitivity analyses were conducted to determine whether modification of the meta-analysis inclusion criteria affected the final results. Limiting the included studies to those
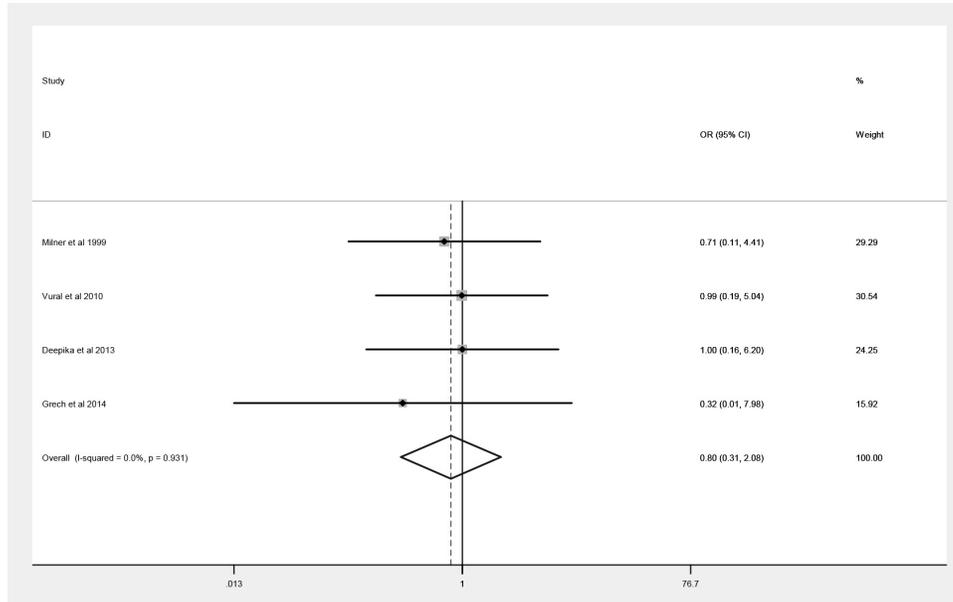
Figure 2. Forest plot of the association between polycystic ovary syndrome and the *TNF-α* 308G/A polymorphism (AA *vs* GG). OR = odds ratio, CI = confidence interval.
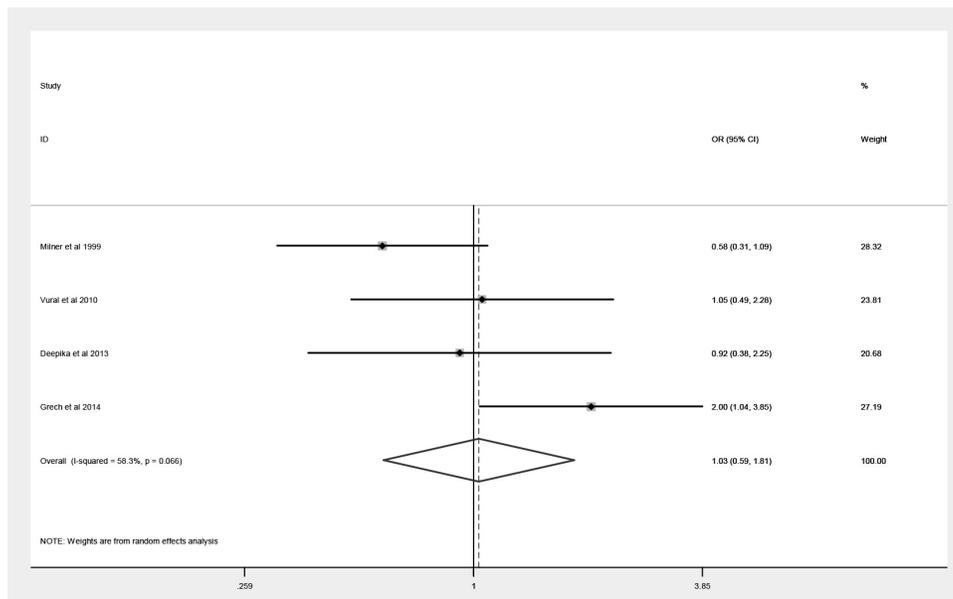

Figure 3. Forest plot of the association between polycystic ovary syndrome and the *TNF-α* 308G/A polymorphism (AG *vs* GG). OR = odds ratio, CI = confidence interval.
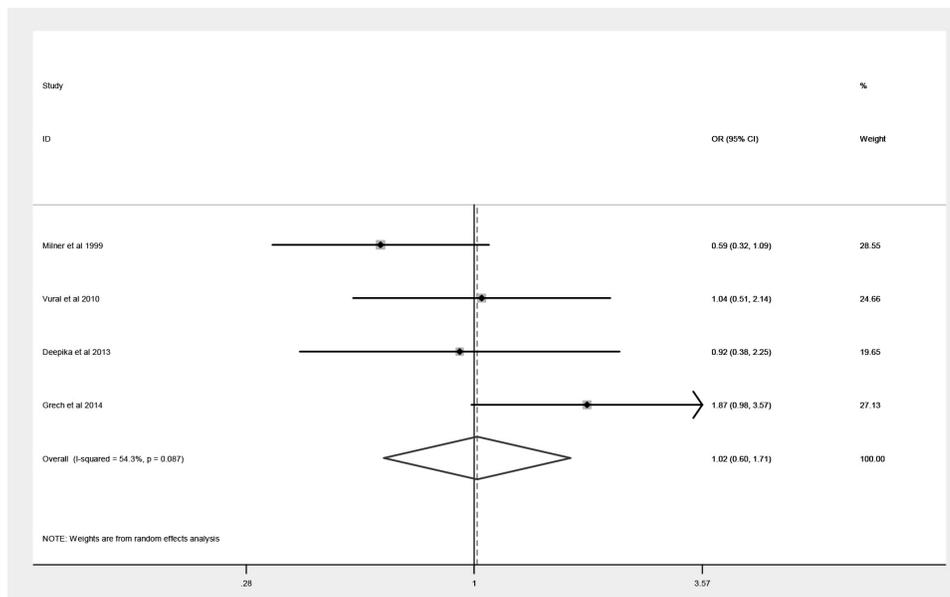
Figure 4. Forest plot of the association between polycystic ovary syndrome and the *TNF-α* 308G/A polymorphism (dominant model). OR = odds ratio, CI = confidence interval.
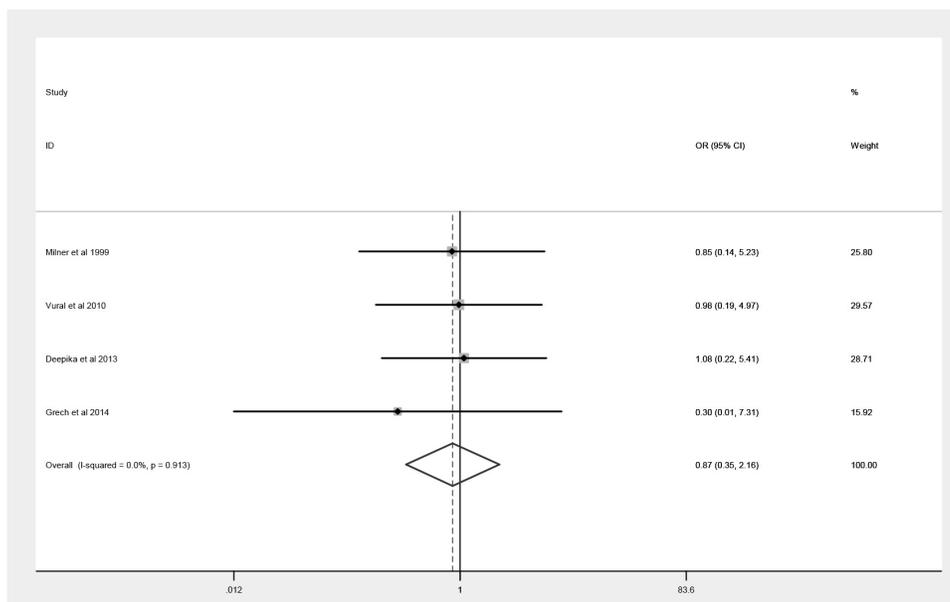

Figure 5. Forest plot of the association between polycystic ovary syndrome and the *TNF-α* 308G/A polymorphism (recessive model). OR = odds ratio, CI = confidence interval.

containing data conforming to HWE did not considerably alter pooled ORs (AA *vs* GG: OR = 0.74, 95%CI = 0.24-2.27; AG *vs* GG: OR = 1.07, 95%CI = 0.51-2.24; dominant model: OR = 1.04, 95%CI = 0.53-1.71; recessive model: OR = 0.87, 95%CI = 0.35-2.16). This suggests that the results of the current meta-analysis are statistically robust.

A Begg's funnel plot and Egger's regression test were used to assess potential publication bias. Neither of these measures revealed obvious levels of such bias in the overall analysis (Figures 6 and 7).

Table 2. Summary odds ratios and 95% confidence intervals concerning the association between the *TNF-α* 308G/A polymorphism and polycystic ovary syndrome risk.

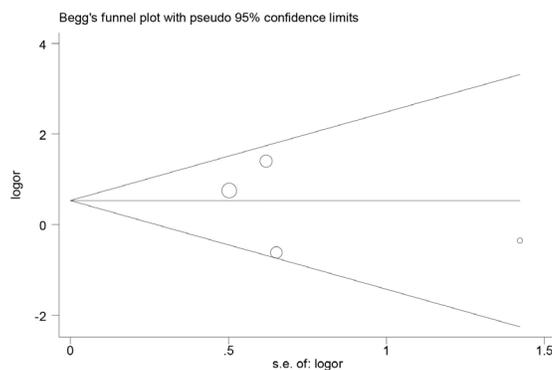| Genetic model	Type of model	Test of heterogeneity		Test of association	
		I^2 (%)	P	OR	95%CI
AA *vs* GG	Fixed	0.0	0.93	0.80	0.31-2.08
AG *vs* GG	Random	58.3	0.07	1.03	0.59-1.81
Dominant	Random	54.3	0.09	1.02	0.60-1.71
Recessive	Fixed	0.0	0.91	0.87	0.35-2.16

OR = odds ratio, CI = confidence interval.

Figure 6. Begg's funnel plot as a test of publication bias. SE = standard error, OR = odds ratio.
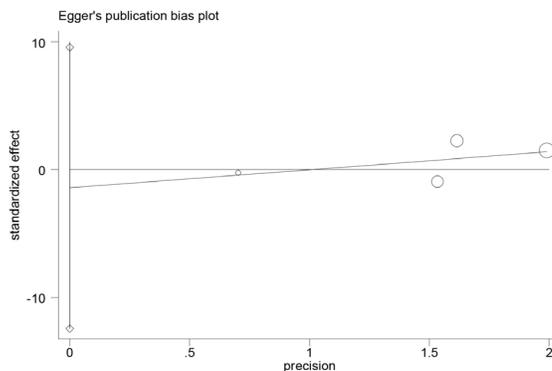

Figure 7. Egger's funnel plot as a test of publication bias.

DISCUSSION

PCOS is a heterogeneous endocrine disorder characterized by oligoovulation or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries. Although evidence exists supporting the involvement of genetic and environmental factors in the development of PCOS, its exact etiology remains unknown. Data from different familial and monozygotic twin studies suggest that genetic factors play an important role (Vink et al., 2006). Recently, several investigations have indicated that the *TNF-α* 308G/A polymorphism may be significantly related to increased PCOS risk. However, these results have been inconsistent and controversial. Therefore, we performed this meta-analysis of all eligible studies to provide a more comprehensive and reliable conclusion by reevaluating the association between this polymorphism and PCOS risk.

Four studies were included in our investigation, consisting of 503 PCOS cases and 649 controls. Based on these data, the present meta-analysis showed no significant correlation between the *TNF-α* 308G/A polymorphism and PCOS. However, previous meta-analyses have demonstrated this variation to be associated with increased susceptibility to hepatocellular carcinoma, gastric cancer, and type 2 diabetes mellitus (Hu et al., 2014; Rokkas et al., 2014; Zhao et al., 2014; Wang, et al., 2015). Such inconsistent results may have been caused by the *TNF-α* 308G/A polymorphism performing divergent roles in different cell types or tissues. Another reason for these conflicting findings may involve gene-gene and gene-environment interactions. In addition, departures from HWE among control groups may reflect genotyping errors (Munafò et al., 2005). Nevertheless, sensitivity analysis showed that pooled ORs were not materially altered by exclusion of a study including data not conforming to HWE. This suggests that the overall results of this meta-analysis were statistically robust.

Several limitations of the current analysis should be acknowledged when interpreting the results. First, it focused exclusively on polymorphisms, yet numerous factors act individually and in concert to influence PCOS risk, including lifestyle and environment, as well as genetics. The articles included in our meta-analysis did not report data concerning these issues, making it impossible for us to assess their influence in the study groups. Second, only studies published in English were included, representing a possible source of publication bias. Third, the sample size of this meta-analysis was not sufficiently large, particularly for subgroup analyses.

In conclusion, our meta-analysis suggests that the *TNF-α* 308G/A polymorphism may be not associated with PCOS risk. Further large-scale and well-designed studies taking into account different ethnic groups remain to be carried out to confirm the results of our work.

Conflicts of interest

The authors declare no conflict of interest.

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