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Significant association between methylenetetrahydrofolate reductase gene C677T polymorphism with polycystic ovary syndrome risk

A meta-analysis update

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Abstract

The methylenetetrahydrofolate reductase (MTHFR) may play a pathological role in polycystic ovary syndrome (PCOS). However, the conclusions of published reports on the relationship between the MTHFR C677T polymorphism and PCOS risk remain controversial.

To derive a more precise estimation we performed a metaanalysis based on 22 studies that together included 2405 cases and 2419 controls. PubMed, EMBASE, WanFang and the Chinese National Knowledge Infrastructure databases were used to retrieve articles up to up to October 28, 2019. The crude odds ratios (ORs) with 95% confidence intervals (95% CI) were calculated to evaluate the association.

Metaanalysis results showed a significant association between the MTHFR C677T polymorphism and PCOS risk in 3 genetic models (allele model: OR = 1.40, 95% CI = 1.27–1.53; dominant model: OR = 1.47, 95% CI = 1.17–1.85); homozygous model: OR = 1.90, 95% CI = 1.55–2.32). Moreover, significant associations were observed when stratified by ethnicity, source of controls, etiology, and genotype methods.

This metaanalysis suggests that the T-allele of the MTHFR C677T polymorphism is associated with an increased risk of PCOS, especially in Asians further studies with larger population sizes are needed to confirm these results.

Abbreviations: 95% CI = 95% confidence intervals, CAD = coronary artery disease, CNKI = Chinese National Knowledge Infrastructure, Hcy = homocysteine, HWE = Hardy-Weinberg equilibrium, LDR = ligase detection reaction, MTHFR = methylenetetrahydrofolate reductase, OR = odds ratio, PCOS = polycystic ovary syndrome, PCR = polymerase chain reaction.

Keywords: meta-analysis, methylenetetrahydrofolate reductase, polycystic ovary syndrome, polymorphism

1. Introduction

Polycystic ovary syndrome (PCOS), the most common endocrinopathy in reproductive-aged women of reproductive age, is characterized by hyperandrogenism, ovulatory dysfunction and infertility,[1] with a prevalence up to 12% to 18% depending on diagnostic criteria like clinical hyperandrogenism, oligoanovulation and polycystic ovaries.[2] The syndrome is linked to metabolic disorders, such as insulin resistance, obesity, and diabetes.[3] Etiology of PCOS remains largely unknown, however, complex polygenic disorder with environment and individual were believed the prominent contributing factors.[5] Additionally, several studies have report a high risk of premature coronary artery disease (CAD) in patients with PCOS.[4] Genetic factors such as vitamin B12 and folate are involved in the regulation of homocysteine (Hcy) metabolism pathway which related to CAD in PCOS.[6] Methylenetetrahydrofolate reductase (MTHFR) plays an essential role in folate metabolism, DNA methylation, and RNA synthesis.[7–8] By regulating enzymatic activity, MTHFR catalyzes the conversion of 5, 10-methylenetetrahydrofolate into 5- methylenetetrahydrofolate irreversibly which is the main form of folic acid in plasma and tissues. Low folate concentrations also tend to be correlated with raised plasma Hcy levels as it is a cofactor in the re-methylation of Hcy.[9] Reduced activity of MTHFR is the most common cause of hyper Hcy. This makes MTHFR an important gene for investigation in PCOS as decreased efficiency of folate/Hcy pathway could increase the risk. Single gene polymorphisms in the MTHFR gene can change the expression and activity of the protein it encodes.[10] Among them, The C677T polymorphism is the most common one which results in a variant of MTHFR enzyme and increase circulating total Hcy levels at a homozygous state.[11,12]

Editor: Jianxun Ding.

Special Fund for Xinglin Scholar Program Hospital of Chengdu University of Traditional Chinese Medicine, Grant No.077035014.

The authors have no conflicts of interest to disclose.

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How to cite this article: Li Y, Zhu H, Liu M, Zeng Z, Zeng Y, Xu X, Ye M. Significant association between methylenetetrahydrofolate reductase gene C677T polymorphism with polycystic ovary syndrome risk: A meta-analysis update. Medicine 2020;99:4(e18720).

Received: 4 August 2019 / Received in final form: 21 November 2019 / Accepted: 11 December 2019

http://dx.doi.org/10.1097/MD.0000000000018720

Up to now, a total of 20 epidemiological studies have evaluated the association between the MTHFR gene polymorphism C677T and risk of PCOS in diverse ethnicities.[13–31] However, the results have been inconsistent. Five meta-analyses have summarized the associations between MTHFR gene polymorphism C677T and risk of PCOS come to opposite conclusions.[22,32–35] The main factor that would contribute to the discrepancy is that the previous metaanalyzes with relatively small sample size may lead to a lower statistical power. Since that data, several more studies have emerged. Therefore, we aimed to perform an updated metaanalysis to investigate the associations between MTHFR gene polymorphism C677T and risk of PCOS in order to get a more precise and reliable assessment of the association.

2. Methods

2.1. Search strategy

To identify eligible studies for this metaanalysis, PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), Embase (http://www.embase.com), WanFang and the Chinese National Knowledge Infrastructure databases were used to retrieve articles up to up to October 28, 2019 without any language limitation. The following terms and keywords were used: "MTHFR" (or "methylenetetrahydrofolate reductase"), "polymorphism" (or "variant") and "PCOS" (or "polycystic ovary syndrome"). We have also manually searched the reference lists of the retrieved articles for potential papers. Ethical approval is not necessary since this study is a metaanalysis.

2.2. Selection criteria

The included studies met the following inclusion criteria:

(1) Full-text publications;
(2) The association between MTHFR gene polymorphism C677T and risk of PCOS was examined based on case-control design;
(3) Provide sufficient data about MTHFR C677T genotypes and genotype distributions to estimate the odds ratio (OR) with 95% confidence intervals (95% CI); Studies that met the exclusion criteria were excluded if they were overlapped data, reviews, reports, comments, letters, and so on.

2.3. Data extraction

The data from all eligible studies were extracted by 2 authors independently (Li and Zhu). The following information was extracted: first author, year of publication, country, ethnicity, source of controls, total sample size, genotype frequencies in cases and controls, *P*-value for Hardy-Weinberg equilibrium (HWE), genotyping methods.

2.4. Statistical analysis

Analyzes were calculated using Stata software version 12.0 (Stata Corp., College Sta-tion, TX) and all *P* values were 2-sided. The pooled ORs and 95% CI were used to assess the strength of association between MTHFR C677T polymorphism and PCOS under 4 genetic models, including allele model (T vs C), dominant model (TT+CT vs CC), recessive model (TT vs CT+CC) and homozygous model (TT vs CC). The significance of pooled ORs was examined by *Z*-test, and $P < .05$ was considered as statistically significant. Heterogeneity assumption was checked by Cochran *Q*-statistic and I^2 statistic test was calculated to quantify the proportion of the total variation across studies due to heterogeneity.[36] Subgroup analysis was also performed by ethnicity, etiologies, genotype methods and source of controls to investigate the possibility of heterogeneity. The fixed-effects model (the Mantel–Haenszel method) is used when the effects are assumed to be homogenous ($P > .05$ of *Q* test and $I^2 < 50\%$). Otherwise, the random effects model (the Der Simonian and Laird method) is used when they are $P < .05$ of *Q* test and $I^2 > 50\%$.[37,38] The Chi-squared test was used to calculated HWE of the genotype frequencies of controls. A value of $P < .05$ signified a departure from HWE. Sensitivity analysis was performed to examine stability of our results by omitting each study in each turn. Publication bias was measured by funnel plots and quantified by the Begg and Egger tests (significance level was set at 0.05).

3. Results

3.1. Study characteristics

Our search identified 18 studies including 2196 cases and 2201 controls from15 publications relevant to the role of MTHFR C677T polymorphism on PCOS susceptibility. Two publications[17,22] respectively included 2 and 3 different diseases which giving 5 studies altogether (Fig. 1). Table 1 describes the detailed characteristics of each studies included in our metaanalysis.

3.2. Meta-analysis results and heterogeneity analysis

The findings with regard to association between MTHFR C677T polymorphism and PCOS risk are presented in Table 2. For the overall analysis, our metaanalysis revealed a significant main effects on PCOS risk in 3 genetic models (allele model: OR = 1.40, 95% CI = 1.27–1.53; dominant model: OR = 1.47, 95% CI = 1.17–1.85); homozygous model: OR = 1.90, 95% CI = 1.55–2.32) (Fig. 2-A;B;D). The results of different ethnic subgroups were also found positive correlations among Asians (allele model: OR = 1.48, 95% CI = 1.33–1.64; dominant model: OR = 1.57, 95% CI = 1.23–1.99; recessive model: OR = 1.51, 95% CI = 1.25–1.83; homozygous model: OR = 2.15, 95% CI = 1.71–2.69) and Turkey population (allele model: OR = 1.89, 95% CI = 1.18–3.03; dominant model: OR = 2.96, 95% CI = 1.49–5.90) (Fig. 2-A;B;D), but no significant associations were found in all Caucasians genetic models (Fig. 2-A;B;C;D). In further stratified analysis by HWE, a significant association was observed in studies in HWE in three genetic models (allele model: OR = 1.34, 95% CI = 1.15–1.98; dominant model: OR = 1.49, 95% CI = 1.16–1.93; recessive model: OR = 1.55, 95% CI = 1.29–1.85; homozygous model: OR = 2.55, 95% CI = 1.66–2.84). In addition, significant effect on genotype method of polymerase chain reaction (PCR), restriction fragment length polymorphism in all genetic models and real-time PCR in allele models, PCR- ligase detection reaction (LDR) in three genetic models. However, no significant elevated risks of Bio-Rad variant under all models. Furthermore, we also found significant risks in the stratified analysis by source of controls.

Subgroup analysis by ethnicity, genotype methods, source of controls and HWE was conducted to detect sources of heterogeneity. And we found significant heterogeneity under the

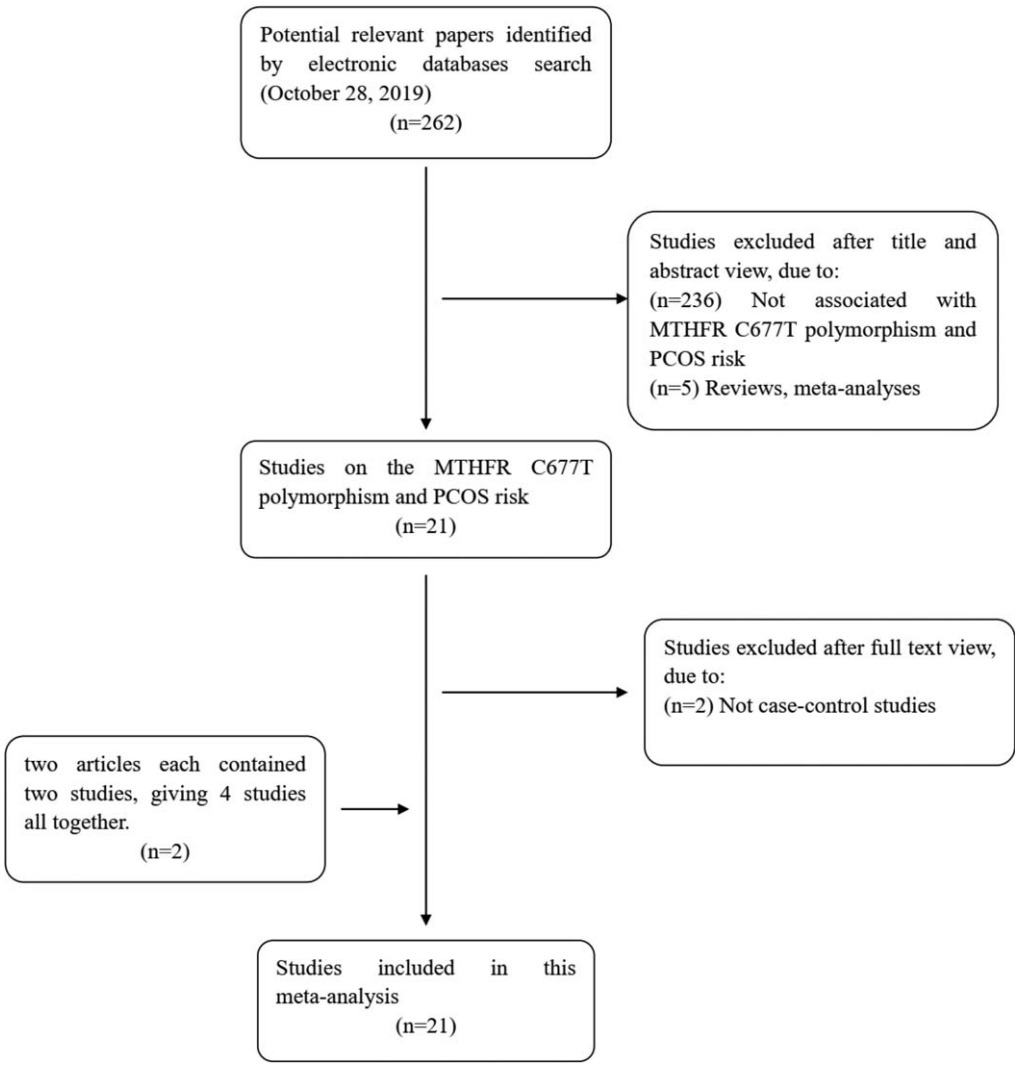

Figure 1. Flow chart of describing the study inclusion/exclusion.

recessive model might be related to the Caucasian subjects, studies not in HWE, genotype method of Bio-Rad Variant, TaqMan,Squencing ($P < .05$).

3.3. Sensitivity analyses

We performed a sensitivity analysis by deletion of 1 single study at a time to explore the influence of each individual study on the overall pooled ORs. And the estimate of results was not influenced excessively by omitting any single study under the allele model (T vs C) of MTHFR C677T (Fig. 3), which indicated that the results of our metaanalysis were statistically reliable.

3.4. Publication bias

The Begg rank correlation and Egger linear regression tests were conducted to access the publication bias. The funnel plots of Begg test seemed to show no evident asymmetry (Fig. 4), further validated by Egger test ($P > .05$).

4. Discussion

At present, epidemiological literature regarding the effect of PCOS as a risk factor for MTHFR C677T gene polymorphism remains inconsistent and inconclusive. Five metaanalyses have summarized the associations, Bagos PG et al (2009) and Lee et al (2014) concluded a negative result based on 6 and 9 eligible studies respectively, similarly, S. Justin Carlus et al suggested that MTHFR C677T was not clinically important in PCOS in most of the populations based on 13 studies. However, Li-yuan Fu et al in their meta-analysis of 10 studies indicated that the 677T allele increases PCOS susceptibility, and its seems to be more intense in Europeans than in Asians. More recently, in 2017 Lihong Wang et al suggested that the T allele is strongly associated with the risks for PCOS in the Middle Eastern populations while protective in Caucasian populations. Nevertheless, the credibility of Lihong Wang analysis should be re-examined as some eligible studies have been left out. While in our meta-analysis, which was based on collecting 7 more studies than previous analyses, we found that T allele likely had an increased PCOS risk compared with the

Table 1

The characteristics of studies included in the meta-analysis.

First author	Year	Country	Ethnicity	Source of control	Case no.	Control no.	Cases			Controls			HWE	Methods
							CC	CT	TT	CC	CT	TT		
Glueck	1999	USA	Caucasian	Hospital	41	234	14	23	4	119	89	26	0.13	PCR-RFLP
Sills	2001	USA	Caucasian	Population	36	18	25	9	2	8	9	1	0.44	PCR-RFLP
Tsanadis	2002	Greece	Caucasian	Hospital	30	45	12	14	4	20	19	6	0.66	PCR-RFLP
Orio	2003	Italy	Caucasian	Population	70	70	16	41	13	17	38	15	0.46	PCR-RFLP
Palep-Singh	2007	UK	Caucasian	Population	25	16	11	12	2	10	5	1	0.73	PCR-RFLP
Palep-Singh	2007	UK	Asian	Population	21	9	14	7	0	9	0	0	-	PCR-RFLP
Choi	2009	Korea	Asian	Population	227	115	67	125	35	33	67	15	0.04	PCR-RFLP
Karadeniz	2010	Turkey	Turkey	Population	86	70	15	65	6	35	28	7	0.69	Real-time PCR
Jain	2012	India	Asian	Population	92	95	76	16	0	82	13	0	0.47	PCR-RFLP
Idali	2012	Iran	Asian	Population	71	100	36	31	4	66	25	9	0.01	PCR-RFLP
Kazerooni	2013	Iran	Asian	Hospital	120	60	102	14	4	52	6	2	0.01	TaqMan
S.Justin (Indo-Europen Population)	2014	India	Asian	Population	93	100	77	16	0	83	16	1	0.82	Sequencing
S.Justin (Dravidian Population)	2014	India	Asian	Population	168	156	132	33	3	126	29	1	0.62	Sequencing
Naghavi A	2015	Iran	Asian	Population	112	196	61	38	13	136	51	9	0.15	PCR-RFLP
Qi wei	2015	China	Asian	Population	115	58	14	60	41	21	23	14	0.14	PCR-RFLP
Jiang	2015	China	Asian	Population	90	122	13	37	40	13	56	53	0.36	TaqMan
Geng	2016	China	Asian	Hospital	184	236	51	79	54	102	96	38	0.06	PCR-RFLP
Katarzyna	2016	Poland	Caucasian	Population	168	99	87	52	29	53	37	9	0.49	Real-time PCR
J.B. Wu	2016	China	Asian	Hospital	244	257	94	106	44	122	104	31	0.23	PCR-RFLP
Szafarowska	2016	Poland	Caucasian	Hospital	76	56	33	39	4	19	30	7	0.36	PCR-RFLP
Xianting Jiao	2018	China	Asian	Hospital	336	307	52	162	122	96	139	72	0.12	PCR-LDR

HWE = Hardy-Weinberg equilibrium; LDR = ligase detection reaction; PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism.

C allele, and the association was more pronounced in the Asians and Turkey population but not in Caucasians, suggesting genetic diversity among different ethnicities. Further studies need to evaluate the association of MTHFR C677T polymorphism with PCOS in Turkish populations as only 1 study on a Turkish population.

In the subgroup analysis by HWE, we demonstrated a significant association in studies in HWE but negative results in studies not in HWE. Combined with sensitivity analysis we can find that the studies showing deviation from the HWE influenced the results of meta-analysis but not significantly, as small size of controls not in HWE. Likewise, we detected similar significant association in subgroup analysis for genotype methods and source of controls. The statistical significance of MTHFR C677T polymorphism with PCOS risk suggesting that this polymorphism may be a potential biomarker which have been expected to

Table 2

Meta-analysis of the association between MTHFR C677T polymorphism and PCOS.

Subgroups	NO.	T vs C			TT+CT vs CC			TT vs CT+CC			TT vs CC		
		OR (95%CI)	P_h	P_{OR}	OR (95%CI)	P_h	P_{OR}	OR (95%CI)	P_h	P_{OR}	OR (95%CI)	P_h	P_{OR}
Overall	21	1.40 (1.27–1.53)	.005	.000	1.47 (1.17–1.85)*	.000*	.001*	1.24 (0.91–1.69)*	.001*	.167*	1.90 (1.55–2.32)	.027	.000
Ethnicity													
Asian	13	1.48 (1.33–1.64)	.036	.000	1.57 (1.23–1.99)*	.011*	.001*	1.51 (1.25–1.83)	.079	.000	2.15 (1.71–2.69)	.030	.000
Caucasian	7	1.06 (0.86–1.30)	.223	.570	1.09 (0.82–1.44)	.154	.569	0.87 (0.36–2.11)	.002	.758	1.14 (0.72–1.81)	.484	.565
Turkey	1	1.89 (1.18–3.03)	NA	.008	2.96 (1.49–5.90)	NA	.002	0.42 (0.13–1.35)	NA	.147	2.00 (0.57–6.96)	NA	.276
Genotype method													
PCR-RFLP	14	1.31 (1.08–1.59)*	.010*	.005*	1.48 (1.13–1.92)*	.012*	.004*	1.44 (1.15–1.81)	.212	.002	1.77 (1.38–2.28)	.042	.000
Real-time PCR	2	1.49 (1.10–2.00)	.195	.009	2.20 (0.51–9.41)*	.001*	.288*	1.28 (0.43–3.84)*	.111*	.027*	1.97 (0.99–3.93)	.980	.053
TaqMan	2	0.98 (0.69–1.41)	.653	.921	0.88 (0.49–1.61)	.435	.689	1.04 (0.62–1.76)	.994	.881	0.80 (0.37–1.74)	.761	.578
Sequencing	2	1.10 (0.74–1.66)	.612	.631	1.10 (0.71–1.70)	.797	.674	1.38 (0.27–1.72)	.302	.704	1.40 (0.27–7.24)	.302	.691
PCR-LDR	1	1.79 (1.43–2.23)	NA	.000	1.68 (1.14–2.47)	NA	.008	1.26 (0.89–1.77)	NA	.186	3.13 (2.00–4.89)	NA	.000
Source of control													
Population	14	1.56 (1.15–2.13)	.187	.005	1.29 (1.13–1.47)	.050	.000	1.44 (1.04–1.98)*	.001*	.028*	1.28 (0.98–1.66)	.499	.000
Hospital	7	1.77 (1.11–2.84)*	.034*	.017*	1.38 (1.10–1.74)*	.025*	.005*	1.57 (1.15–2.15)*	.041*	.004*	1.66 (1.33–2.08)	.189	.066
HWE													
Yes	17	1.34 (1.15–1.98)*	.005*	.000*	1.49 (1.16–1.93)*	.000*	.002*	1.55 (1.29–1.85)	.233	.000	2.05 (166–2.54)	.033	.000
No	4	1.16 (0.90–1.49)	.453	.248	1.30 (0.92–1.84)	.199	.138	1.03 (0.61–1.77)	.611	.901	1.05 (0.58–1.89)	.897	.880

95%CI = 95% confidence interval, HWE = Hardy-Weinberg equilibrium, LDR = ligase detection reaction, NA = data not available, OR = odds ratio, PCR = polymerase chain reaction, P_h = *P* value of heterogeneity test, P_{OR} = pool *P* value, RFLP = restriction fragment length polymorphism.

* Estimates for random-effects model; otherwise, fixed-effects model was used.

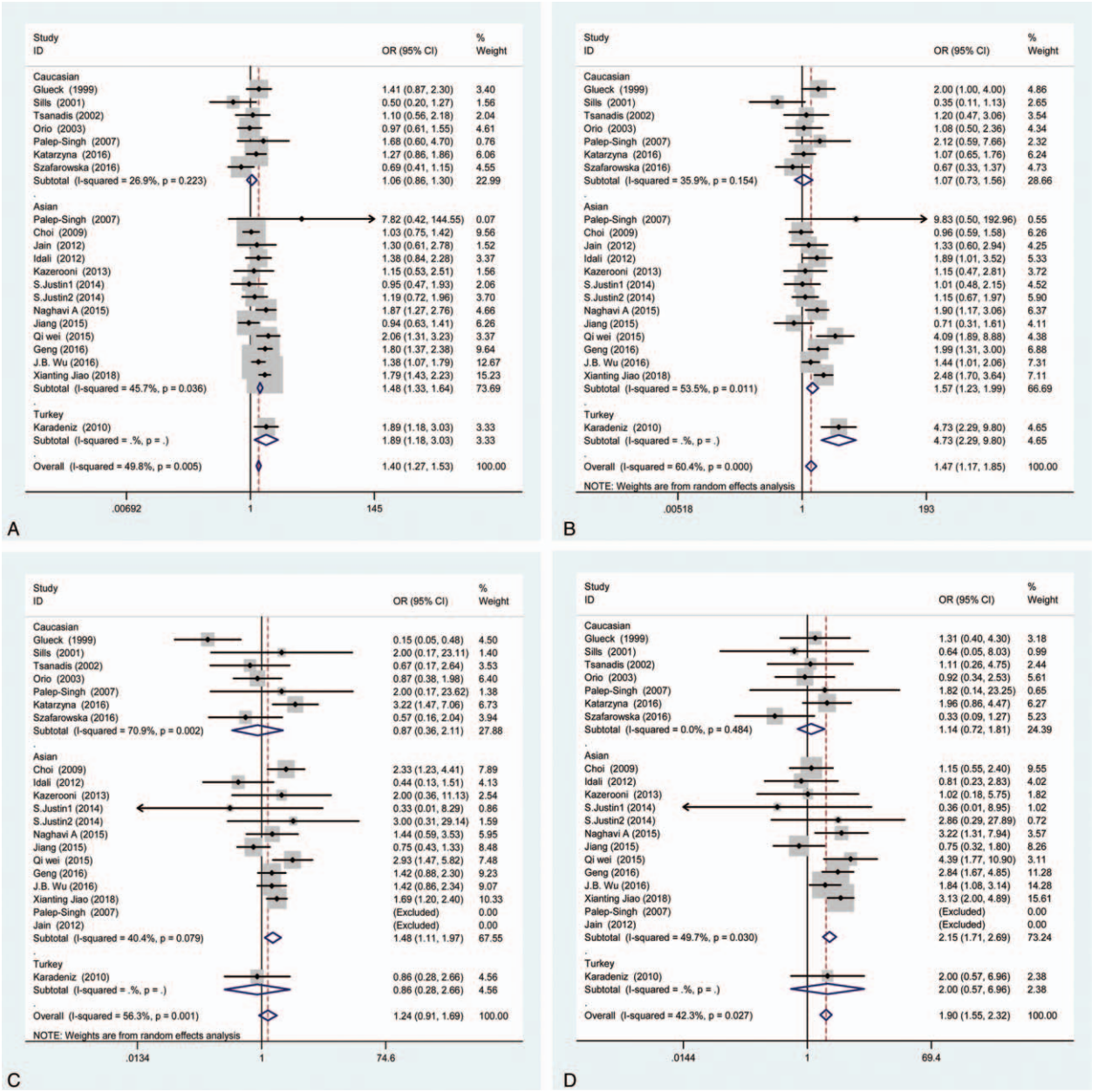

Figure 2. Forest plots of studies with all samples under allele model (A), dominant model (B), recessive model (C), homozygous model (D).

make early diagnosis, predict patient outcome, or direct optimal therapy for the individual patient.

There are still some limitations in interpreting the current results. First, the interactions between gene-gene, gene-environment, and even different polymorphic loci of the same gene may modulate POCS risk, as there is no original data for further analysis in our included studies, which limited our evaluation of potential interactions. Second, in the stratified analysis by ethnicity and genotype method, only 1 study was included in Turkey population, Bio-Rad Variant and PCR-LDR method, the limited sample sizes might weaken the metaanalysis results. Finally, there is the potential for publication bias, as no attempts were made to identify unpublished articles and only studies in English or Chinese were included in this analysis.

In conclusion, this meta-analysis demonstrates that the T-allele of MTHFR C677T polymorphism contribute to an increased risk of PCOS, especially among Asians but not Caucasians. Larger sample sizes study with more detailed individual data of gene-gene and functional studies on MTHFR C677T polymorphism are needed to strengthen our current results.

Author contributions

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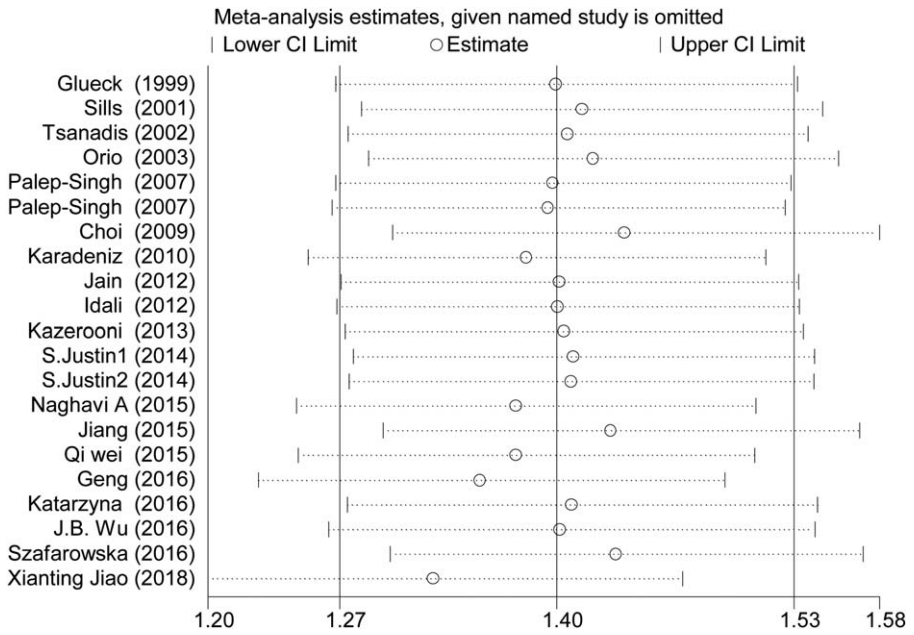

Figure 3. Sensitivity analysis of the correlation between MTHFR C677T polymorphism and susceptibility to PCOS. MTHFR = methylenetetrahydrofolate reductase, PCOS = polycystic ovary syndrome.

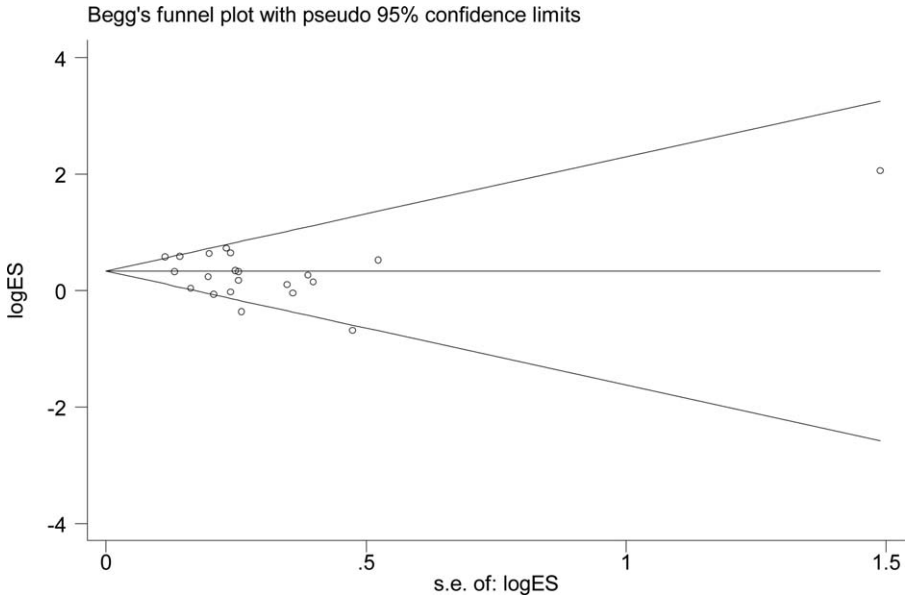

Figure 4. Funnel plots for studies investigating the effect of MTHFR C677T genetic polymorphisms on PCOS risk. MTHFR = methylenetetrahydrofolate reductase, PCOS = polycystic ovary syndrome.

Funding acquisition: Yin Li, Hongqiu Zhu, Min Liu, Zhulan Zeng, Yanling Zeng, Xinlei Xu, Min Ye.
Investigation: Yin Li, Hongqiu Zhu, Min Liu, Zhulan Zeng, Yanling Zeng, Xinlei Xu, Min Ye.
Methodology: Yin Li, Hongqiu Zhu, Min Liu, Zhulan Zeng, Yanling Zeng, Xinlei Xu, Min Ye.
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Resources: Yin Li, Hongqiu Zhu, Min Liu, Zhulan Zeng, Yanling Zeng, Xinlei Xu, Min Ye.
Software: Yin Li, Hongqiu Zhu, Min Liu, Zhulan Zeng, Yanling Zeng, Xinlei Xu, Min Ye.
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