Meta-Analysis of Observational Studies in Epidemiology

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Associations between tumor necrosis factor-α and interleukin-6 polymorphisms and unexplained recurrent spontaneous abortion risk

A meta-analysis

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Abstract

To evaluate the associations between Tumor necrosis factor-α (TNF-α)(-238G>A) and Interleukin-6 (IL-6)(-174G>C) polymorphism and risk of unexplained recurrent spontaneous abortion (URSA).

Correlated case-control studies were collected by computer retrieval. A meta-analysis was conducted by Stata 12.0 software to analysis the strength of association between polymorphism of TNF-α -238G>A and IL-6 -174G>C and URSA.

Twenty-one articles with twenty-two studies were included, of which 12 and 10 studies were respectively related to mutation of TNF-α -238G>A, IL-6 -174G>C and URSA. The integrated results showed that the TNF-α-238G>A gene mutation was significantly correlated with the risk of URSA under homozygote model (AA vs GG;OR 1.533,95% CI 1.022–2.301) and recessive model (AA vs GG+AG;OR 1.571,95%CI 1.050–2.350)($P < .05$). There was no association between URSA and TNF-α -238G>A under heterozygote model (AG vs GG;OR 0.963,95% CI 0.816–1.137), dominant model (AA+AG vs GG; OR 1.031,95%CI 0.880–1.209) and additive model (A vs G;OR 1.046,95%CI 0.909–1.203)($P > .05$). The results of subgroup analysis based on ethnicity showed that -238G>A was significantly correlated with the risk of URSA in Asians under all gene models except for heterozygote model (AG vs GG; OR 1.129,95% CI 0.857–1.487) ($P < .05$). In Caucasians, it was dominant model (AA+AG vs GG; OR 1.430,95%CI 1.040–1.965) ($P < .05$) rather than others that showed relationship with URSA. From the integrated results, association was manifested between -174G>C and URSA under all gene models ($P < .05$) except for recessive model (CC vs GG+CG, OR 1.166, 95%CI 0.938–1.449) ($P > .05$), which is identical to subgroup analysis based on ethnicity.

It is of great guiding significance for screening out and preventing URSA among high-risk women to test on TNF-α -238G>A and IL-6 -174G>C under gene models mentioned above which are highly associated with the risk of URSA, which can act as biological markers for URSA.

Abbreviations: HLA = MHC I class human leukocyte antigen, HWE = Hardy-Weinberg equilibrium, IL-6 = Interleukin-6, RSA = Recurrent Spontaneous Abortion, SNP = single nucleotide polymorphism, TNF-α = Tumor necrosis factor-α, URSA = Unexplained Recurrent Spontaneous Abortion.

Keywords: interleukin-6, meta-analysis, polymorphism, tumor necrosis factor-α, unexplained recurrent spontaneous abortion

1. Introduction

Recurrent spontaneous abortion (RSA) is defined as 2 or more times of pregnancy loss in the first 20 weeks of pregnancy which can be clinically detected in.[1] It is a common complication of pregnancy, which accounts for 2% to 4%[2] of the women in childbearing age. In addition to clear etiologies, such as uterine anatomical defects, chromosome aberration, hormone disorders, blood system diseases,[3] there are still 60% causes that remain to be explored,[4] which is mainly associated with immune

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XZ and YJ contributed equally to this work and are co-first authors and contributed equally.

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abnormalities and defined as unexplained recurrent spontaneous abortion (URSA). Successful pregnancy is considered as a semi-allogenic process. Many studies have shown that the balance of Th1/Th2 cells is critical for maternal immune tolerance and pregnancy maintenance. The pro-inflammatory cytokine TNF-α produced by monocytes/ macrophages is often been associated with increased risk for URSA when considering its multifunctional role in lipid metabolism, coagulation, insulin resistance, et al.[5] On the contrary, IL-6, a Th2 cytokine, is of great significance in promoting embryo implantation, down-regulating cell-mediated immune response to maintain pregnancy.[6] The evidence was demonstrated by current clinical observation in which higher serum levels of TNF-α and lower levels of IL-6 were detected in URSA groups.[7,8] As we know, genes are transcribed and translated into proteins that perform vital activities in human body. Mutations in gene sites affect the levels of transcription products and thus their function. At present, there are many studies on the relationship between single nucleotide polymorphism (SNP) and URSA, but few studies definitely illuminate the correlation of SNP in inflammatory factor and patients with URSA. We hypothesized that mutations in the TNF-α and IL-6 are associated with risk of URSA. So we looked extensively at the literature and we find that at present the most common polymorphism that have been investigated are SNP at the promoter region such as -238G/A, -308G/A of TNF-α and -174G>C of IL-6, but the conclusions are contradictory.[9,10] In order to compare different research results more scientifically and objectively, meta-analysis on this issue is coming to be widely carried out. Dong[11] conducted an meta-analysis which only studied-308G/A loci, showing that TNF-α -308G/A polymorphism was not associated with RSA risk. At present, studies on the -238G/A loci are relatively fewer than -308G/A and the results are equally controversial. There are only 4 articles being included in the latest meta -analysis on -238G/A loci,[12] with a set of data being extracted wrongly which does not accord with the original. It will seriously affect the authenticity of the results. When it comes to IL-6, significant associations were found between RSA and IL-6 174G>C genetic polymorphisms in Shi's study but without analysis in different gene models.[13] Therefore, on this basis, we carry out a meta-analysis on association of -238G/A of TNF-α and -174G/C of IL-6 and URSA from all eligible investigations in the latest years involving more extensive countries and regions so as to clarify the relationship between SNPs of these 2 cytokine and URSA, and to provide evidence and research direction for clinical gene screening and gene target therapy.

2. Materials and methods

2.1. Search strategy

Our study followed the Meta-analysis of Observational Studies in Epidemiology guidelines.[14] Studies were searched in the following databases: the China National Knowledge Infrastructure (CNKI),China Wanfang Database, China Weipu Database, Chinese biomedical literature database and PubMed, EMBASE, Wiley, IEEE, PROQUEST, Cochrane library, Web of Science, Science direct for relevant studies published in Chinese or English from the inception to Jun 2018. The following key words were "Tumor necrosis factor-α" or "TNF-α" or "238G>A", "Interleukin-6" or "IL-6","-174G>C", "polymorphism" or "mutation" or "variant", "recurrent miscarriage" or "recurrent abortion" or "recurrent fetal death", "recurrent pregnancy wastage" or "recurrent fetal loss". These keywords are combined according to the retrieval method of each database. Besides, we reviewed the references of the retrieved articles to search for further relevant studies. Furthermore, all magazines were retrieved from the first issue, and the relevant conference literature was tracked. If necessary, contact the correspondent author to obtain information not found by the above retrieval strategy.

2.2. Inclusion and exclusion criteria

Studies that meet the following criteria will be adopted:

(1) The literature must be a case–control study published at home or abroad, with good balance and comparability.
(2) Languages are limited to Chinese or English.
(3) The research should involve gene polymorphism of TNF-α-238G>A or IL-6-174G>C and URSA.
(4) Patients with RSA all experienced at least 2 spontaneous abortion in the first 2 trimesters of pregnancy, and the controls were participants having experienced at least 1 live birth and without the history of abortion.
(5) Each genotype distribution and individual number in the case and control groups should be listed in the literature. Or the number needed can be calculated by the frequency of each genotype given.

Studies with the following characteristics will be excluded:

(1) Not associated with TNF-α-238G>A or IL-6-174G>C polymorphism and URSA;
(2) Not a case–control study;
(3) The case group did not exclude the clinical abortion factors;
(4) The data of genotype frequency and allele frequency in the literature are incomplete or unclear.

2.3. Data extraction and quality evaluation

The 2 researchers (Zhao and Jiang) were responsible for screening and eliminating the studies that did not meet the above-mentioned inclusion criteria. The quality of the included case–control studies was assessed by the Newcastle–Ottawa Scale.[15] It includes 3 aspects: study object selection, group comparability and exposure factor measurement. In brief, 9 points was assigned to each study: 4 for selection, 2 for comparability, and 3 for outcomes. If the final score is greater than 6, it was regarded as high quality. Organize each article that are included in and extract relevant data: The first author's name, years of publication, country and region, genotype frequencies in RSA and control group, Minimum number of abortions, the evidence of Hardy–Weinberg equilibrium (HWE) in controls and Quality score of case-control study were showed in the table (Fig. 1).

2.4. Statement

The ethical approval was not necessary. Because this study is about associations between tumor necrosis factor-α and Interleukin-6 polymorphisms and URSA Risk: A meta-analysis. This paper is not a clinical trial study, ethical approval and informed consent are not required. All included articles have passed ethical approval and informed consent.

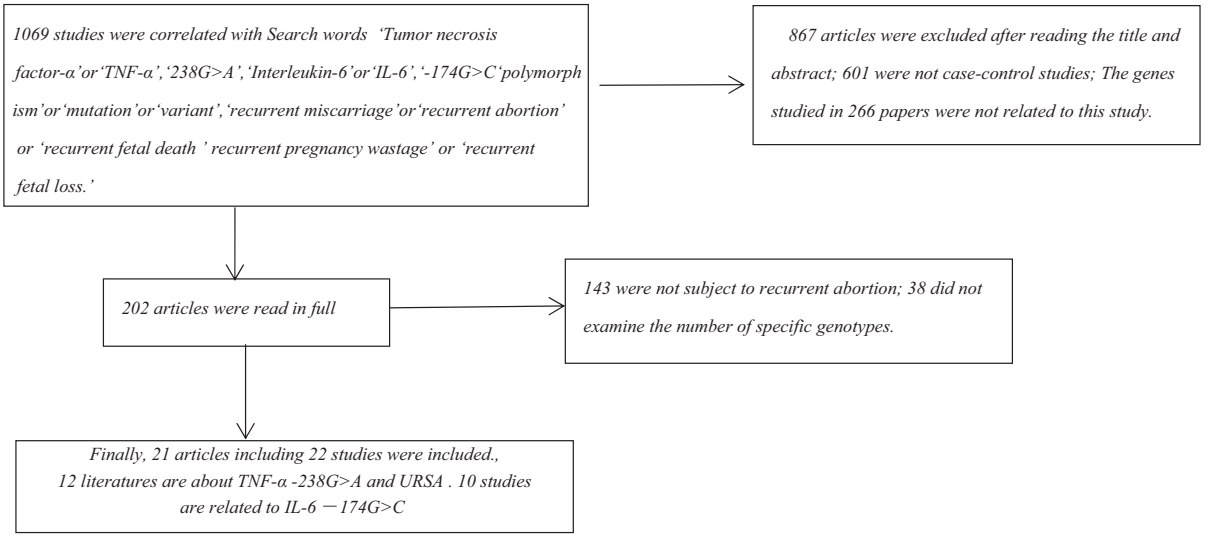


Figure 1. Article screening flowchart.

2.5. Statistical analysis

All the data were analyzed using Stata 12.0 software and the charts related were drawn below. Based on the odds ratio (OR) with a corresponding 95% confidence interval (CI), we calculated the pooled odds which were used to analyze the effect on the association. While crossing these studies, Q test and I^2 were used to test the heterogeneity of the included literature firstly. It suggested that there was heterogeneity between the studies when $I^2 > 50\%$, and the random effect model was used, or if not, the fixed effect model was used instead. Subgroup analysis was carried out when there is a need to find the potential source of heterogeneity. In order to evaluate the stability of the combined results, a sensitivity analysis was conducted for the meta-analysis results after each removal of a case-control study. The Begg funnel plot was used to assess potential publication bias.

3. Results

3.1. Characteristics of the included studies

Overall, a total of 21 out of 1069 articles were selected for the final meta-analysis.[16–36] Among the included articles,12 articles[16–27] demonstrated the relationship between TNF-α-238G>A and URSA with 2713 cases and 2793 controls. Ten studies[24,28–36] reported the association between IL-6-174G>C gene mutation and URSA with 2287 cases and 3506 controls. The baseline characteristics of the studies related to mutation of TNF-α(238G>A) IL-6 (174G>C) are respectively shown in Tables 1 and 2. All of the 21 articles were published before Jun 2018. In addition, 19 manuscripts were published in English, and 2 manuscripts were published in Chinese.

3.2. Results of the overall meta-analysis

3.2.1. Meta-analysis of TNF-α 238G>A polymorphism and URSA risk. Twelve articles were related to 238G>A and the risk of URSA. The results showed that the polymorphism of TNF-α-238G>A gene was significantly correlated with the risk of URSA under homozygote model (AA vs GG;OR 1.533,95% CI 1.022–2.301) and recessive model (AA vs GG+AG;OR 1.571,95%CI 1.050–2.350) ($P < .05$). There was no association between URSA and 238G>A under heterozygote model (AG vs GG;OR 0.963,95%CI 0.816–1.137), dominant model (AA+AG vs GG; OR1.031,95%CI 0.880–1.209) and additive model (A vs G;OR1.046,95%CI 0.909–1.203) ($P > .05$). (Table 3)

3.2.2. Heterogeneity test and Subgroup analysis. I^2 of all models were greater than 50% except the recessive model, indicating that the included studies show heterogeneity. Therefore, we conducted sensitivity analysis and found that the article of Ma[26] and Gupta[21] contributed a lot to the heterogeneity (Sensitivity analysis was shown in Fig. 2). As a result, we excluded the 2 papers and conducted a subgroup analysis on the basis of racial classification. Among the remaining researches, 8 were Asians,[18,19,21–24,26,27] 4 were Caucasians,[16,17,20,25] TNF-α -238G>A was significantly correlated with the risk of URSA in Asians under all gene models except heterozygote model (AG vs GG; OR 1.129,95% CI 0.857–1.487) ($P < .05$). In Caucasians, it was dominant model (AA+AG vs GG; OR 1.430,95%CI 1.040–1.965) ($P < .05$) rather than others that had relationship with URSA (Table 4).

3.2.3. Meta-analysis of IL-6 -174G>C polymorphism and URSA risk. Ten articles[24,28–36] were related to -174G>C and the susceptibility of URSA. Correlation was showed between -174G>C and URSA under homozygote model (CC vs GG; OR1.268,95%CI 1.008–1.596), heterozygote model (CG vs GG; OR 0.640,95%CI 0.570–0.718),dominant gene model (CC+CG vs GG;OR 1.237,95%CI 1.094–1.398), and additive gene model in Asians (T vs C) ($P < 0.05$), except for recessive gene model (CC vs GG+CG,OR 1.166,95%CI 0.938–1.449) ($P > .05$). (Table 5)

3.2.4. Sensitivity analyses and subgroup analysis. I^2 of all models were greater than 50% except for the recessive model, indicating that the included studies had heterogeneity, of which Ma,[29] paper contributed a lot to the heterogeneity of the paper through sensitivity analysis (Fig. 3). So, we excluded this paper and conducted a subgroup analysis on the basis of racial classification. Among all the researches, 5[24,29,30,33,36] were Asians, 4[31,33,34,35] were Caucasians, and the results were showed Table 6.

Table 1

Characteristics of studies on the association between TNF-α 238G>A gene polymorphisms of URSA risk.

The first author	Publication date	Country/ city	Total of cases					Total of controls					Definition of IRM	HWE inspection	Quality score
			AA	GA	GG	G	A	AA	GA	GG	G	A			
Baxter et al[16]	2001	UK	760	3	73	149	3	1380	12	126	264	12	≥3	0.86	8
Zammiti et al[17]	2009	Tunisia	37220	88	264	616	128	2747	52	215	482	66	≥3	<0.05	8
Finan et al[18]	2010	Bahrain	2044	52	148	348	60	2480	48	200	448	48	≥3	0.82	9
Liu et al[19]	2010	China	1320	4	128	260	4	1520	17	135	287	17	≥2	0.86	7
Palmirotta et al[20]	2010	Italy	1000	16	84	184	16	1000	6	94	194	6	≥2	0.38	8
Gupta[21]	2012	Indian	20016	63	121	305	95	30033	113	154	421	179	≥3	0.07	6
Alkhuriji et al[22]	2013	Saudi	653	7	55	117	13	650	8	57	122	8	≥3	<0.05	7
Lee1 et al[23]	2013	Korea	3571	26	330	686	28	2360	8	228	464	8	2	0.53	8
Liu et al[24]	2015	China	28414	30	240	510	58	2840	35	249	533	53	3	<0.05	7
Piosik et al[25]	2013	Denmark.	480	1	47	95	1	910	4	87	178	4	3	0.94	8
Jianting[26]	2017	China	7752	41	732	1527	45	8053	57	745	1647	63	3	0.09	6
Rahmani et al[27]	2017	Iran	1000	4	96	196	4	1001	2	97	196	4	3	0.84	8

The results showed that the polymorphism of IL-6 -174G>C was significantly correlated with the risk of URSA under all models ($P<.05$) except for recessive model in Asians and Caucasians ($P>.05$).

3.2.5. Publication bias. We analyzed the Publication bias of articles on the relationship between -238G>A, -174G>C and URSA risk. The gene funnel plot analysis of the two groups showed asymmetry indicating the possibility of publication bias (Figs. 4 and 5).

4. Conclusion

An increasing number of genetic association researches are conducted to explore the genetic background of URSA.[37] And many scholars have focused on single nucleotide polymorphism

Table 2

Characteristics of studies on the association between IL-6 174G>C gene polymorphisms of URSA risk.

The first author	Publication date	Country/ city	Total of cases					Total of controls					Mini no. of RPL	HWE inspection	Quality score
			CC	CG	GG	C	G	CC	CG	GG	C	G			
Liu et al[24]	2015	China	28418	95	171	131	437	28410	63	211	83	485	3	0.22	9
Jianting et al[28]	2017	China	77543	248	484	334	1216	80551	291	463	393	1217	3	0.41	6
Zhanet al[29]	2016	China	2280	7	221	7	449	2130	6	207	6	420	3	0.14	7
Chen et al[30]	2016	China	600	1	59	1	119	600	3	57	3	117	3	0.95	7
Dina et al[31]	2016	Egypt	14210	47	85	67	217	1424	33	105	41	243	3	0.33	8
Bohiltea et al[32]	2014	Romania	690	9	60	9	129	640	8	56	8	120	3	0.56	8
Parveen et al[33]	2013	Indian	20013	67	120	93	307	30010	67	223	87	513	3	0.39	8
Demirturk et al[34]	2014	Turkey	1135	36	72	46	144	1132	11	100	15	211	3	0.85	7
Drozdzik et al[35]	2013	Germany	15731	81	45	143	171	15828	69	61	125	191	3	0.62	7
Stonek et al[36]	2008	Austria	25942	122	95	206	312	1367212	618	537	1042	1692	3	0.79	9

Table 3

Meta-analysis of TNF-α 238G>A polymorphism and URSA risk.

	I^2	Model	OR	95%CL		*P*	z
AA vs GG	59.9%	FEM	1.533	1.022	2.301	<.05	2.06
AG vs GG	64.8%	FEM	0.963	0.816	1.137	.660	0.44
AA+AG vs GG	69.6%	FEM	1.031	0.880	1.209	.702	0.38
AA vs GG+AG	55.0%	FEM	1.571	1.050	2.350	<.05	2.20
A vs G	71.2%	FEM	1.046	0.909	1.203	.528	0.63

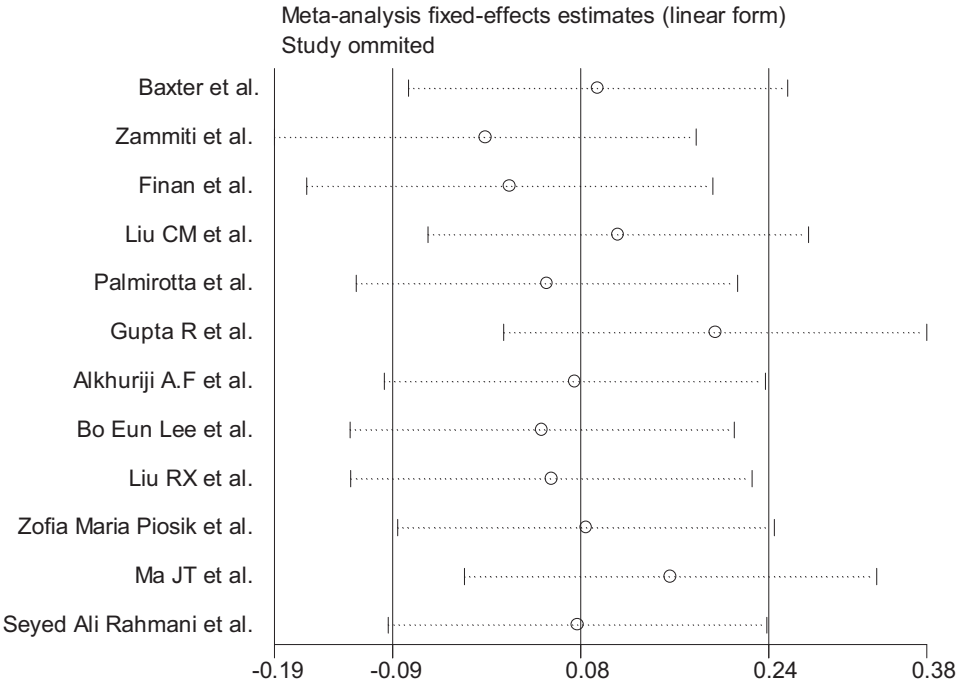


Figure 2. Sensitivity analyses for TNF-α -238G>A and URSA.

Table 4

Table of subgroup analysis results after Ma JT Gupta R was eliminated.

	I^2	Model	OR	95%CL		*P*	z
AA vs GG in Asians	22.6%	FEM	7.155	2.342	21.853	<.05	3.45
AA vs GG in Caucasians	0.0%	FEM	2.327	0.966	5.606	.06	1.88
AG vs GG in Asians	60.2%	REM	1.129	0.857	1.487	.389	0.86
AG vs GG in Caucasians	52.9%	REM	1.343	0.963	1.873	.083	1.74
AA+AG vs GG in Asians	55.2%	REM	1.321	1.013	1.724	<.05	2.05
AA+AG vs GG in Caucasians	53.6%	REM	1.430	1.040	1.965	<.05	2.20
AA vs GG+AG in Asians	23.3%	FEM	7.054	2.314	21.503	<.05	3.44
AA vs GG+AG in Caucasians	0.0%	FEM	2.167	0.903	5.200	.083	1.73
A vs G in Asians	59.5%	REM	1.295	1.021	1.643	<.05	2.13
A vs G in Caucasians	51.6%	REM	1.458	1.096	1.941	.102	2.59

Table 5

Meta-analysis of IL-6 -174G>C polymorphism and URSA risk.

	I^2	Model	OR	95%CL		*P*	z
CC vs GG	52.4%	REM	1.268	1.008	1.596	<.05	2.03
CG vs GG	94.8%	REM	0.640	0.570	0.718	<.05	7.60
CC+CG vs GG	80.4%	REM	1.237	1.094	1.398	<.05	3.40
CC vs GG+CG	23.0%	FEM	1.166	0.938	1.449	.167	1.38
C vs G	82.7%	REM	1.187	1.077	1.308	<.05	3.46

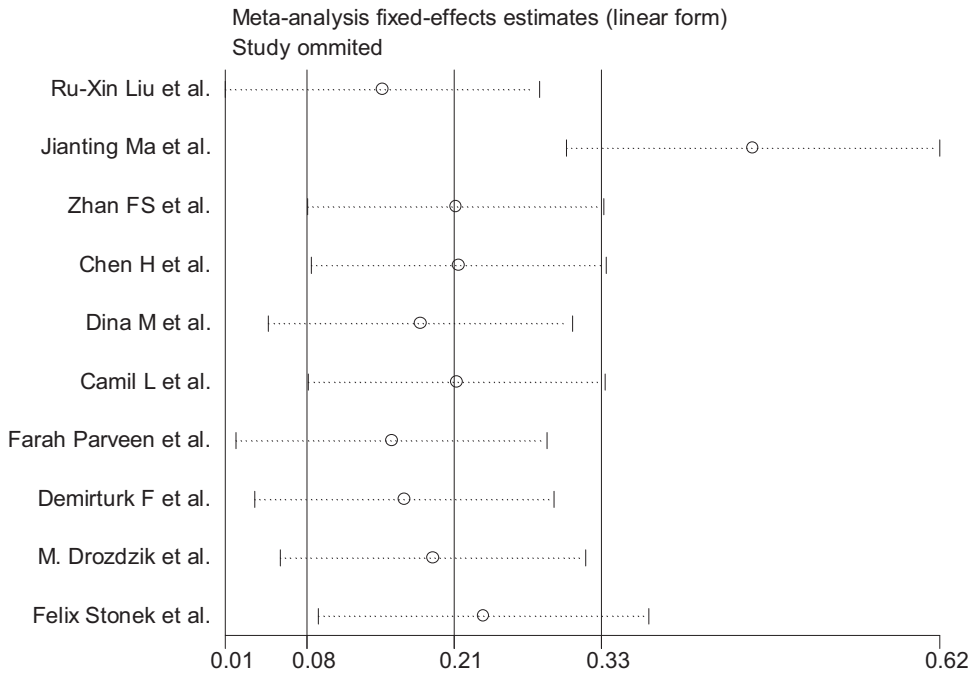


Figure 3. Sensitivity analyses for IL-6 -174G>C and URSA.

of Th1/Th2 cytokines as their important roles in regulating the maternal immune balance during pregnancy. Among them, the relationship between gene promoter polymorphism of TNF-α, IL-6 and URSA have been extensively detected. Considering the shortcomings of current studies that I mentioned at the beginning, we decided to conduct a meta-analysis on -238G>A locus of TNF-α and -174G/C locus of IL-6. Gene of TNF-α is located within the human leukocyte antigen class III region in chromosome 6p21.3 consisting of 4 exons and 3 introns, close to the gene of MHC I class human leukocyte antigen (HLA) -27B.[38] The -238 site in TNF-α promoter was polymorphic characterized as different alleles, including G/G, G/A and A/A. Several studies have illustrated the correlation between -238G>A polymorphism and URSA but presented contradictory results. Our meta-analysis indicated that the polymorphism of TNF-α -238G>A gene was significantly correlated with the risk of URSA under homozygote model (AA vs GG;OR 1.533,95% CI 1.022–2.301) and recessive model (AA vs GG+AG;OR 1.571,95%CI 1.050–2.350)($P<.05$). There was no association between URSA and -238G>A under heterozygote model (AG vs GG; OR 0.963,95% CI 0.816–1.137), dominant model (AA+AG vs GG; OR1.031,95%CI 0.880–1.209) and additive model (A vs G;OR 1.046,95%CI 0.909–1.203). ($P>.05$). Considering the heterogeneity, sensitivity analysis and subgroup analysis based on ethnicity were conducted and the results showed that TNF-α -238G/A was significantly correlated with the risk of URSA in Asians under all gene models except heterozygote model (AG vs GG; OR 1.129,95% CI 0.857–1.487) ($P<.05$). While in Caucasians, it was dominant model (AA+AG vs GG;OR 1.430,95%CI 1.040–1.965) ($P<.05$) rather than others that had relationship with URSA. Human IL-6 gene is located on chromosome 7p15–21, consisting of 4 introns and 5 exons.[39] -174G/C is a familiar mutation site that transforms from guanine (G) to cytosine (C)at nucleotide position 174. In the previous study in vitro, the mutation of -174G/C could affect the IL-6 transcription. However, whether it could affect the serum level of IL-6 and the outcome of pregnancy was still not conclusive. Our meta-analysis confirmed the association between -174G/C gene and URSA under all gene models ($P<.05$) except for recessive gene model ($P<.05$) no matter in the overall results or in the

Table 6

Table of subgroup analysis results after Ma JT was eliminated.

	I^2	Model	OR	95%CL		*P*	z
CC vs GG in Asians	50.0%	FEM	1.413	1.024	1.949	<.05	2.10
CC vs GG in Caucasians	0.0%	FEM	1.919	1.133	3.251	<.05	2.43
CG vs GG in Asians	96.4%	REM	0.425	0.362	0.499	<.05	10.46
CG vs GG in Caucasians	47.8%	FEM	1.518	1.126	2.046	<.05	2.74
CC+CG vs GG in Asians	59.2%	REM	1.451	1.205	1.748	<.05	3.92
CC+CG vs GG in Caucasians	60.0%	REM	1.980	1.480	2.648	<.05	4.60
CC vs GG+CG in Asians	34.1%	FEM	1.254	0.929	1.694	.139	1.48
CC vs GG+CG in Caucasians	3.9%	FEM	1.435	0.887	2.321	.141	1.47
C vs G in Asians	68.5%	REM	1.316	1.141	1.517	<.05	3.77
C vs G in Caucasians	77.9%	REM	1.695	1.353	2.123	<.05	4.59

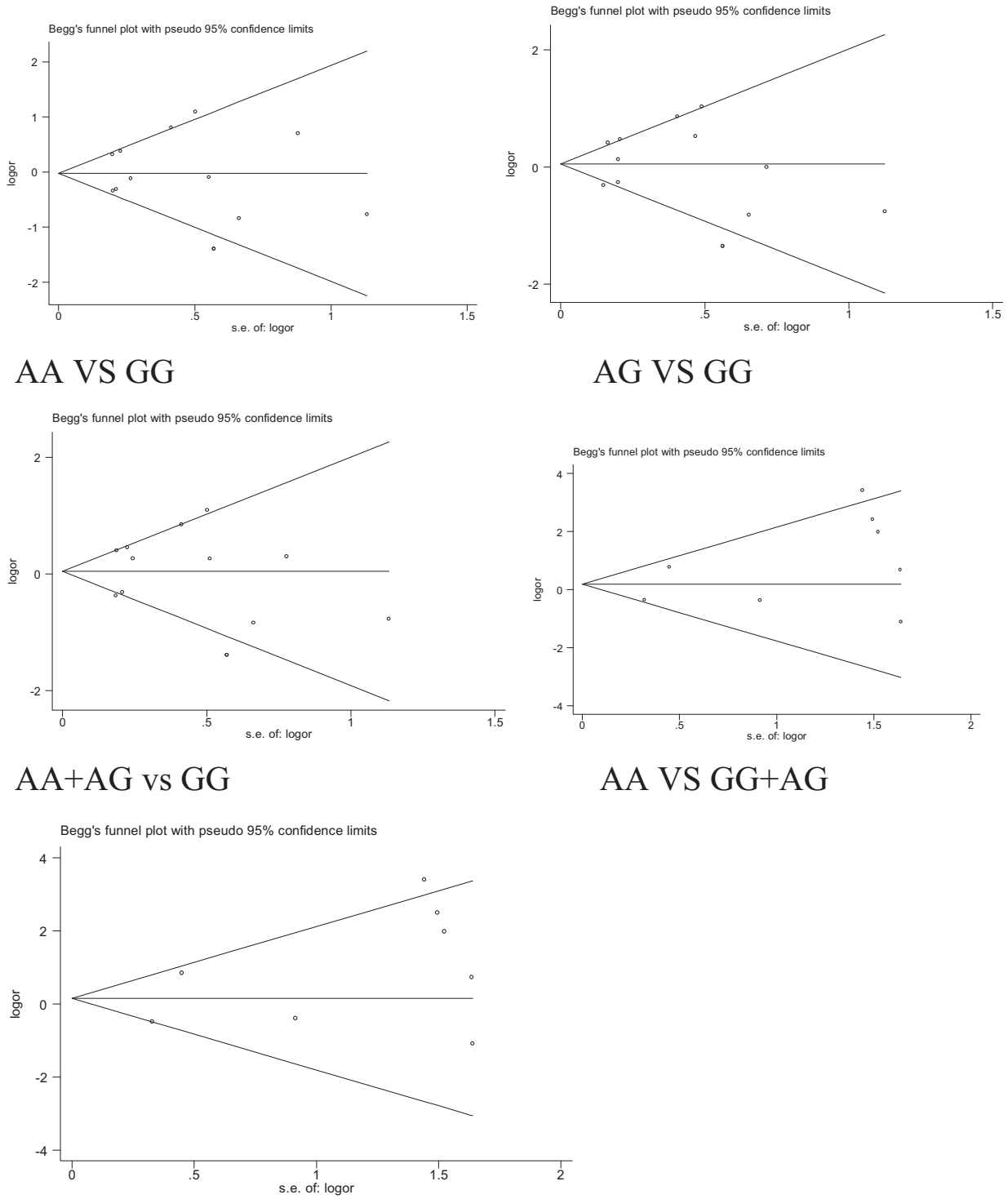
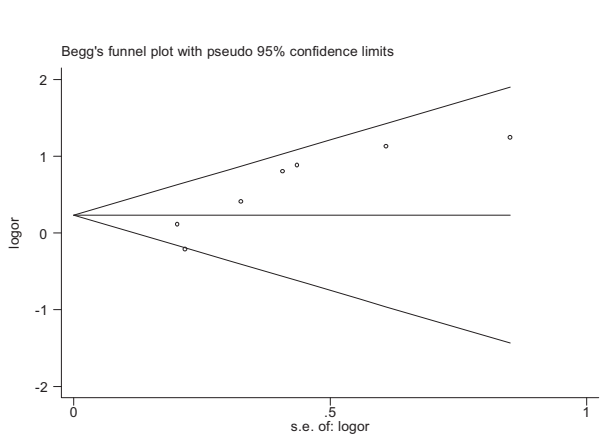


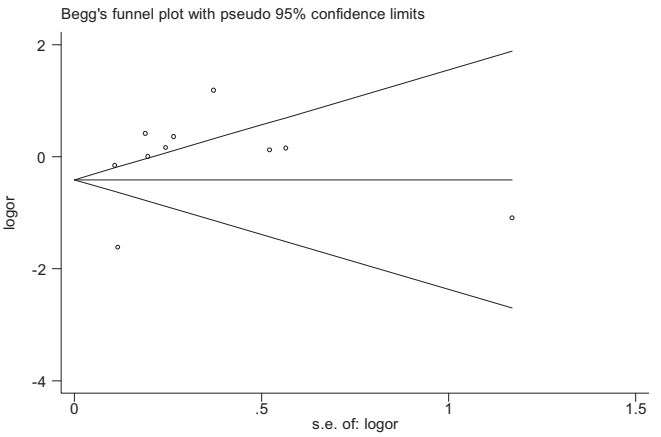
Figure 4. the publication bias of articles on the relationship between TNF-α -238G>A and URSA risk was shown in the funnel figure.

subgroup analysis, of which was inconsistent with Lee's research.[40] This may be due to the fact that sample sizes of many studies included in Lee's meta-analysis were relatively small.

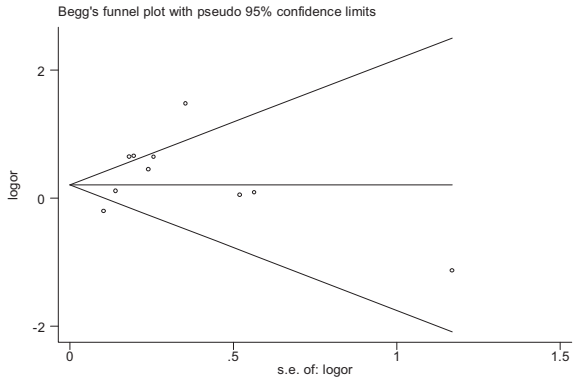
So far, our paper has included the most extensive studies, along with the most comprehensive genetic grouping and the most in-depth analysis when comparing with previous meta-analyses. This may help to draw more scientific and conclusive conclusions. Our research reveals the relationship between gene mutation and onset of URSA, which has a profound impact on future treatment direction. In the future, medical researchers may focus on research that can inhibit site mutations that can lead to various



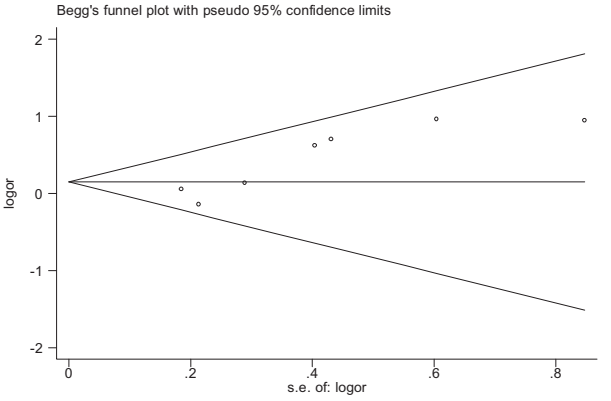
CC VS GG



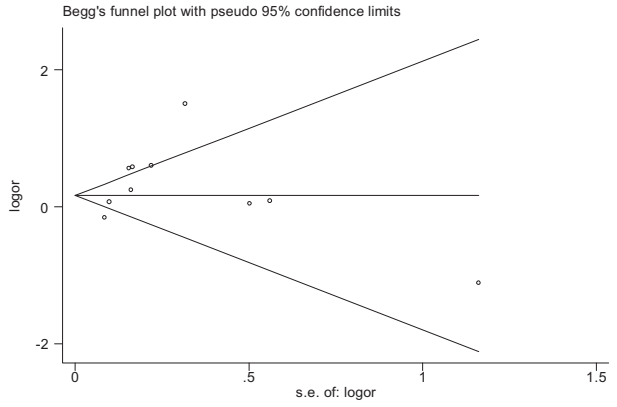
CG VS GG



CC+CG VS GG



CC VS GG+CG



C VS G

Figure 5. the publication bias of articles on the relationship between IL-6 -174G>C and URSA risk was shown in the funnel figure.

disease. At present, literature has shown that traditional Chinese medicine can inhibit harmful gene mutations in some diseases so as to treat diseases,[41,42] but there is still a lack of such research in the field of RSA, which may become the research direction of our team in the future.

Although heterogeneity is very common in genetic association meta-analyses, but we cannot ignore it. This may be arisen from the differences in ethnicity, source of control, HWE, or the times of abortion. Besides substantial heterogeneity, another limitation in our meta-analysis was the asymmetry in the funnel plots which

suggests that the number of eligible studies included in total is also not enough. Therefore, more relevant case-control studies are required to be conducted and then included in the meta-analysis so as to get a more scientific result.

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