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Meta-Analysis Review

Possible Association of Three Polymorphisms in Cytokine TNF-α (238G/A, 308G/A, 1031T/C) with Polycystic Ovary Syndrome: A Systematic Review and Meta-Analysis

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ABSTRACT

Background: Polycystic ovarian syndrome (PCOS) cases have recently increased drastically among women during ovulation. The etiology of this endocrine disorder remains complex due to its multiple links that affect women of all ethnicities and races. Recent studies have implicated tumor necrosis factor-alpha (TNF-α) in PCOS pathophysiology. This study examines the associations of *TNF-α* polymorphisms 238G/A, 308G/A, and 1031T/C with PCOS. **Methods:** We searched the Google Scholar, PubMed, EMBASE, Scopus, and Science Citation Index databases to identify suitable case-control studies and literature reviews for the statistical analysis. The obtained data were evaluated using the Review Manager 5.4 software. An odds ratio and 95% confidence interval were calculated for each genetic model. **Results:** Twenty-three studies met the eligibility criteria, comprising 3294 cases and 3288 controls. Meta-analysis showed no significant association between *TNF-α* polymorphisms 238G/A and 308G/A and PCOS risk. However, *TNF-α* polymorphism 1031T/C was significantly associated with PCOS risk. **Conclusion:** This meta-analysis indicates that *TNF-α* polymorphisms 238G/A and 308G/A may not be associated with PCOS risk, while *TNF-α* polymorphism 1031T/C appears associated with PCOS risk. However, a larger sample size is required to evaluate this association.

Key words: PCOS, TNF-Alpha, Meta, Necrosis factor, Gene polymorphism

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INTRODUCTION

Tumor necrosis factor-alpha (*TNF-α*), frequently found bound to the promoter region of genes implicated in various diseases, is a cytokine that promotes inflammation. Its gene is located in the class III region of the histocompatibility complex at chromosome 6p21.3, encoding a 157 amino acid (17 kDa) protein that forms a homotrimer[1]. It helps activate various inflammatory molecules, such as chemokines and cytokines. TNF-α greatly contributes to cellular homeostasis, differentiation, proliferation, and immune responses and regulates metabolite function in the body. It also helps in various biological activities such as enhancing neutrophil phagocytic ability and preventing liver cells from producing acute phase proteins, inhibiting or destroying tumor cells and viral replication[2]. Therefore, the dysregulated production or function of TNF-α causes various inflammatory diseases, including inflammatory bowel diseases, systemic lupus, multiple sclerosis, and rheumatoid arthritis. TNF-α is also known to induce hemorrhagic necrosis in murine Meth A sarcomas[3]. Around 43 single nucleotide polymorphisms (SNPs) have been identified in the promotor region of *TNF-α* (https://shorturl.at/ioDX9). However, some studies have reported conflicting results regarding their association with changes in TNF-α levels.

TNF-α has soluble and transmembrane forms that bind to outer membrane-bound receptors on the target cells. Specifically, the membrane-bound metalloproteinase TNF-α converting enzyme (TACE) is required to synthesize soluble TNF-α from transmembrane TNF-α[4]. TNF-α binds to type-1 and type-2 receptors. Type-1 receptors are TNF receptor superfamily member 1A (TNFRSF1A/TNFR1/CD120a) and CD4 molecule (CD4/p55)[5]. Type-2 receptors are TNFRSF1A and TNF receptor superfamily member 1B (TNFRSF1B/TNFR2/p75/CD120b). The TNFR1 receptor is important in regulating inflammatory pathways and is mainly expressed in human tissues. The TNFR2 receptor greatly affects tumor cell development by promoting immune escape[6]. Both TNFR1 and TNFR2 receptors induce cellular signals for biological activities, including inflammation and cell death. Several distinct signaling complexes designated as I, IIa, IIb, and IIc all induce unique cell re-

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sponses. These responses are controlled by the transmembrane and soluble forms of TNF-α, which bind to the death domain adaptor protein to trigger cell apoptosis or growth via the TNFR1 receptor[7].

During complex I assembly, TNFR1 stimulates and attaches to the TNFR1-associated death domain (TRADD) protein, followed by the assembly and cooperation of many parts, including TNF receptor-associated factors 2 (TRAF2) and 5 (TRAF5), ubiquitin-conjugating enzyme E1 (UBE1), receptor-interacting threonine/serine protein kinase 1 (RIPK1), and cellular inhibitor of apoptosis proteins 1 (cIAP1) and 2 (cIAP2). Therefore, complex I activates the nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways, leading to cell proliferation, tissue degeneration, cell survival, and inflammation[8].

Unlike complex I, complexes IIa, IIb, and IIc are assembled in the cytoplasm rather than at the plasma membrane. Pro-Caspase 8 (pro-CASP8), Fas-associated protein with death domain (FADD), TRAF2, RIPK1, and cIAP1/2 are all components of complex IIa. The complex IIb components are arranged in the same manner as those of complex IIa, except for a protein called receptor-interacting serine/threonine kinase 3 (RIPK3)[9]. An adaptor protein complex, called the apoptosome, helps complexes IIa and IIb induce apoptosis by aiding CASP8 activation. Necrosomal complex IIc is generated when RIPK1 and RIPK3 bind without being cleaved. This complex triggers necroptosis and inflammation by activating mixed lineage kinase domain (MLKL)[10]. In general, cell stimulation, migration, and replication are predominantly triggered by TNFR2, whereas cytotoxicity and inflammation are triggered by TNFR1.

TNF-α is known for its crucial role in autoimmune disorders, including psoriasis, noninfectious uveitis (NIU), psoriatic arthritis, and rheumatoid arthritis. Psoriatic arthritis affects 1% of the population, with physical characteristics such as swollen toes and fingers and inflamed joints. Activated dendritic cells (DCs), T helper 17 (Th17) cells, and macrophages are primarily involved in the pathogenesis of psoriatic arthritis, which is triggered by the overproduction of interleukin (IL)-23 and TNF-α[11,12]. IL-23 promotes the differentiation of naive T cells into Th17 cells, which overproduce IL-17. Then, inflammatory cells such as DCs become activated after stimulation by TNF-α and IL-17. TNF-α promotes anti-apoptosis and keratinocyte proliferation through the transforming growth factor (TNF)-ß signaling pathway, increasing the recruitment of inflammatory cells and resulting in the formation of microabscesses in psoriasis[13]. The psoriasis lesions are mainly subdivided into five types: guttate, erythrodermic, inverse, plaque, and pustular. The dysregulation of skin immune responses is reflected by angiogenesis and epidermal hyperplasia on the lesions[14]. Most clinical psoriasis subtypes have a common inflammatory mechanism that contributes to psoriasis development[15]. DCs become activated by IL-12 and induce differentiation of IL-23 into Th17 cells and naive T cells into T helper 1 (Th1) cells, which secretes TNF-α and interferon (IFN), while Th17 cells secrete abundant IL-17. Therefore, epidermal alterations and keratinocyte hyperproliferation, including hypogranulosis, parakeratosis, and acanthosis, are caused by TNF-α, IFN-γ, and IL-17[16].

Another autoimmune disorder related to the eye is NIU[17]. In addition to causing blindness or visual impairment, NIU has been associated with the development of visual distribution, cataracts, retinal detachment, and glaucoma. Many cytokines, including IL-10, IL-12, IL-23, and IL-6, are generated by macrophages[18]. TNF-α and other cytokines help activate DCs. Excess IL-12 production by activated DCs causes naive T cells to differentiate into Th1 cells[19]. Overproduction of IL-6 and TGF-β by DCs is a key factor in Th17 cell development. Activated Th1 and Th17 cells penetrate the choroid layers that supply blood to the retina. Migration of Th1 and Th17 cells can stimulate the retinal vasculature, attracting non-specific blood-circulating leukocytes. NIU reflects inflammation that causes uvea destruction and leads to edema[20].

In the modern lifestyle, many women of premenopausal age are affected by polycystic ovary syndrome (PCOS) caused by endocrine disorders associated with genetic factors such as premature fetal development, early follicle maturity, and a family history of PCOS. It occurs in about 1 in 13 premenopausal women[21]. Stein and Leventhal first described PCOS in 1935 in a female patient with oligo-ovulatory infertility[22]. This condition is mainly caused by altered ovulation and hyperandrogenism, leading to complications such as ovarian enlargement, infertility, endometrial cancer, and other diseases. In India, its prevalence is about 8.25%–22.5% based on lifestyle and food habits[23]. In 1990, diagnostic criteria were produced for PCOS at conferences funded by the National Institutes of Child and Health (NICH) and Human Development (HD)[24]. In 2003, Rotterdam proposed classification criteria at the conference organized by the American Society of Reproductive Medicine (ASRM) and the European Society of Human Reproduction and Embryology (ESHRE), which was primarily used to classify PCOS[25]. Another conference organized by the PCOS Society in 2006 proposed diagnostic features for PCOS[26]. Individuals affected by PCOS are mostly obese due to androgen overexpression, which increases adipose tissue in the abdominal region. The classification criteria proposed at various conferences are listed in **Table 1**.

Genic SNPs and single nucleotide variants influence steroidogenesis, ovarian theca cell activity, and the release of hormones from the hypothalamus and pituitary gland[27,28]. Epigenetic factors such as intrauterine exposure and excess androgen in the maternal environment can also cause stable, heritable phenotypes that contribute to PCOS. Hyperandrogenism, abnormal steroid production, insulin resistance, and central obesity are all symptoms of a malfunctioning hypothalamic-pituitary-ovarian axis, which results in PCOS. Hyperandrogenism is caused by excess androgen secretion by the theca cells in the ovaries in response to adipose tissue development, leading to the formation of small multiple antral follicles and a sex hormone imbalance, causing endometrial carcinoma. Oocyte quality and endothelial function are both adversely affected by chronic oxidative stress and proinflammatory cytokines, which indicate infertility. However, prescreening and diagnosis are crucial in preventing PCOS and helping prevent metabolic abnormalities. Physical and mental well-being and a healthy lifestyle and environment play significant roles in overcoming the PCOS burden.

Several studies have looked at the association of TNF-α with various autoimmune diseases. However, SNP-based studies have been limited to only one or two genes, and their results have been limited[29]. Qualified data still needs to be incorporated to improve results. It has been demonstrated that TNF-α levels are elevated in the serum and follicular fluid of women with PCOS. Therefore, this study aimed to assess the associations of *TNF-α* polymorphisms 1031T/C, 308G/A, and 238G/A with PCOS.

METHODS

Literature search

A literature search was conducted in all available public and scientific databases, including Embase, NCBI, Google Scholar, Medline, and Science Direct, from inception to April 2023 using the following keywords to identify all articles on the association of 238G/A, 308G/A, and 1031 T/C with PCOS: TNF-alpha, TNF-alpha with polycystic ovarian syndrome, PCOS-TNF-alpha, and PCOS. Only English-language articles were considered. In order to avoid duplicated studies, authors' names were searched and screened in all the databases to identify appropriate studies, titles, abstracts, and full texts. In addition, the reference lists of the identified articles were also screened.

Selection criteria

The studies had to meet all of the following inclusion criteria: (i) evaluate the relationship between the *TNF-α* 238G/A, 308G/A, and 1031T/C polymorphisms and PCOS; (ii) include patients with PCOS and controls with eligible genotypic and phenotypic distributions of *TNF-α* 238G/A, 308G/A, and 1031T/C polymorphisms; (iii) both cases and controls are of the same ethnicity; and (iv) the full text was available in English. The exclusion criteria were as follows: (i) no control group; (ii) low 95% confidence interval or odds ratio (OR); (iii) studies with overlapping data; and (iv) animal studies. Most studies on Caucasians have not examined the association between PCOS and *TNF-α* 238G/A, 308G/A, and 1031T/C polymorphisms. We identified very few studies on Caucasians, and many were excluded due to the lack of proper inclusion criteria.

Data extraction

The following data were extracted from the articles selected for inclusion: publication year, first author, origin, ethnicity, number of cases, genotyping methods, and controls registered. The conflict and disagreement from the selected articles were removed from the study.

Statistical analysis

The data were analyzed using the Review Manager 5.4 and MetaGenyo software. In order to determine whether the study was significant, we determined whether the *p*-value was significant at $p < 0.005$ using genetic variations such as allele comparison, dominant regression, over-dominant, and recessive. The consistency of the findings across all studies was evaluated using the inconsistency index (I^2), which ranges from 0 to 100. The inconsistency index is crucial in determining the homogeneity (0% significance) and heterogeneity indications, which are responsible for most variations[30]. The degree of heterogeneity among the studies was assessed using Q-statistics and the chi-square test. The *z*-test was used to calculate ORs, and shared results among studies were considered statistically significant at $p < 0.05$. A sensitivity analysis was performed to assess the relative contributions of the included studies to the total estimates, removing one study at a time. Funnel plots and Egger's linear regression test were used to detect

Table 1: The classification criteria proposed at various conferences on PCOS

Year Proposed	Proposed By	Features
1990	NICH and HD	Hyperandrogenism, oligo-ovulation, thyroid, hyperprolactinemia, and inherited adrenal hyperplasia
2003	ASRM and ESHRE	Hyperandrogenism and oligo-ovulation
2006	Androgen and PCOS Societies	Clinical and biochemical analysis of hyperandrogenism and oligo-ovulation

publication bias. The log standard error was plotted against the odds ratios for each study. The heterogeneity between the eligible analyses performed using Egger's test, Q-test, and inconsistency index statistics was considered statistically significant if $p < 0.005$.

RESULTS

This study aimed to identify associations between *TNF-α* gene polymorphisms and PCOS. The searches of the various databases, including Google Scholar, NCBI, and Science Direct, identified six studies with 768 cases and 678 controls for the *TNF-α* 238G/A polymorphism[31–35,47], 11 studies with 1646 cases and 1578 controls for the *TNF-α* 308G/A polymorphism[31,32,34,36–41,43,48], and six studies with 880 cases and 1032 controls for the *TNF-α* 1031T/C polymorphism[31,32,39,44–46]. Based on the data collected from these 23 studies, PCOS was associated with the *TNF-α* 238G/A, 308G/A, and 1031T/C polymorphism (**Figure 1**). The data selected for the analysis is shown in **Table 2**. Among the selected studies, 22 were conducted in Asians and one in Caucasians. Begg's funnel plot, funnel plot, and Egger's test were performed for statistical evidence, and heterogeneity was observed from all selected articles.

Quantitative data analysis

The meta-analysis included 23 total studies to assess the association of PCOS with *TNF-α* 238G/A, 308G/A, and 1031T/C polymorphisms. We combined all the collected data with 3288 controls and 3294 cases. The *TNF-α* 238G/A polymorphism showed no significant associations in the allelic, recessive, dominant, and over-dominant models ($p > 0.05$; **Table 3**). In addition, subgroup analyses with allelic, recessive, dominant, and over-dominant models were nonsignificant ($p > 0.05$). Similarly, the *TNF-α* 308G/A polymorphism showed no significant associations in the allelic, dominant, overdominant, and recessive models ($p > 0.05$; **Table 4**). Again, subgroup analyses with the allelic, dominant, recessive, and over-dominant models were nonsignificant ($p > 0.05$). However, the TNF-α 1031T/C polymorphism showed significant associations with the allelic, recessive, and over-dominant models ($p < 0.05$; **Table 5**).

Publication Bias

Each variable was checked for apparent publication bias due to sample size constraints and reporting bias. A forest plot represented the heterogenicity, and a sensitivity analysis was conducted to identify studies contributing to the total estimates. Statistically constant results were obtained from the collected data (**Figures 2, 3, 4, 5, 6 and 7**), and significant results (**Figures 8, 9 and 10**) were obtained when the funnel plot was statistically analyzed (**Figures 11, 12 and 13**).

DISCUSSION

PCOS is the most commonly occurring endocrine disorder among adult women. In PCOS, ovarian dysfunction causes other metabolic disorders, which may result in chronic inflammation. TNF-α, an inflammatory cytokine found in the human ovaries, ovarian follicular fluid, and oocytes, causes many inflammatory disorders and impacts follicular atresia, physiological ovulation dysfunction, ovarian apoptosis, anovulation, and steroid secretion. The pathophysiological and etiological mechanisms of PCOS are highly complex. Inflammation and immune-regulatory genes are responsible for PCOS development and progression. However, many studies have not succeeded in identifying a significant relationship between inflammatory pathways and PCOS development.

Our study comprised 2764 cases with PCOS and 3218 controls obtained from published studies exploring associations between PCOS and *TNF-α* 238G/A, 308G/A, and 1031T/C polymorphisms. In our study, the *TNF-α* 238G/A polymorphism showed no association with PCOS in all genetic models (allele, recessive, over-dominant, and dominant), suggesting that *TNF-α* 238G/A may not contribute to PCOS development. There is no conclusive proof associating the *TNF-α* 308G/A polymorphism with PCOS. However, the *TNF-α* 1031T/C polymorphism was significantly associated with PCOS in the allelic, recessive,

Table 2: Attributes the studies to examine the relationship between *TNF-Alpha* gene polymorphism and PCOS

Contents	Study	Ethnicity	AA_Cases/	GA_Cases/	GG_Cases	Total Cases/Contro	Hardy-Weinberg equilibrium p-value
TNF-Alpha 238G/A	Sampurna *et al.* 2021 [31]	Asian	32/30	44/45	24/25	100/100	0.3283
		Asian	2/0	50/83	148/117	200/200	0.0002
	Kordestani *et al.* 2018 [33]	Asian	1/3	6/6	104/96	111/105	0
	Wen *et al.* 2013 [34]	Asian	0/0	7/4	137/68	144/72	0.8084
	Xie *et al.* 2016 [35]	Asian	0/0	3/3	99/93	102/96	0.8764
	Kordestani *et al.* 2018 [33]	Asian	1/3	6/6	104/96	111/105	0
TNF-Alpha 308G/A	Sampurna *et al.* 2021 [31]	Asian	32/30	44/45	24/25	100/100	0.3283
	Bhatnagar *et al.* 2019 [32]	Asian	2/0	50/83	148/117	200/200	0.0002
	Sampurna *et al.* 2021 [31]	Asian	25/30	50/47	25/23	100/100	0.5798
	Azeez *et al.* 2021 [36]	Asian	1/1	25/15	32/14	58/30	0.2054
	Alwan *et al.* 2021 [37]	Asian	9/7	15/13	56/50	80/70	0.0007
	Li *et al.* 2017 [38]	Asian	3/1	33/24	357/356	393/381	0.387
	Bhatnagar *et al.* 2019 [32]	Asian	0/2	79/63	121/135	200/200	0.0671
	Deepika *et al.* 2013 [39]	Asian	3/3	10/10	270/293	283/306	0
	Mao *et al.* 2000 [40]	Asian	1/4	29/13	88/37	118/54	0.0889
	Milner *et al.* 1999 [41]	Caucasiar	2/3	23/42	59/63	84/108	0.1939
	Peng *et al.* 2010 [42]	Asian	1/2	11/27	118/146	130/175	0.557
	Vural *et al.* 2010 [43]	Asian	3/3	16/15	78/77	97/95	0.0549
	Wen *et al.* 2013 [34]	Asian	0/0	14/7	89/52	103/59	0.6281
TNF-Alpha 1031T/C	Sampurna *et al.* 2021 [31]	Asian	38/35	38/40	24/25	100/100	0.055
	Bhatnager *et al.* 2019 [32]	Asian	0/2	48/69	152/129	200/200	0.0272
	Deepika *et al.* 2013 [39]	Asian	6/5	170/139	107/162	283/306	0
	Yun *et al.* 2011 [44]	Asian	2/0	71/22	144/122	217/144	0.321
	Hazwanie *et al.* 2015 [45]	Asian	3/91	8/45	1/9	12/145	0.2922
	Alkhuriji *et al.* 2020 [46]	Asian	1/9	17/46	50/82	68/137	0.4666

and over-dominant models but not in the dominant model. Therefore, these results suggest that the *TNF-*α 1031T/C polymorphism may not be associated with PCOS.

Our meta-analysis has generated some important findings, but it is not without limitations. Since we only considered research studies published in English that were abstracted and indexed in online databases, we might have missed relevant studies published in other languages. We also did not investigate the possible influence of gene-environment interactions, and a subgroup analysis was not performed because there was a dearth of appropriate studies and data. Nonetheless, our meta-analysis does have some strengths. Firstly, our qualitative and quantitative analysis, such as funnel plot and Egger's linear regression, did not demonstrate clear evidence of publication bias. Therefore, we can conclude that our findings have strong statistical support. Secondly, a strict data extraction and analysis procedure was performed

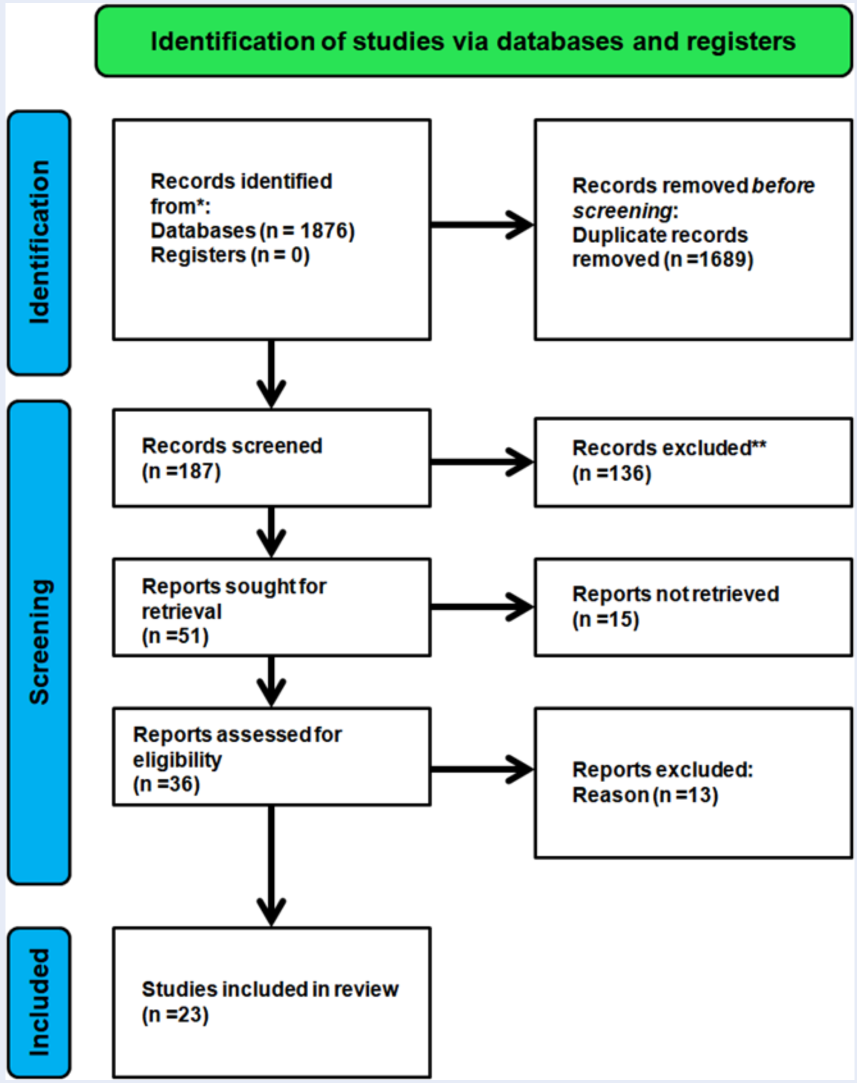

Figure 1: **A flow diagram of article identification, screening, and inclusion**.

Table 3: **Summary estimates for Odd ratios and 95% confidence interval in different ethnicity for TNF-α 238G/A**

Model	Ethnicity	Number of studies	Test of association				Test of heterogeneity		
		-	Odd ratio	95% confidence interval	p-value	Model	p-value	I^2	p-value (Egger's test)
Allele contrast (A *vs.* a)	Overall	6	1.2860	[1.0001; 1.6537]	0.049953	Fixed	0.3215	0.1457	0.957
Recessive model (AA *vs.* Aa+aa)	Overall	6	1.5287	[1.1134; 2.0989]	0.008691	Fixed	0.3803	0.0463	0.2936
Dominant model (AA+Aa *vs.* aa)	Overall	3	0.9344	[0.5287; 1.6516]	0.815448	Fixed	0.3419	0.0683	0.8213
Overdominant (Aa *vs.* AA + aa)	Overall	5	0.6539	[0.4800; 0.8908]	0.007073	Fixed	0.2978	0.1835	0.3097

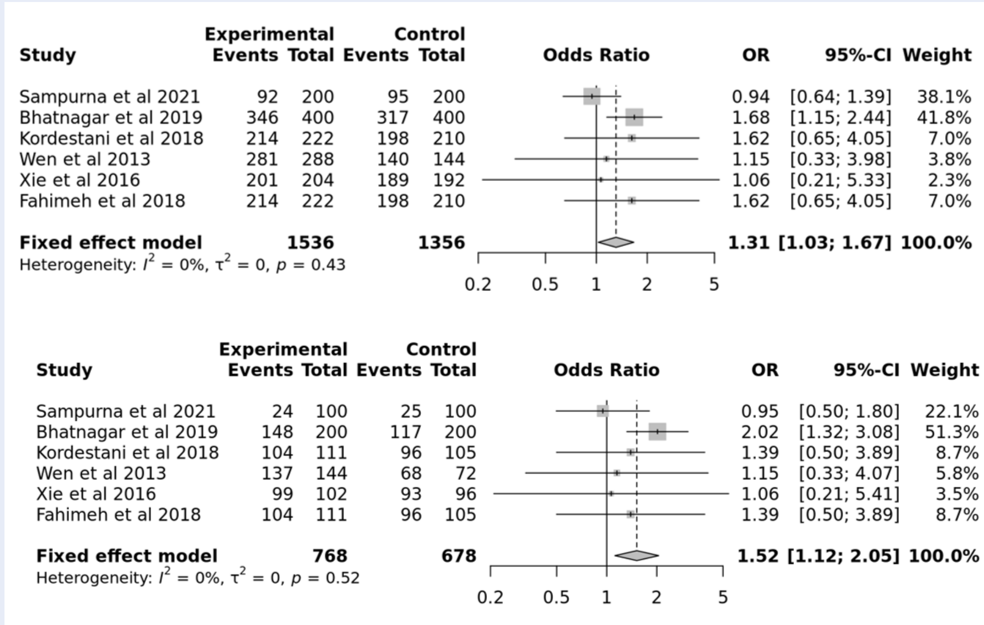

Figure 2: **Forest plot displaying the relationship between TNF-α 238G/A gene polymorphism and PCOS using an allelic and recessive model.**

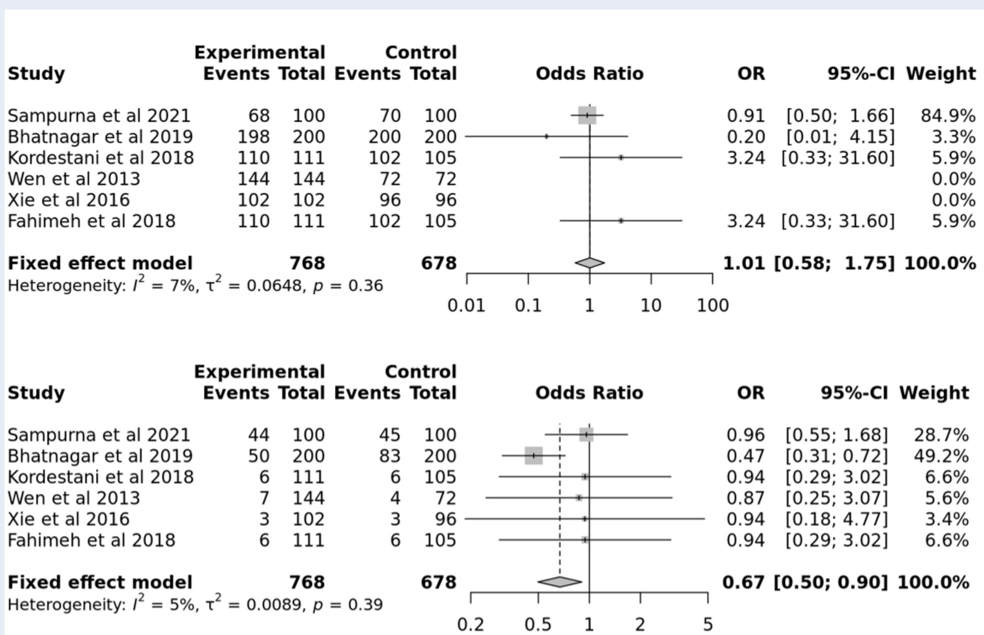

Figure 3: **Forest plot displaying the relationship between TNF-α 238G/A gene polymorphism and PCOS using dominant and over-dominant model.**

Table 4: Summary estimates for Odd ratios and 95% confidence interval in different ethnicity for TNF-α 308G/A

Model	Ethnicity	Number of studies	Test of association				Test of heterogeneity		Publication bias
	-	-	Odd ratio	95% confidence interval	p-value	Model	p-value	I^2	p-value (Egger's test)
Allele contrast (A *vs.* a)	Overall	11	1.0494	[0.8912; 1.2356]	0.562921	Fixed	0.2733	0.1789	0.3883
Recessive model (AA *vs.* Aa+aa)	Overall	11	1.0159	[0.8364; 1.2338]	0.873805	Fixed	0.3078	0.143	0.1283
Dominant model (AA+Aa *vs.* aa)	Overall	10	1.2340	[0.8015; 1.9000]	0.339546	Fixed	0.7397	0	0.4684
Overdominant (Aa *vs.* AA + aa)	Overall	11	1.0380	[0.8515; 1.2654]	0.711939	Fixed	0.3892	0.057	0.1445

Table 5: Summary estimates for Odd ratios and 95% confidence interval in different ethnicity for TNF-Alpha 1031T/C

Model	Ethnicity	Number of studies	Test of association				Test of heterogeneity		Publication bias
	-	-	Odd ratio	95% confidence interval	p-value	Model	p-value	I^2	p-value (Egger's test)
Allele contrast (A *vs.* a)	Overall	6	1.0641	[0.6570; 1.7235]	0.800633	Random	0	0.8621	0.3096
Recessive model (AA *vs.* Aa+aa)	Overall	6	0.912	[0.4971; 1.6733]	0.766151	Random	0	0.8524	0.5966
Dominant model (AA+Aa *vs.* aa)	Overall	6	1.5185	[0.6634; 3.4755]	0.322802	Random	0.097	0.4635	0.3609
Overdominant (Aa *vs.* AA + aa)	Overall	6	1.3060	[0.7446; 2.2907]	0.35179	Random	0	0.8456	0.9502

to draw satisfactory and reliable conclusions from the study.

CONCLUSIONS

In conclusion, our meta-analysis assessed possible associations of *TNF-α* 238G/A, 308G/A, and 1031T/C polymorphisms with PCOS using valuable statistical data from significant and nonsignificant studies. Overall, our meta-analysis showed that the *TNF-α* 238G/A and 308G/A polymorphisms may not be associated with PCOS, whereas the *TNF-α* 1031T/C polymorphism may be associated with PCOS. Future studies should explore possible associations and interactions between *TNF-α* polymorphisms and PCOS using large datasets comprising gene and environment data to confirm our findings.

ABBREVIATIONS

PCOS: Polycystic ovarian syndrome, **TNF-α**: Tumor necrosis factor-alpha, **SNPs**: single nucleotide polymorphisms, **TACE**: TNF-α converting enzyme

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AUTHOR'S CONTRIBUTIONS

All authors significantly contributed to this work, read and approved the final manuscript.

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None.

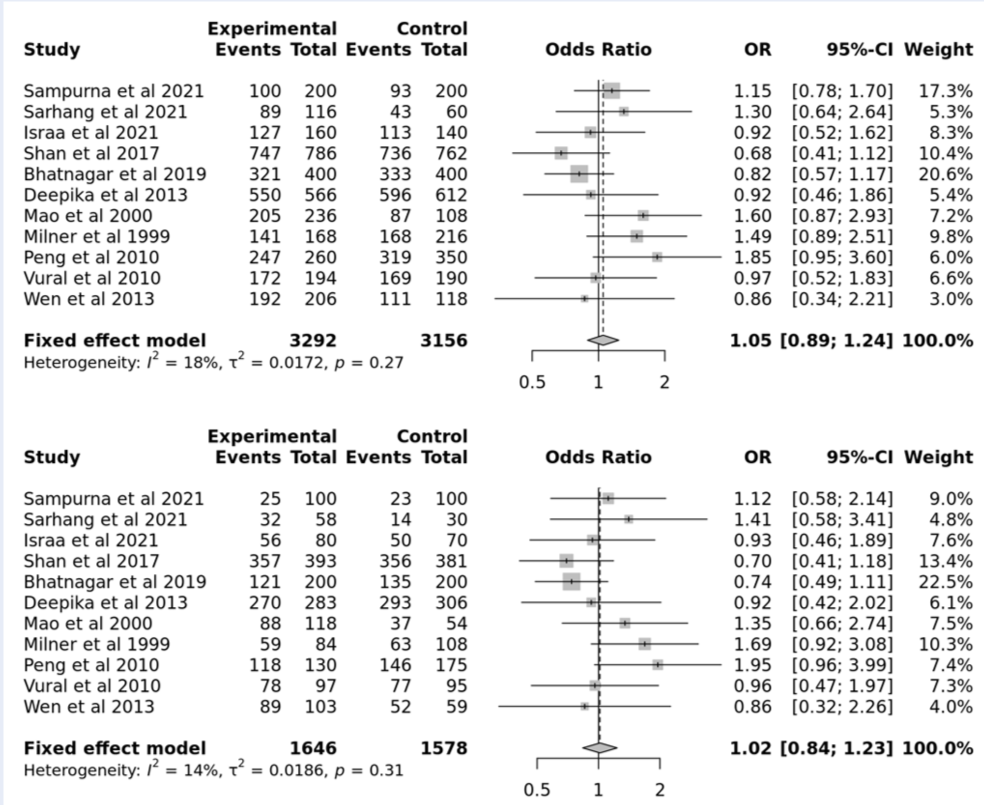

Figure 4: **Forest plot displaying the relationship between TNF-α 308G/A gene polymorphism and PCOS using an allelic and recessive model.**

AVAILABILITY OF DATA AND MATERIALS

Data and materials used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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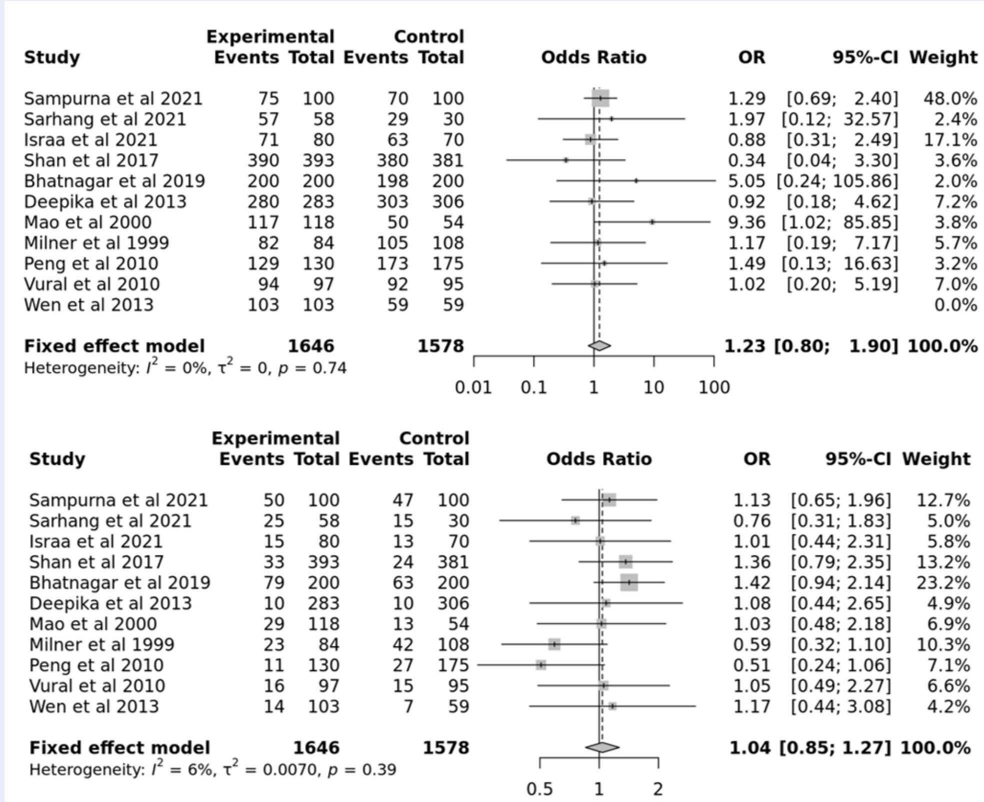

Figure 5: **Forest plot displaying the relationship between TNF-α 308G/A gene polymorphism and PCOS using dominant and over-dominant model**.

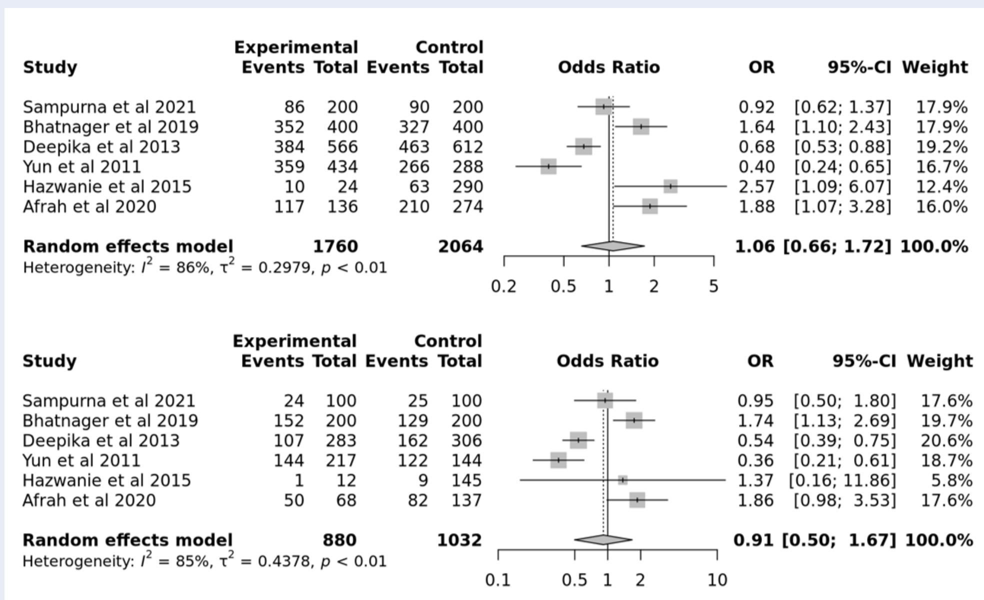

Figure 6: **Forest plot displaying the relationship between TNF-α 1031T/C gene polymorphism and PCOS using an allelic and recessive model**.

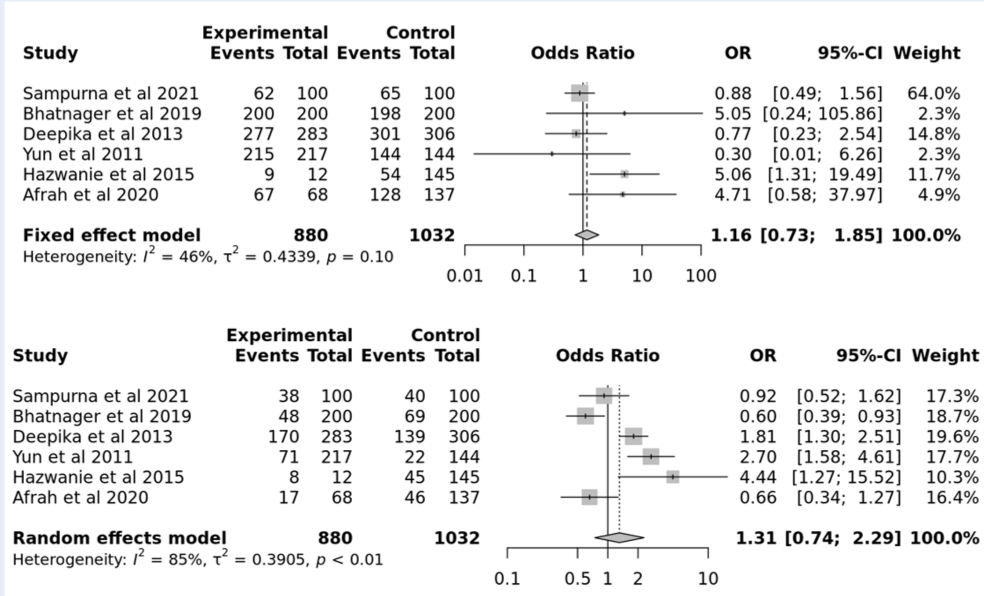

Figure 7: **Forest plot displaying the relationship between TNF-α 1031T/C gene polymorphism and PCOS using dominant and over-dominant model**.

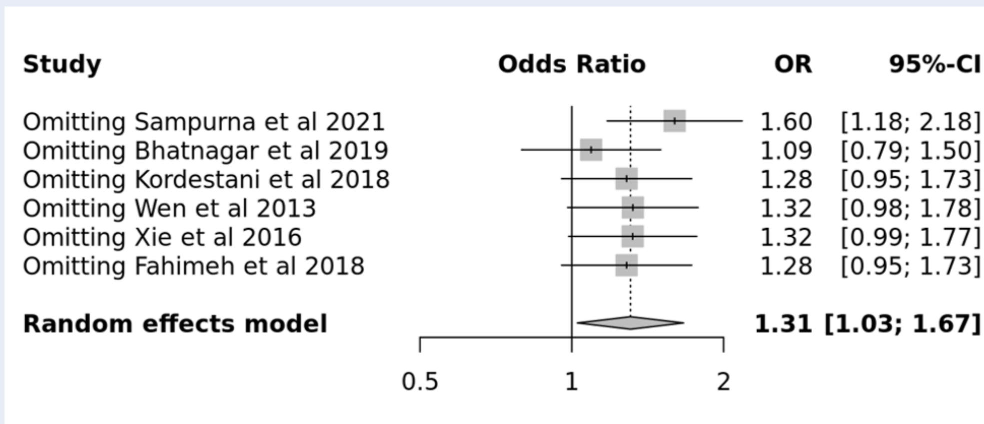

Figure 8: **Sensitivity analyses to investigate the association between TNF-α 238G/A gene polymorphism and PCOS risk using allelic model**.

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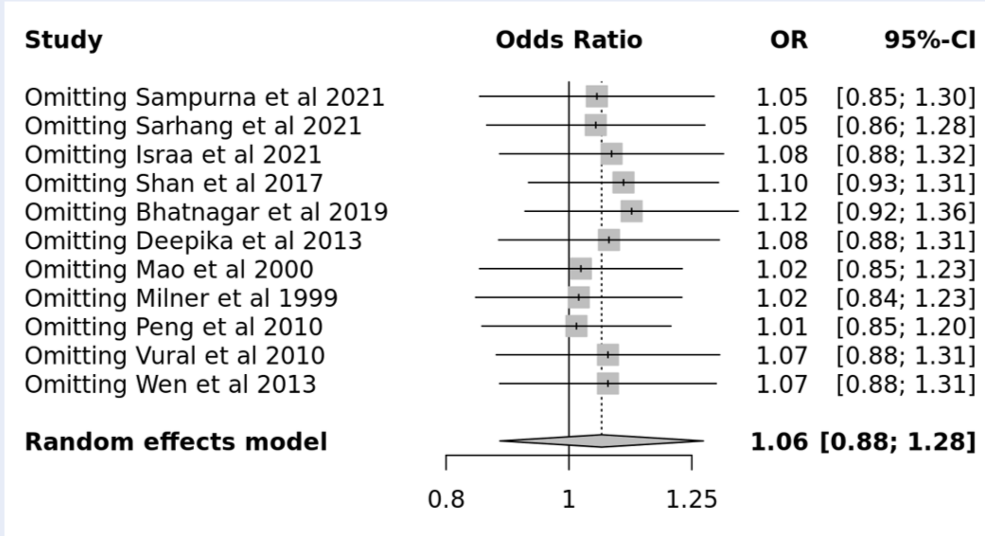

Figure 9: **Sensitivity analyses to investigate the association between TNF-α 308G/A gene polymorphism and PCOS risk using allelic model**.

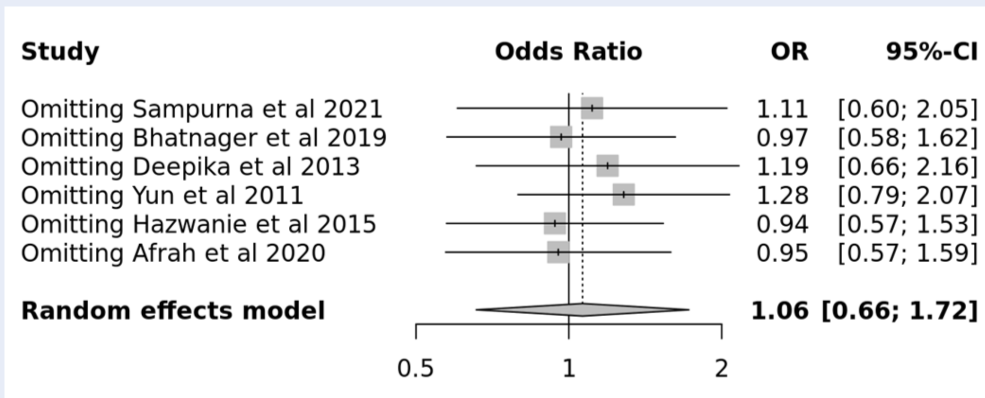

Figure 10: **Sensitivity analyses to investigate the association between TNF-α 1031T/C gene polymorphism and PCOS risk using allelic model**.

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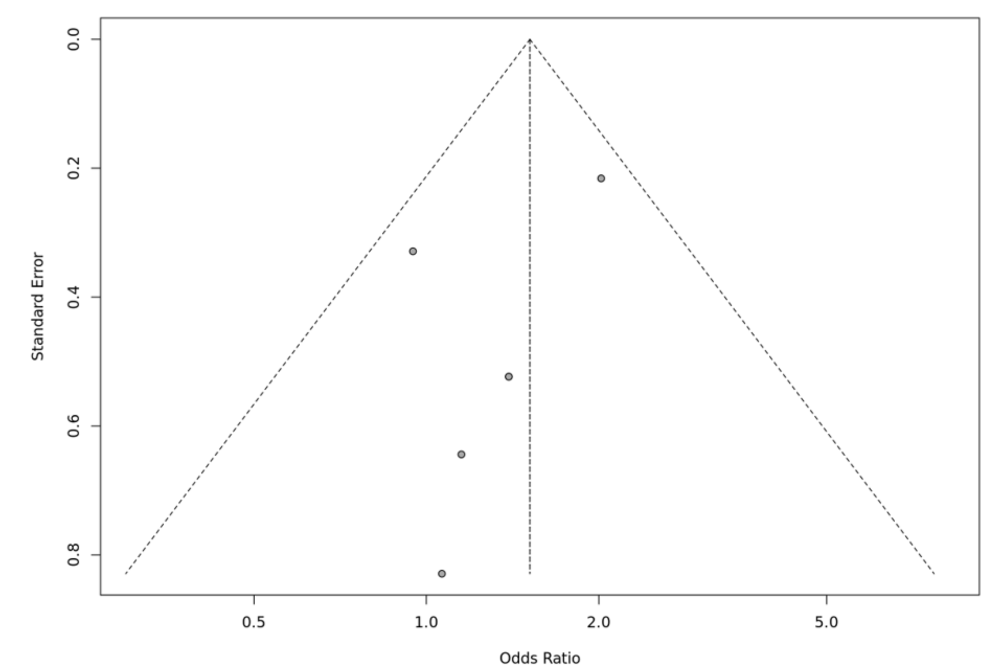

Figure 11: **Examining publication bias in the association betweenTNF-α 238G/A gene polymorphism and PCOS risk using allelic model**.

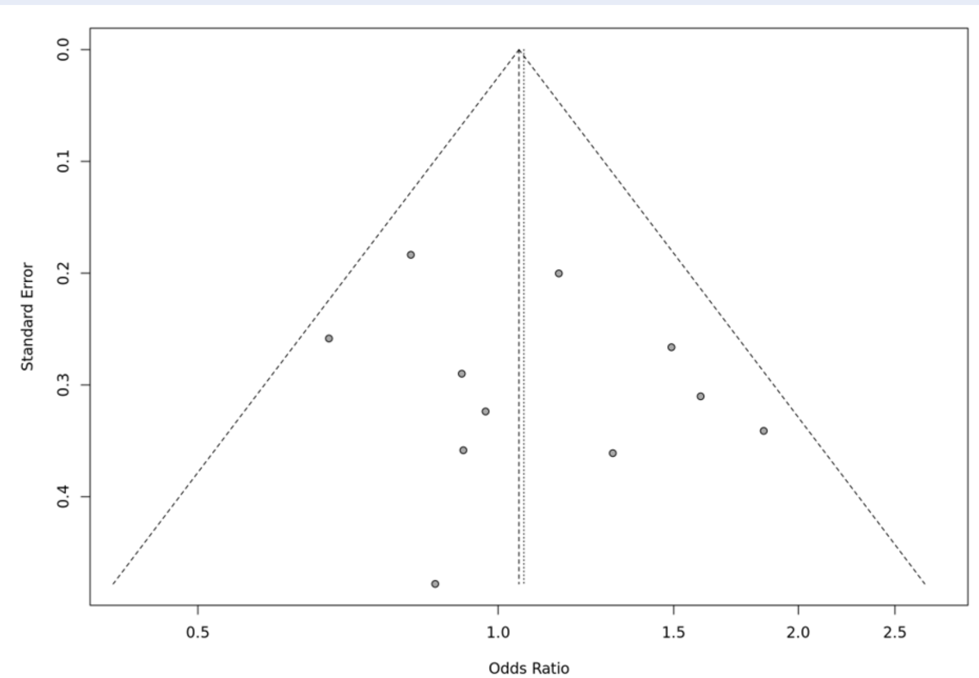

Figure 12: **Examining publication bias in the association between TNF-α 308G/A gene polymorphism and PCOS risk using allelic model**.

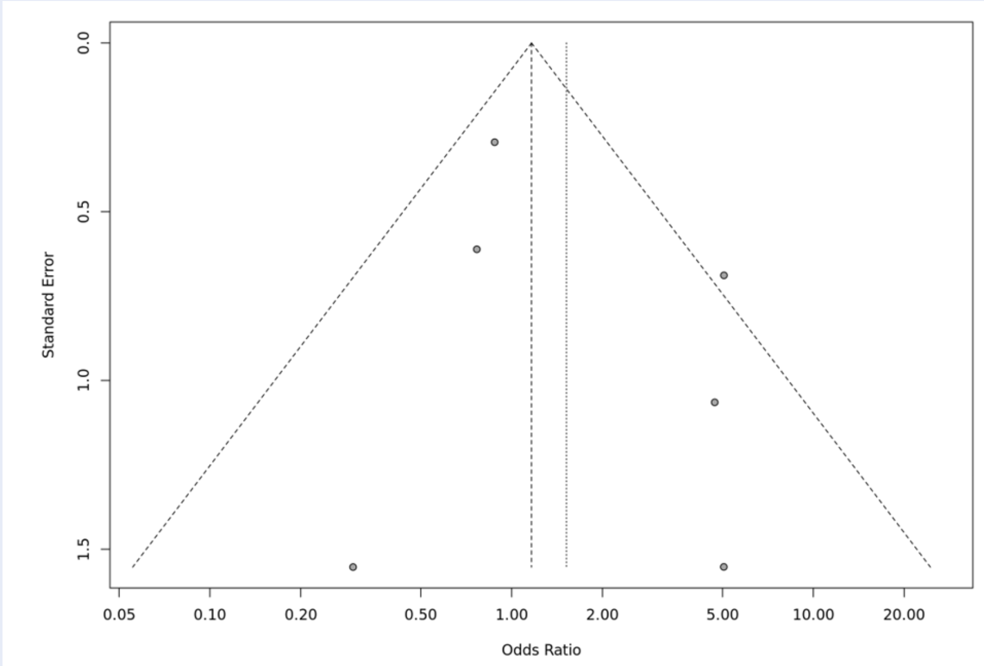

Figure 13: Examining publication bias in the association between TNF-α 1031T/C gene polymorphism and PCOS risk using allelic model.

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