

# Association between gene polymorphism of folate metabolism and recurrent spontaneous abortion in Asia

## A Meta-analysis

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### Abstract

To evaluate the association between gene polymorphisms of MTHFR (C677T, A1298C) and MTRR (A66G), and the recurrent spontaneous abortion (RSA) risk in Asia.

Related case-control studies were collected, selected, and screened. A meta-analysis was conducted by Stata 12.0 software to assess the association between polymorphisms of target genes and RSA.

Altogether 30 studies examining the relationship between genetic polymorphism of folate metabolism and RSA risk were included, among which 20 studies were related to MTHFR C677T, 11 to MTHFR A1298C and 6 to MTRR A66G. The studies suggested that MTHFR C677T polymorphism was closely connected with RSA risk under all models ( $P < .05$ ). Furthermore according to the subgroup analysis of ethnicity, the correlation between C677T polymorphism and RSA was stronger in north of China when compared with south of China and other Asian countries ( $P > .05$ ). For MTHFR A1298C, it was closely related to RSA risk in all gene models except for (AC vs AA) ( $P < .05$ ). However, when it comes to MTRR A66G, there was no significant correlation between gene A66G polymorphism and RSA risk except for the additive gene model (G vs A) ( $P < .05$ ).

The present evidence shows that the correlation between gene polymorphisms and RSA risk can be found in MTHFR C677T, A1298C (except for heterozygote model) and MTRR A66G (only in additive genotypes), and the detection of the correlated gene polymorphisms mentioned above is of certain guiding significance for preventing RSA and screening high-risk groups.

**Abbreviations:** Ala = alanine, Hcy = homocysteinemia, MTHFR = methylenetetrahydrofolate reductase, MTRR = methioninesynthase reductase, RSA = recurrent spontaneous abortion, SNPs = single nucleotide polymorphisms, URSA = unexplained recurrent spontaneous abortion, Val = valine.

**Keywords:** methylenetetrahydrofolate reductase (MTHFR), methioninesynthase reductase (MTRR), polymorphism genetic, abortion, meta-analysis

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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## 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.<sup>[1]</sup> It is a common complication of pregnancy, which accounts for 5% of the women in childbearing age.<sup>[2]</sup> In addition to clear etiology such as uterine anatomical defects, chromosome aberration, hormone disorders, blood system diseases<sup>[3]</sup> et al, there are still 60% causes remaining to be explored<sup>[4]</sup> which is defined as unexplained recurrent spontaneous abortion (URSA). It is believed that hyperhomocysteinemia caused by abnormal metabolism of folic acid is one of the independent risk factors for many pregnancy-related disorders,<sup>[5,6]</sup> because of its facilitation to embolization in placenta arteries,<sup>[7]</sup> which can further affect embryo's blood supply and result in villi necrosis, lapoptosis of placental trophoblast, thus leading to the lower secretion of maternal gonadotropin,<sup>[8]</sup> which finally results in the recurrent embryo loss.

Crucially, MTHFR and MTRR are key enzymes in folate/homocysteine pathway.<sup>[9]</sup> Some single nucleotide polymorphisms (SNPs) in genes involved in the folate metabolic pathway have been considered to be closely related to a low level of folate and a high level of homocysteine such as polymorphisms of MTHFR and MTRR,<sup>[10]</sup> which is also the most concerned by scholars in

home and abroad. Over the last 10 years, a number of molecular epidemiological studies have been conducted worldwide to study the correlation between MTHFR or MTRR polymorphisms and RSA risks, but the results remain inconsistent.<sup>[11,12]</sup>

The inconsistency of results may be due to differences in race, regional, eating habits, as well as the fact that the sample size is too small to objectively reflect the relevance. In order to compare the different results of the researches more scientifically and objectively. Meta-analysis on this issue being widely carried out also draws conflicting conclusion.<sup>[13,14]</sup> Moreover the articles included in the latest meta-analysis by previous researchers were 8 years ago, of which the research scope only focused on 1 or 2 gene loci in the area of China.<sup>[15]</sup> Therefore, based on this situation, we carry out a meta-analysis including the genotype data from all eligible investigations in the latest years involving as extensive countries and regions in Asia as possible, as well as covering 3 gene loci (C677T and A1298C of MTHFR and A66G of MTRR) so as to provide a more accurate evaluation of the connection between folate metabolism polymorphisms and RSA susceptibility.

## 2. Materials and methods

### 2.1. Search strategy

This research was carried in adherence to the Meta-analysis of Observational Studies in Epidemiology guidelines<sup>[16]</sup> and we searched the following academic databases: China National Knowledge Infrastructure (CNKI), China Wanfang Database, PubMed, EMBASE, Cochrane library, Web of Science and Wiley for relevant studies since the establishment of the library to November of 2019. The following search words were combined according to different retrieval methods of each database: “methylenetetrahydrofolate reductase”, “MTHF”, “C677T”, “A1298C”, “methionine synthase reductase”, “MTRR” or “A66G”, “Unexplained recurrent spontaneous abortion” or “recurrent pregnancy loss”. In addition, we screened the bibliography of the included studies for any additional relevant study. Furthermore all magazines were retrieved from the first issue, and the relevant conference literature was tracked.

### 2.2. Statement

The ethical approval is not necessary, as this study is about the association between gene polymorphism of folate metabolism and RSA: a meta-analysis. It is not a clinical trial study, thus ethical approval and informed consent are not required. All the articles included in this meta-analysis have passed ethical approval and informed consent.

### 2.3. Inclusion and exclusion criteria

Studies that meet the following criteria will be adopted:

1. The literature must be a case-control study published with good balance and comparability.
2. Languages are limited to Chinese or English.
3. The research should involve RSA risk and gene polymorphisms about MTHFR C677T, A1298C or MTRR A66G loci.
4. Patients with RSA should have experienced 2 or more times abortion in the first trimester that have ruled out definite etiologies and the control group should have at least 1 successful pregnancy.

5. Distribution each genotype and individual number in the case and control groups should be listed in the literature.

Studies with the following characteristics will be excluded:

1. Those are not associated with MTHFR C677T A1298C, MTRR A66G polymorphism and URSA;
2. Those are not case-control studies;
3. Those that case groups did not exclude the clinical abortion factors;
4. Frequency of genotype or allele in the literature are incomplete or unclear.

### 2.4. Data extraction and quality evaluation

The selection procedure was independently replicated by 2 reviewers (X-X.Z and Y-L.P) to avoid biasing. Firstly, the title and summary of the studies were viewed. Then they read the full text to eliminate studies that did not accord with the above-mentioned inclusion criteria. We attempted to contact the corresponding author to obtain important information that was missing. If that failed, the literature would be deleted. We used the nine - star Newcastle - Ottawa scale to evaluate the quality of the studies.<sup>[17]</sup> It included 3 aspects: study object selection, group comparability, and exposure factor measurement. In brief, a maximum of 9 points was assigned to each study: 4 for selection, 2 for comparability, and 3 for outcomes. A final score above 6 was regarded as high quality. They extracted relevant data from each article: The first authors name, years of publication, country and region, genotype frequencies in the observation group, and control group, the number of miscarriages defining RSA, Hardy-Weinberg equilibrium and Quality score of case-control study were showed in the table (Fig. 1).

### 2.5. Statistical analysis

All the data were analyzed by Stata 12.0 software and the flow charts were drawn below. Based on the odds ratio (OR) with a corresponding 95% confidence interval (CI), we counted the pooled odds to analyze the effect on the association. While crossing these studies, Q test and  $I^2$  were first used to test the heterogeneity of the included literatures. When  $I^2 > 50\%$ , it proved that there was heterogeneity between the studies, and the random effect model was used, and if not, the fixed effect model was applied instead. In order to search for the sources of heterogeneity, we mainly conducted subgroup analysis according to different countries and regions. 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 utilized as a criterion for evaluating publication bias.

## 3. Results

### 3.1. Characteristics of the included studies

Overall, a total of 30 out of 1207 articles were selected for the final meta-analysis.<sup>[18-47]</sup> Among the included articles, 20 studies described the relationship between polymorphisms of MTHFR C677T and RSA with 2413 cases and 2923 controls involving 10 countries. Eleven articles<sup>[18,21,22,27,30,38-43]</sup> demonstrated the association between MTHFR A1298C and RSA with 1889 cases and 1947 controls involving 5 countries. Six literatures described the relationship between A66G and URSA with 918 cases and

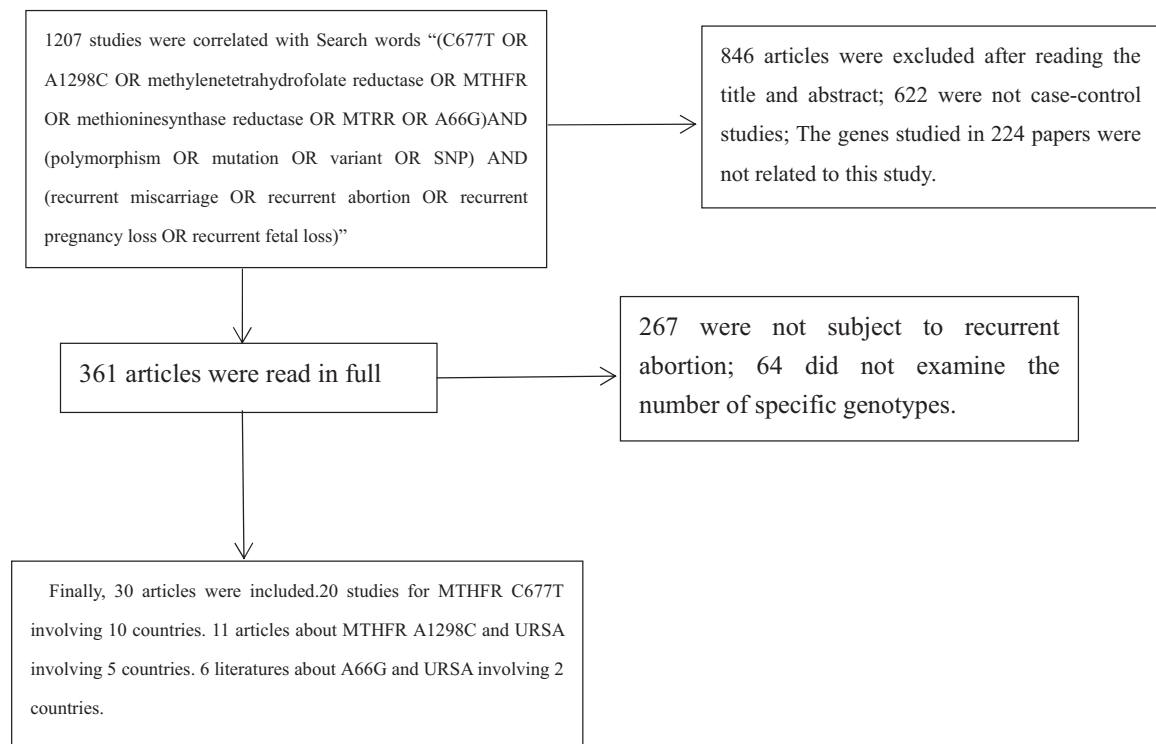


Figure 1. Article screening flowchart.

942 controls involving 2 countries. The baseline characteristics of the participants by MTHFR C677T, A1298C, and MTRR A66G were respectively shown in Tables 1–3. All of the 30 articles were published before June 2019. In addition, 13 manuscripts were published in English, and 17 manuscripts were in Chinese, as shown in Tables 1–3.

#### 4. Results of the overall meta-analysis

##### 4.1. Meta-analysis of MTHFR C677T polymorphism and URSA risk

Twenty articles were related to C677T and RSA risk. The results showed that the polymorphisms of MTHFR C677T were significantly correlated with RSA under dominant gene model (TT+CT vs CC; OR 1.781, 95% CI 1.574–2.014), recessive gene model (TT vs CC+CT; OR 2.155, 95% CI 1.798–2.583), heterozygote gene model (CT vs CC; OR 1.600, 95% CI 1.401–1.826), homozygote gene model (TT vs CC; OR 2.813, 95% CI 2.300–3.442) and additive gene model (T vs C; OR 1.686, 95% CI 1.539–1.848) (Table 4).

#### 5. Sub-group analysis

##### 5.1. Subgroup analysis of (TT vs CC) genotypes in China and other countries

As shown in Table 2, compared with other genetic models, odds ratio in homozygote gene model (TT vs CC) (OR = 2.81) was the highest, which means that population with homozygous genotype TT were more likely to suffer from RSA. We made an in-depth analysis of the results. Since 11 of the 20 articles were from China and 9 were from other countries, we conducted subgroup analysis of (TT vs CC) genotypes according to countries. The pooled OR

was 3.420 in China [95% CI = (2.579–4.536)], and 2.273 in other countries [95% CI = (1.700–3.039)]. It can be concluded that population in China contributed more to the correlation between TT genotype and RSA (Fig. 2).

Of the 11 literatures from China, 6 were carried out in the south and 5 were in the north. Subgroup analysis was conducted on (TT vs CC) genotype in terms of RSA susceptibility according to the population from south or north of China. The pooled OR was 4.580 [95% CI (2.896–7.244)] and 2.852 [95% CI = (1.990–4.088)] respectively in north and south of China. It was obvious that the population in the north of China contributed more to high probability of TT genotype and RSA (Fig. 3).

##### 5.2. Test for heterogeneity

The results of the meta-analysis presented in the above table showed that  $I^2$  was <50%, indicating that the included studies had clinical significance and statistical homogeneity.

##### 5.3. Publication bias evaluation

In the evaluation process, we found that there were various differences in subjects included in the literatures such as countries, regions, races, and miscarriage times, etc. Two groups of gene funnel plot analysis showed asymmetry indicating the possibility of publication bias (Fig. 4).

##### 5.4. Meta-analysis of MTHFR A1298C polymorphism and URSA risk

Eleven articles were associated with A1298C and the chance of RSA. The results showed that the polymorphism of MTHFR A1298C was closely related to RSA under dominant model (CC +

**Table 1****Characteristics of studies on the association between C677T gene polymorphisms of MTHFR and URSA risk.**

The first author	Publicati-on date	Country/city	Total of cases					Total of controls					Mini NO. of RPL	HWE inspec-tion	Quality score
			TT	CT	CC	T	C	TT	CT	CC	T	C			
Al-Achkar et al <sup>[18]</sup>	2017	Syria	100					106					2	0.064	9
Farahmand et al <sup>[19]</sup>	2016	Iran	18	41	41	77	123	1	39	66	41	171	2	0.000	7
			330					350							
Gao <sup>[20]</sup>	2016	Taiyuan. China	36	114	180	186	474	35	85	230	155	545	2	0.629	8
			71					80							
Hu et al <sup>[21]</sup>	2014	Shenzhen, China	8	27	36	22	49	1	20	59	13	67	2	0.473	9
			52					16							
Huang et al <sup>[22]</sup>	2017	Shenzhen, China	9	14	29	32	72	1	4	11	6	26	2	0.292	7
			83					90							
Dissanayake et al <sup>[23]</sup>	2012	Sri Lanka	25	39	19	89	77	12	48	30	72	108	2	0.581	7
			200					171							
Jeddi-Tehrani et al <sup>[24]</sup>	2011	Iran	3	39	158	311	355	2	27	142	31	45	2	0.009	8
			100					100							
Kaur et al <sup>[25]</sup>	2013	India	15	42	43	157	128	9	25	66	43	72	2	0.000	8
			107					593							
Li et al <sup>[26]</sup>	2017	Yanan, China	5	16	86	1035	188	21	109	463	151	26	2	0.000	9
			50					50							
Luo et al <sup>[27]</sup>	2014	Hangzhou, China	18	25	7	62	38	15	13	22	45	55	2	0.177	8
			125					135							
Ma et al <sup>[28]</sup>	2008	Taiyuan, China	15	70	40	100	150	10	65	60	85	185	2	0.162	7
			60					60							
Mao et al <sup>[29]</sup>	2015	Wenzhou, China	16	32	12	64	56	7	34	19	48	72	2	0.001	8
			100					100							
Parveen et al <sup>[30]</sup>	2013	India	31	41	28	52	48	26	29	45	38	62	2	0.381	8
			200					300							
Settin et al <sup>[31]</sup>	2011	Egypt	20	70	110	482	290	14	90	196	118	110	2	0.000	7
			70					136							
Shi et al <sup>[32]</sup>	2017	Yantai, China	4	26	40	202	106	1	68	67	70	34	2	0.205	9
			69					169							
Tang et al <sup>[33]</sup>	2016	Guiyang, China	26	35	8	45	24	31	92	46	76	93	2	0.042	7
			100					50							
Torabi et al <sup>[24]</sup>	2012	Iran	25	37	38	87	113	9	16	25	34	66	2	0.009	9
			100					100							
Unfried et al <sup>[35]</sup>	2002	Austria	15	42	43	157	128	9	25	66	43	72	2	0.711	8
			133					74							
Wang et al <sup>[36]</sup>	2011	Shandong, China	23	46	64	92	174	4	24	46	32	116	2	0.009	8
			159					127							
Zhan et al. <sup>[37]</sup>	2017	Anhui, China	59	82	18	200	118	21	78	28	120	134	2	0.072	8
			120					98							
			41	48	31	130	110	11	32	55	54	142			

**Table 2****Characteristics of studies on the association between A1298C gene polymorphisms of MTHFR and URSA risk.**

The first author	Public-ation date	Country/city	Cases					Controls					No. of RPL	HWE	Quality score
			AA	AC	CC	A	C	AA	AC	CC	A	C			
Al-Achkar et al <sup>[18]</sup>	2017	Syria	101					106					2	0.053	9
Cao <sup>[38]</sup>	2014	Suzhou, China	53	40	8	126	56	65	40	1	170	42	2	0.462	7
			82					166							
Gao et al <sup>[39]</sup>	2015	Zhengzhou, China	49	31	2	129	35	132	31	3	295	37	2	0.078	7
			378					423							
Hu et al <sup>[21]</sup>	2014	Shenzhen, China	180	118	80	478	278	210	165	48	585	261	2	0.248	8
			52					16							
Huang et al <sup>[22]</sup>	2017	Shenzhen, China	33	12	7	78	26	12	3	1	27	5	2	0.605	8
			83					90							
Kyu Hwang et al <sup>[40]</sup>	2017	Korea	52	28	3	132	34	64	23	3	151	29	2	0.672	9
			302					315							
Zhu <sup>[41]</sup>	2015	Zhengzhou, China	209	86	7	504	100	210	93	12	513	117	2	0.017	8
			118					174							
Luo et al <sup>[27]</sup>	2014	Hangzhou, China	48	58	12	154	82	76	88	10	240	108	2	0.068	8
			125					135							
Parveen et al <sup>[30]</sup>	2013	India	82	40	3	204	46	78	54	3	210	60	2	0.133	9
			200					300							
Xie et al <sup>[42]</sup>	2016	Tianjin, China	88	92	20	268	132	157	127	16	441	159	2	0.253	7
			244					116							
Yousefian et al <sup>[43]</sup>	2014	Iran	165	74	5	404	84	82	29	5	193	39	2	0.317	8
			204					116							
			98	81	25	277	131	68	39	9	175	57			

**Table 3**

**Characteristics of studies on the association between A66G gene polymorphisms of MTRR and URSA risk.**

The first author	Publication date	Country/city	Cases					Controls					No. of RPL	HWE	Quality score	
			AA	AC	CC	A	C	AA	AC	CC	A	C				
Cao <sup>[38]</sup>	2014	Suzhou China	82											2	0.067	7
Chen et al <sup>[44]</sup>	2013	Hainan China	43	28	11	114	50	98	53	15	249	83		2	0.119	7
			59	24	7	80	38	64	19	4	147	27				
Kim et al <sup>[45]</sup>	2013	Korea	353					226						2	0.437	7
			182	146	25	510	196	108	100	18	316	136				
Luo et al <sup>[27]</sup>	2014	Hangzhou China	125					135						2	0.068	8
			22	70	33	114	136	26	78	31	130	140				
Wang et al <sup>[46]</sup>	2009	Nanjing China	50					56						2	0.003	8
			18	28	4	64	36	18	36	2	72	40				
Wang Juan <sup>[47]</sup>	2016	Chong qing China	190					272						2	0.445	7
			86	78	26	258	150	134	110	28	378	166				

AC vs AA; OR 1.189, 95%CI 1.042–1.358), recessive model (CC vs AA + AC; OR 1.706, 95%CI 1.323–2.200), heterozygote model (AC vs AA; OR 1.106, 95% CI 0.962–1.273) homozygote model (CC vs AA; OR 1.710, 95%CI 1.314–2.225) and additive model (C vs A; OR 1.193, 95%CI 1.072–1.328). And there was no significant association between (AC vs AA; OR 1.106, 95%CI 0.962–1.273,  $P = .157$ ). The result was shown in Table 5.

**5.5. Test for heterogeneity**

The results of the meta-analysis presented in the above table showed that  $I^2$  was <50%, indicating that the included studies had clinical significance and statistical homogeneity.

**5.6. Publication bias**

In the same situation as C677T, we analyzed the publication bias of articles on the relationship between A1298C and RSA risk. The 2 groups of gene funnel plot analysis showed asymmetry, indicating the possibility of publication bias (Fig. 5).

**5.7. Meta-analysis of A66G polymorphism and URSA risk**

Six articles were associated with A1298C and RSA. In all genotypes of MTRR A66G polymorphism, it was the additive gene model (G vs A; OR 1.204, 95%CI 1.044–1.390) rather than the dominant (GG + AG vs AA), recessive (GG vs AA + AG; OR 1.290, 95%CI 0.956–1.740), heterozygote (AG vs AA; OR 1.075, 95%CI 0.874–1.322), homozygote gene model (GG

vs AA; OR 1.343, 95%CI 0.966–1.867) that had association with RSA risk. The result was shown in Table 6.

**5.8. Test for heterogeneity**

In the heterogeneity test for the A66G genotypes of each gene model,  $I^2$  of (GG + AG vs AA) and (G vs A) were greater than 50%, indicating that the included studies had heterogeneity. Subgroup analysis was necessary for heterogeneous sources.

**5.9. Subgroup analysis**

Of the 6 articles included, 5 were from China and 1 was from Korea. Therefore, the subgroup analysis of (GG + AG vs AA) and (G vs A) were conducted according to the source of the countries. The meta-analysis of 5 Chinese literature showed that  $I^2$  of (GG + AG vs AA) and (G vs A) groups were both lesser than 50%, indicating that different countries exerted an impact on heterogeneity. And the subgroup analysis showed that the MTRR A66G gene mutation was dramatically connected with RSA risk under the dominant group (GG + AG vs AA)[OR = 1.301, 95%CI = (1.022–1.657)] and additive group (G vs A) [OR = 1.373, 95%CI = (1.157–1.630)]. The results was shown in Table 7, Figures 6 and 7.

**5.10. Publication bias**

We also analyzed the publication bias of articles on the relationship between A66G and URSA risk. The gene funnel plot analysis of the 2 groups showed asymmetry indicating the possibility of publication bias (Fig. 8).

**Table 4**

**Meta-analysis of MTHFR C677T polymorphism and URSA risk.**

	$I^2$	Model	OR 95% CI	P	z
TT vs CC	36.2%	FEM	2.813 2.300 3.442	<.05	10.05
CT vs CC	40.6%	FEM	1.600 1.401 1.826	<.05	6.96
TT + CT vs CC	48.7%	FEM	1.781 1.574 2.014	<.05	9.19
TT vs CC + CT	34.1%	FEM	2.155 1.798 2.583	<.05	8.32
T vs C	48.1%	FEM	1.686 1.539 1.848	<.05	11.20



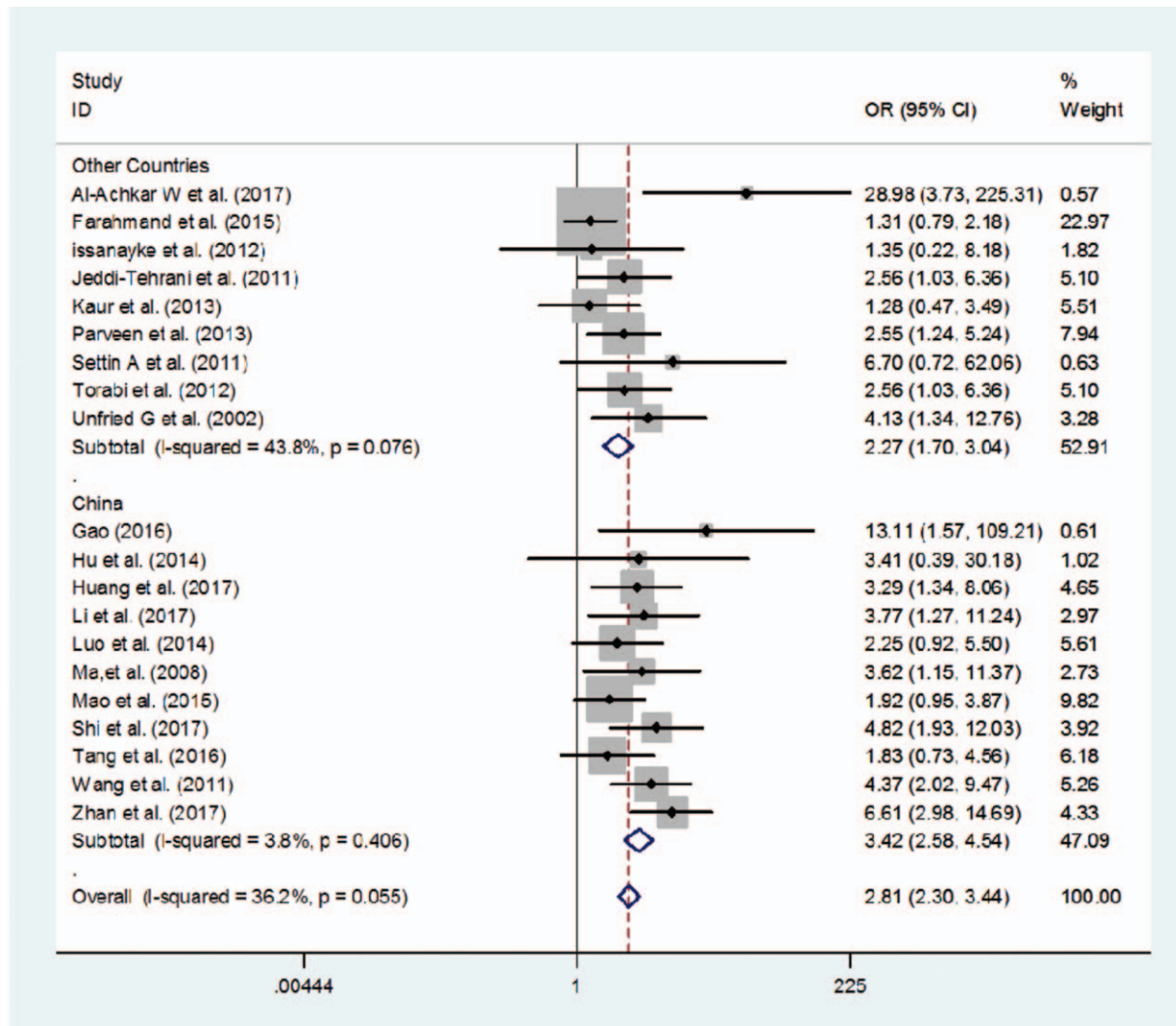


Figure 2. Subgroup analysis of (TT vs CC) genotypes in China and other countries.

## 6. Discussion

At present, disorders of coagulation mechanism in the pathogenesis of RSA have attracted extensive attention in the academic world,<sup>[48]</sup> which can be classified into 2 categories: acquired and hereditary coagulation disorders, and the latter is closely related to gene polymorphisms.<sup>[49]</sup> MTHFR and MTRR are the 2 vital enzymes contributing to the folate metabolism.<sup>[50]</sup> Abnormal activities of these enzymes can lead to hyperhomocysteinemia, which is one of the important reasons for hypercoagulable state, and act as an independent risk for various arteriovenous thromboembolic diseases, including pregnancy loss.<sup>[51]</sup> Thrombus in placenta can limit blood supply to the embryo and cause villi necrosis, which is a momentous component of all RSA etiologies.<sup>[52]</sup> Homocystein(Hcy), catalyzed by methionine synthetase, accompanied with vitamin B12 to receive methyl from 5-methyl tetrahydrofolic acid so as to produce methionine. MTHFR catalyzes the formation of 5, 10 methylenetetrahydrofolate, which can be transformed to 5-methyl tetrahydrofolate.<sup>[53]</sup> Moreover, vitamin B12 needs MTRR to catalyze its methylation to maintain the activity of co-enzyme so that the

catalytic reaction of methionine synthetase is normally carried out.<sup>[54]</sup> MTHFR and MTRR genes show the characteristics of polymorphism, among which the single nucleotide polymorphisms of genes C677T, A1298C in MTHFR, and A66G in MTRR are the most studied. The MTHFR gene is located in chromosome 1q36. 3. At present, there are 15 types of point mutations in human MTHFR, and the most important one is the mutation of 677 sitting on the fifth exon called C677T mutation. The allele "C" is mutated to "T", making the original encoding alanine (Ala) mutate to valine (Val), which can affect the enzymes heat sensitivity.<sup>[55]</sup> A1298C is another important mutation site located in the eighth exon. After mutation, the encoding amino acid is changed from Glu to Ala, leading to the decrease of enzyme activity.<sup>[56]</sup> MTRR can catalyze the conversion of cysteine to methionine. When the "A" lying on the 66th site of MTRR mutates to "G", the amino acid it encodes will transform from methionine to isoleucine, resulting in the decrease of enzyme activity in MTRR.

Chen<sup>[22]</sup> published a meta-analysis in 2016, discussing the relationship between MTHFR mutation and RSA in China, and drew the conclusion that MTHFR C677T gene mutations were

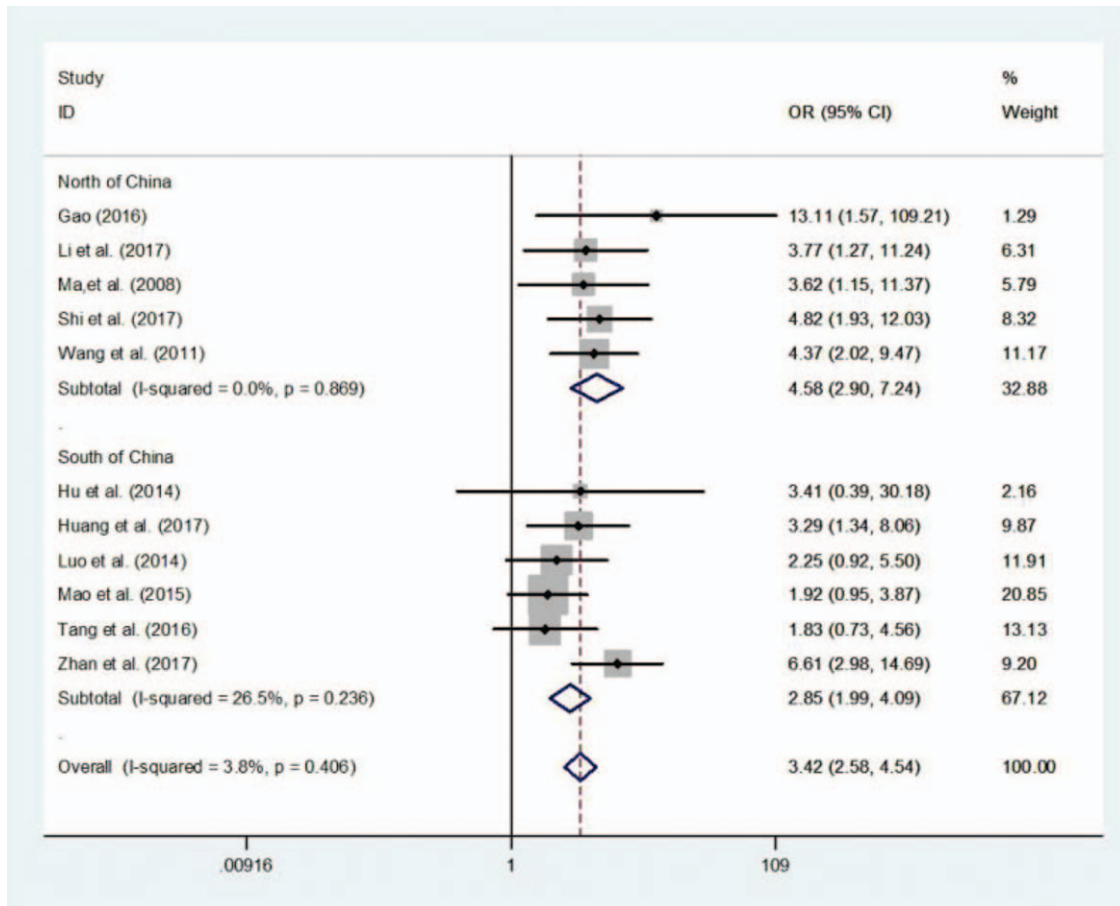


Figure 3. Subgroup analysis of TT vs CC genotype in north and south China.

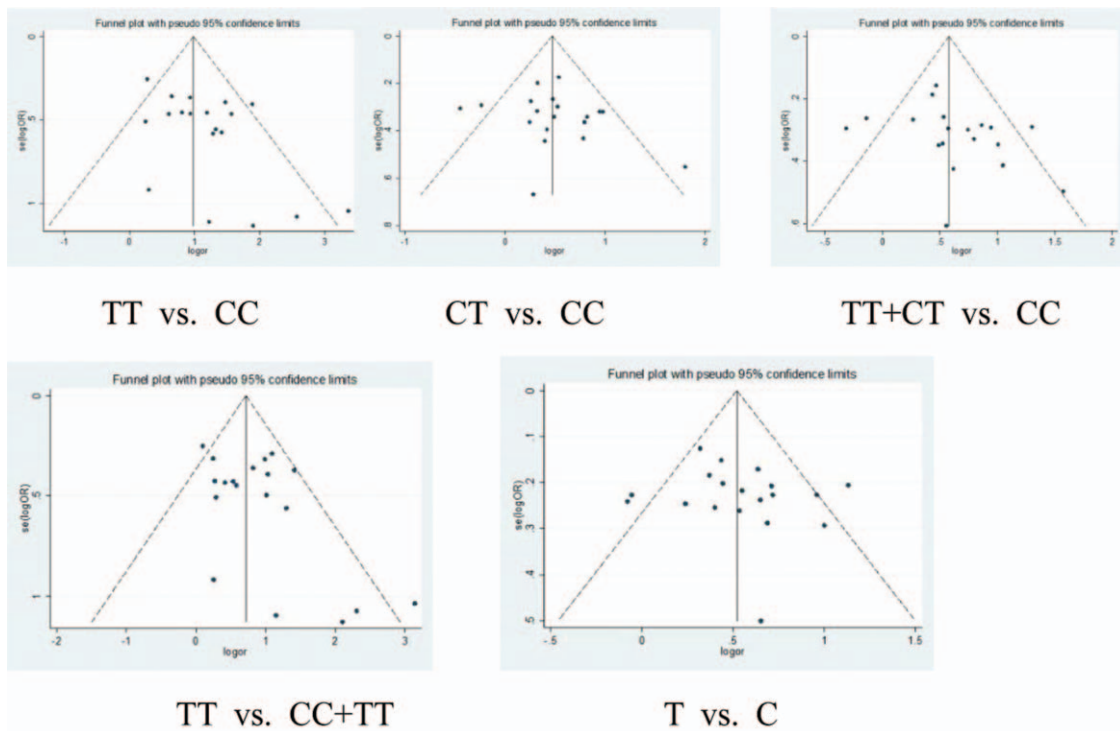


Figure 4. The publication bias of articles on the relationship between C677T and URSA risk was shown in the funnel figures.

**Table 5**  
**Results of MTHFR A1298C polymorphism and URSA risk.**

	$I^2$	Model	OR 95% CI	P	z
CC vs AA	23.0%	FEM	1.710 1.314 2.225	<.05	3.99
AC vs AA	47.0%	FEM	1.106 0.962 1.273	.157	1.42
CC+AC vs. AA	42.5%	FEM	1.189 1.042 1.358	<.05	2.56
CC vs AA+AC	24.6%	FEM	1.706 1.323 2.200	<.05	4.12
C vs A	31.4%	FEM	1.193 1.072 1.328	<.05	3.23

**Table 6**  
**Results of MTRR A66G polymorphism and URSA risk.**

	$I^2$	Model	OR 95% CI	P	z
GG vs AA	6.8%	FEM	1.343 0.966 1.867	.079	1.76
AG vs AA	44.1%	FEM	1.075 0.874 1.322	.492	0.69
GG+AG vs AA	55.8%	REM	1.128 0.928 1.372	.226	1.21
GG vs AA+AG	0.0%	FEM	1.290 0.956 1.740	.095	1.67
G vs A	67.0%	REM	1.204 1.044 1.390	.011	2.54

connected with the onset of RSA in China, while there was no significant correlation between A1298C mutation and RSA susceptibility. However, in this meta-analysis, only 5 studies discussed about A1298C, thus the result were inevitably showed

limitation to some extent, and there was no detailed subgroup analysis of the different regions between the north and south. Moreover 11 out of 21 articles were published before 2010. In recent years, relevant researches have emerged endlessly. Based

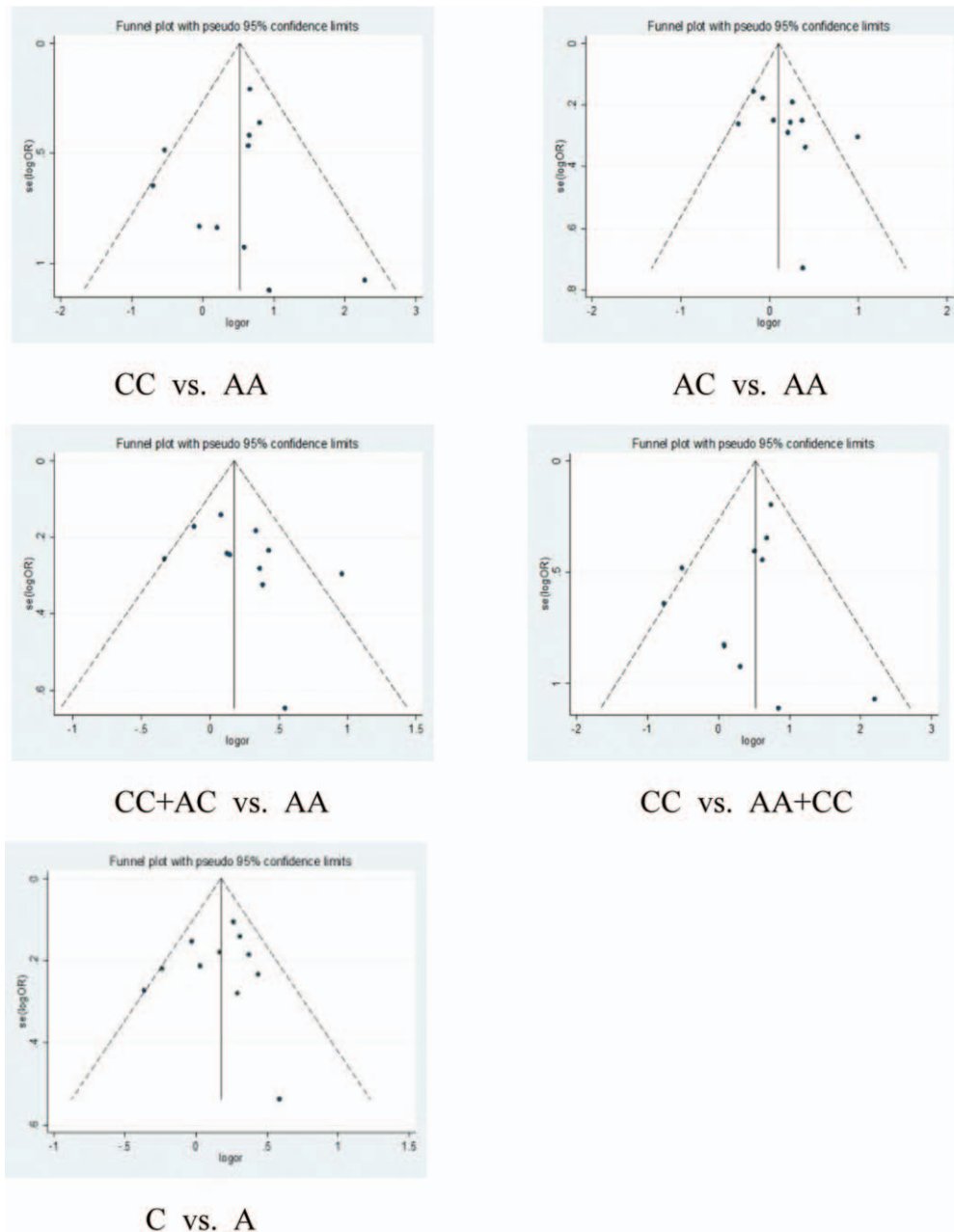


Figure 5. The publication bias of articles on the relationship between A1298C and URSA risk was shown in the Funnel figure.



**Table 7**  
**Results of MTRR A66G mutation and URSA risk in subgroup analysis.**

	<i>I</i> <sup>2</sup>	Model	OR 95% CI	<i>P</i>
GG+AG vs AA	46.6%	FEM	1.301 1.022 1.657	.045
G vs A	48.8%	FEM	1.373 1.157 1.630	.001

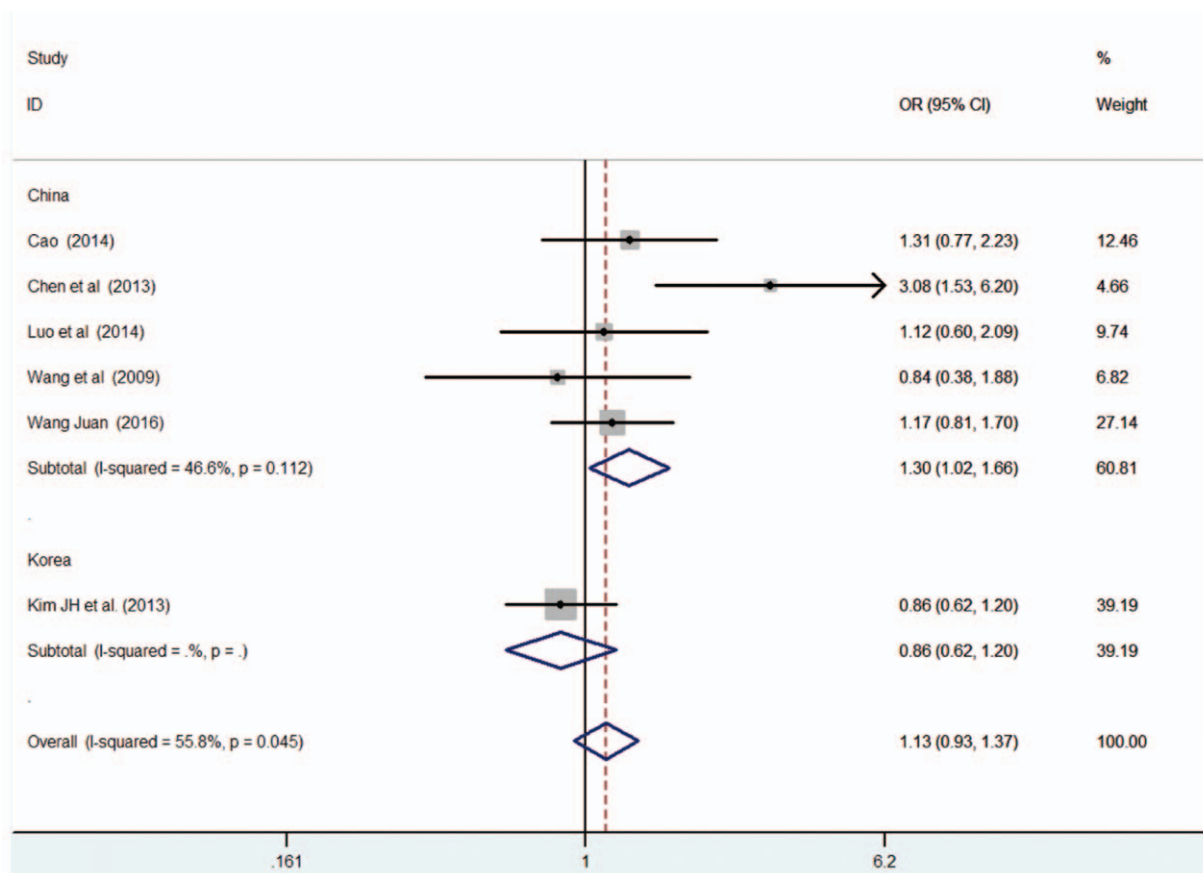
on this, it is necessary to carry out a new research to collect more updated achievements, involving more different provinces in China and more countries in Asia.

In our current meta-analysis, a total of 30 studies were accepted to review the relationship between MTHFR, MTRR gene polymorphism and RSA risk, among which 20 were related to the C677T polymorphism, 11 to A1298C polymorphism and 6 studies to polymorphism of A66G. The results displayed that the mutation of MTHFR C677T was associated with RSA risk in Asian population under dominant group (TT+CT vs CC; OR 1.781, 95% CI 1.574–2.014), recessive group(TT vs CC+CT; OR 2.155, 95%CI 1.798–2.583), heterozygote group(CT vs CC; OR 1.600, 95% CI 1.401–1.826), homozygote group(TT vs CC; OR 2.813, 95% CI 2.300–3.442), and additive group (T vs C; OR 1.686, 95%CI 1.539–1.848), especially in the model of (TT vs CC) (OR=2. 81). Stratified analysis obviously reduces the heterogeneities and the results showed a higher risk of RSA in Chinese [OR=3.420, 95%CI=(2.579–4.536)], indicating that population in China contributed more to the correlation between

genotype of TT and RSA. And we further conducted subgroup analysis on (TT vs CC) and RSA risk according to the population from south or north of China. The pooled OR was respectively 4.580[95%CI (2.896–7.244)] and 2.852[95%CI=(1.990–4.088)] in north and south. The result further showed that the existence of TT genotypes was closely related to the susceptibility to RSA in northern China.

For A1298C, 11 articles were associated with A1298C and RSA risk. The results demonstrated that the polymorphism of MTHFR A1298C was dramatically connected with RSA risk under the dominant model (CC+AC vs AA; OR 1.189, 95% CI 1.042–1.358), recessive model (CC vs AA+AC; OR1. 706, 95% CI 1.323–2.200), heterozygote model(AC vs AA; OR1.106, 95% CI 0.962–1.273) homozygote model (CC vs AA; OR 1.710, 95% CI 1.314–2.225), and additive model (C vs A;OR1.193, 95%CI 1.072–1.328). And there was no significant association between (AC vs AA; OR1.106, 95% CI 0.962–1.273, *P*=.157) and RSA risk.

When it comes to A66G, 6 articles are associated with A1298C and RSA risk. In all genotypes of MTRR A66G polymorphism, it was the additive model (G vs A; OR 1. 204, 95%CI 1.044–1.390) that showed association with the risk of RSA, rather than the dominant (GG+AG vs AA), recessive (GG vs AA+AG; OR 1.290, 95%CI 0.956–1.740), heterozygote (AG vs AA; OR1.075, 95%CI 0.874–1.322) and homozygote gene models (GG vs AA; OR1.343, 95%CI 0.966–1.867). Of the 6 articles included, 5 were from China and 1 was from Korea. Therefore, the subgroup analysis was conducted. After the exclusion of



**Figure 6.** Results of MTRR A66G mutation and URSA risk in subgroup analysis.

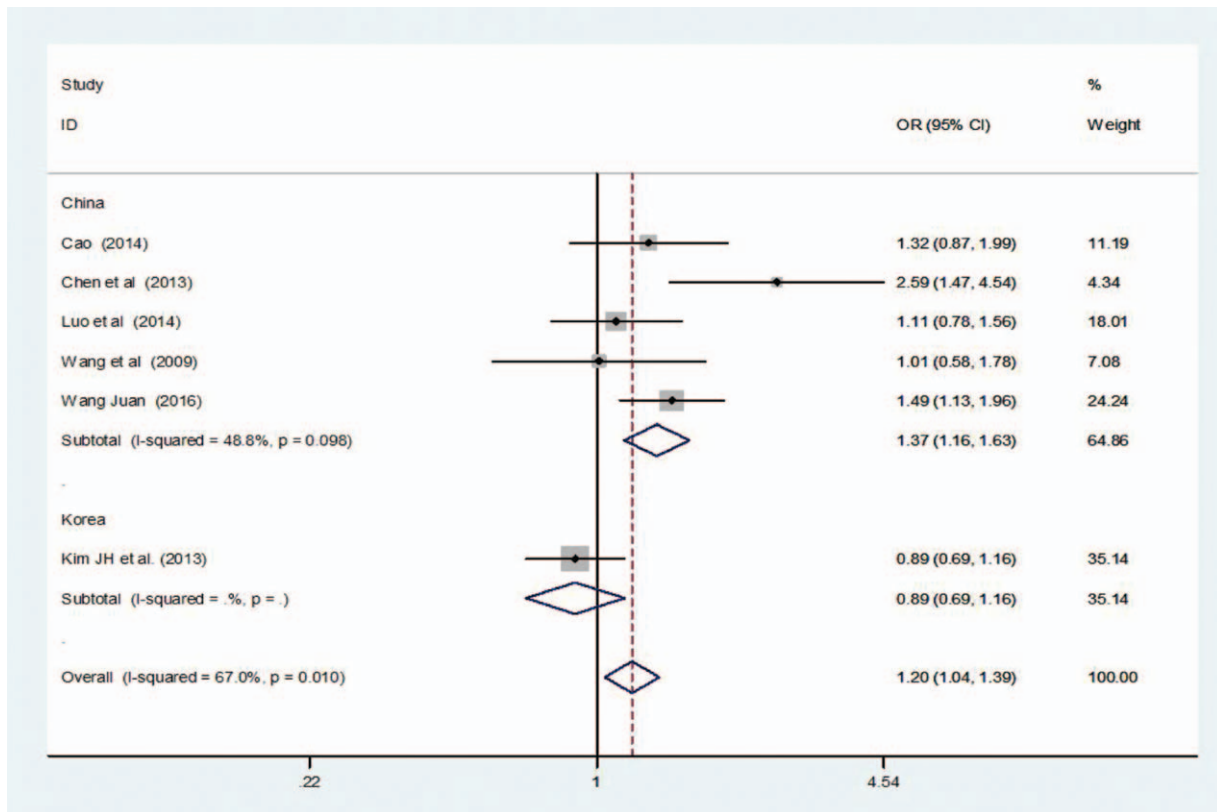


Figure 7. Results of MTRR A66G mutation and URSA risk in subgroup analysis.

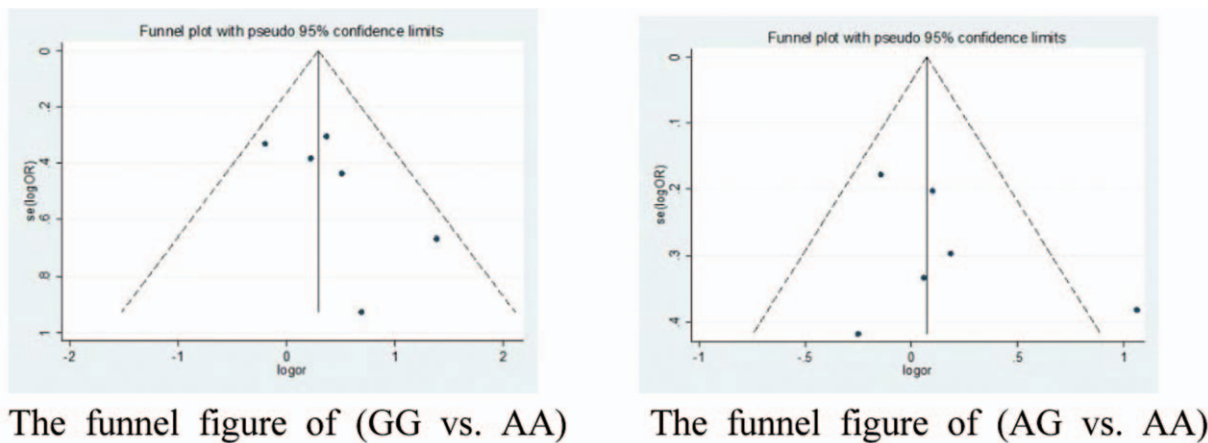


Figure 8. The publication bias of articles on the relationship between A1298C and URSA risk was shown in the funnel figure.

South Korea, the meta-analysis showed that  $I^2$  of (GG+AG vs AA) and (G vs A) gene groups were both lesser than 50%, indicating that different countries contribute to heterogeneity. And the subgroup analysis displayed that the MTRR A66G gene mutation was significantly associated with RSA risk in China under dominant group (GG+AG vs AA)[OR=1.301, 95%CI=(1.022–1.657)], and additive group (G vs A) [OR=1.373, 95% CI=(1.157–1.630)].

The present evidence showed that identification of MTHF C677T and A1298C mutation was of certain practical significance for preventing RSA and screening high-risk groups, especially MTHFR C677T mutation in north of China when compared with other regions and countries in Asia. This may be due to the difference in living environment and diet between north and south China. Furthermore, we found that the risk of RSA was significantly increased in women carrying the mutant

TT or TC of C677T and CC of A1298C. Except for the gene model (G vs A), the genetic A66G polymorphism was not associated with the risk of RSA. Large-scale clinical studies were needed to verify the conclusions in terms of the quantity and quality of the study.

### Author contributions

Xiaoxuan Zhao was responsible for designing and writing this article; Yunlu Ping was in charge of study selection and information extraction; Yang Zhao was responsible for meta-analysis; Lu Chen took charge of reviewing and revising the article; Xiaoling Feng was responsible for the supervision and submission work.

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