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Gene polymorphism associated with endothelial nitric oxide synthase (4VNTR, G894T, C786T) and unexplained recurrent spontaneous abortion risk

A meta-analysis

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Abstract

To evaluate the association between endothelial nitric oxide synthase gene polymorphisms (4VNTR A/B, G894T, C786T) and risk of URSA.

Related case-control studies were collected by computers. A meta-analysis was conducted using Stata 12.0 software to assess the strength of association.

Altogether 37 articles were examining the relationship between endothelial nitric oxide synthase gene polymorphisms and URSA, among which sixteen (16) studies were related to 4VNTR, twelve (12) to G894T, and nine (9) to C786T, the study suggested that 4VNTR A/B polymorphism was closely connected with URSA risk under all gene models except for recessive model (AA vs. BB+AB). The integrated result which indicated the association between G894T gene mutation and URSA risk had been shown under homozygote (TT vs. GG; OR 1.585, 95%CI 1.175–2.138) and recessive models (TT vs. TG+GG; OR 1.530, 95%CI 1.142–2.052). Considering heterogeneity in the remaining gene models, subgroup analysis was performed on ethnicity, and the results showed that it was the dominant (TT+TG vs. GG; OR 1.585, 95%CI 1.175–2.138) and additive models (T vs. G; OR 1.727, 95%CI 1.372–2.175) of G894T in Asians and the heterozygote model (TG vs. GG; OR 1.015, 95%CI 0.846–1.217) in Caucasians that were associated with URSA ($P < .05$). Besides C786T gene was significantly connected with URSA under all models except for additive model (T vs. C).

It is of great guiding significance for screening out and preventing URSA among high-risk women via testing on 4VNTR A/B, G894T, C786T eNOS under gene models mentioned above which are closely associated with URSA.

Abbreviations: eNOS = endothelial nitric oxide synthase, NO = nitric oxide, RSA = recurrent spontaneous abortion, SNPs = single nucleotide polymorphisms, URSA = unexplained recurrent spontaneous abortion, VNTR = variable number of tandem repeats.

Keywords: endothelial nitric oxide synthase, meta-analysis, polymorphism, unexplained recurrent spontaneous abortion

1. Introduction

Recurrent spontaneous abortion (RSA) is a specific medical condition, defined as the occurrence of two or more consecutive pregnancy losses.[1] It is a multifactorial disease with several etiologic factors. In addition to the genetic abnormality (gene mutation and abnormal karyotype of embryo), endocrine disorders, abnormal anatomy of the uterus, infectious or immune factors, and chemical factors from Danni et al,[2] 40%–55% pregnant women suffering from RSA are induced by unclear etiologies named unexplained recurrent spontaneous abortion (URSA).[3] At present, the relationship between endothelial nitric oxide (NO) and URSA has attracted more and more attention from scholars in various countries.[4] NO is released during the conversion of L-arginine to L-citrulline under the catalysis of an enzyme family called NOS.[5] There are three different forms of NO enzymes, which play diverse physiological functions in disparate tissues: neuronal (nNOS), inducible (iNOS), and endothelial (eNOS).[6] eNOS is the most important member in this family when considering its role in regulating the reproductive system during normal pregnancy.[7] Therefore, genetic polymorphism of eNOS, which could be explained in part to alter protein expression and/or bioactivity and then directly affects the level of NO,[8] has become a hot topic among scholars. Moreover, the polymorphisms of three loci being mostly

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investigated are the promoter region single-nucleotide polymorphism (SNP) (C786T, rs2070744), the variable number of tandem repeats (VNTR) in intron 4 4b/4a variant (rs61722009) and G894T within the exon 7 (rs1799983). It has been showed that the three gene loci polymorphisms of eNOS gene tend to alter the expression and activity of eNOS enzyme, and then increased the risk of URSA. But the results are inconsistent.[9,10] This may be due to racial and regional differences as well as the fact that the sample size is too small to objectively reflect the relevance between eNOS gene polymorphism and URSA. In order to compare different research results more scientifically and objectively. Meta-analysis on this issue being widely carried out also drew conflicting results,[11,12] and the reference citation of the latest meta-analysis they utilised were 5 years ago, of which the research scope only focused on two gene loci involving a very small number of countries.[13] Therefore, based on this basis, we carry out a meta-analysis including the genotype data from all eligible investigations in the latest years involving more extensive countries and regions, as well as covering three gene loci to provide a more precise evaluation of the association between polymorphisms of 4VNTR, G894T, and C786T with URSA susceptibility.

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 May 2018. The following key words were combined: "endothelial nitric oxide synthase" or "C786T" or "4 VNTR" or "G894T" and "polymorphism" or "mutation" or "variant," or "SNP" and "recurrent miscarriage" or "recurrent abortion" or "recurrent pregnancy loss" or "recurrent fetal loss." 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 corresponding author to obtain information that was not found by the above retrieval strategies.

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 both at home and abroad, with good balance and comparability.

(2) Languages are limited to Chinese or English.

(3) The research should involve URSA risk and gene polymorphism of 4VNTR, G894T, or C786T loci.

(4) Patients with URSA should have 2 or more times abortion in the first trimester that has ruled out definite etiologies and the controls are participants with at least one live birth and without history of abortion.

(5) Each genotype distribution and individual number in the case and control group should be listed in the literature, or the corresponding number can be calculated by the frequency of each genotype given.

Studies with the following characteristics will be excluded:

(1) Those are not associated with C786T, 4VNTR, or G894T polymorphism and URSA;

(2) Those are not case-control studies;

(3) The case group do not exclude the clinical abortion factor; and

(4) The data of genotype frequency and allele frequency in the literature are incomplete or unclear.

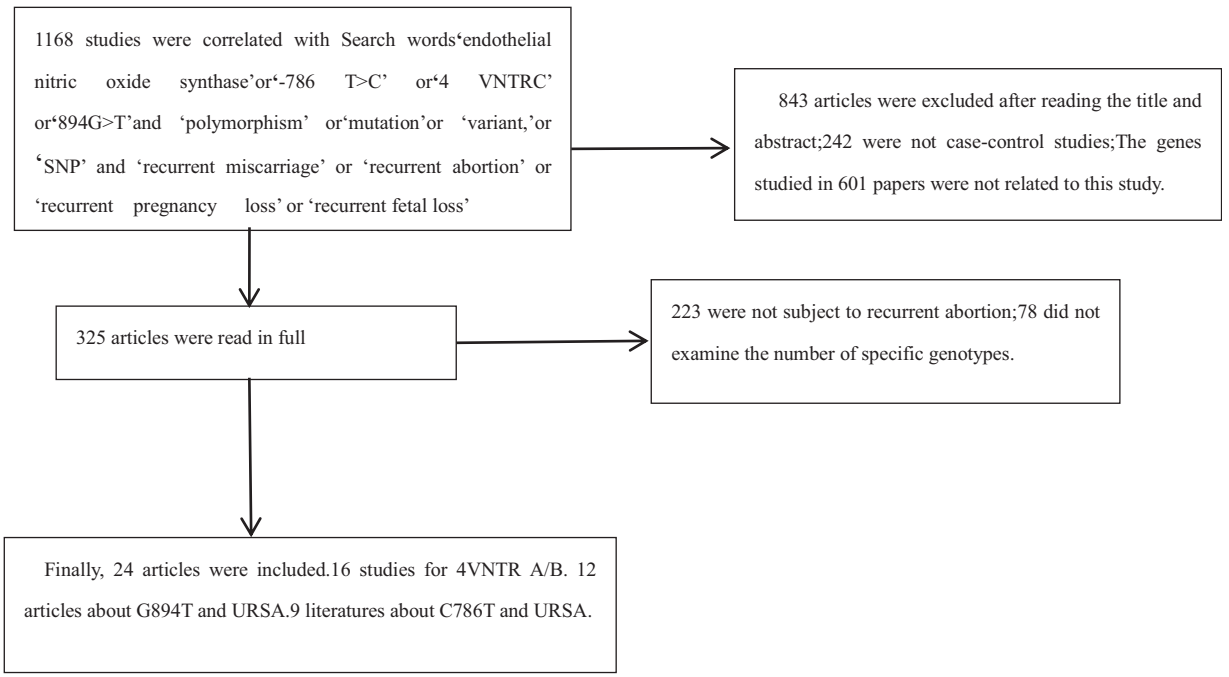


Figure 1. Article screening flowchart.

Table 1

Characteristics of studies on the association between 4VNTR A/B 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
			aa	ab	bb	a	b	aa	ab	bb	a	b			
Almawi et al[15]		France	236					305					3	<0.05	9
	2013		5	114	117	124	348	20	109	176	149	461			
Karvela et al[16]		Greece	126					130					3	0.41	7
	2008		1	30	95	32	220	4	31	95	39	221			
Buchholz et al[17]		Germany	179					126					3	0.58	9
	2004		123	52	4	298	60	82	39	5	203	49			
Walid Zammiti et al[9]		Tunisia	350					200					3	<0.05	7
	2008		22	97	231	141	559	8	46	146	62	338			
Parveen et al[18]		India	200					300					3	<0.05	7
	2011		17	50	133	84	316	12	74	214	98	502			
Al Sallout et al[19]		Gaza Strip	99					99					3	0.82	8
	2010		4	30	65	38	160	0	32	67	32	166			
Oztürk et al[10]		Turkey	54					70					3	0.20	8
	2011		38	16	0	92	16	54	10	6	118	22			
Pereza et al[11]		Gaza	148					149					3	0.10	7
	2010		4	26	118	34	262	3	25	121	31	267			
Shin et al[20]		Korea	340					115					3	0.43	9
	2010		2	63	275	67	613	1	24	90	26	204			
Abulata et al[21]		Egypt	50					50					3	0.19	7
	2015		9	19	22	37	63	0	10	40	10	90			
El-Gharably et al[22]		Gaza	45					45					3	0.88	6
	2013		0	2	43	2	88	0	0	45	0	90			
Makino et al[23]		Japan	85					76					2	0.37	8
	2004		0	15	70	15	155	0	14	62	14	136			
Azani et al[24]		Iran	130					120					3	0.05	7
	2017		9	35	86	53	207	9	30	81	48	192			
Suryanarayana et al[25]		India	145					99					3	0.11	6
	2006		1	43	101	45	245	0	28	71	28	170			
Buchholz et al[26]		Germany	179					126					2	0.58	6
	2004		4	52	123	60	298	5	39	82	49	203			
Makino et al[27]		Japan	85					78					2	0.37	7
	2004		0	15	70	15	155	0	14	64	14	142			

2.3. Data extraction and quality evaluation

The two researchers (Zhao and Li) 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. It consisted of three 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. The final score above 6 was regarded as high quality. They extracted relevant data from each article that included: the first author's name, years of publication, country and region, genotype frequencies in case and control group, the minimum number of abortions, Hardy–Weinberg equilibrium (HWE), and Quality score of case-control studies and were showed in the table (Fig. 1).

2.4. Statement

The ethical approval was not necessary. Because this study is about gene polymorphism associated with endothelial nitric oxide synthase (4VNTR, G894T, and C786T) and unexplained recurrent spontaneous abortion risk: a meta-analysis. This paper is not a clinical trial study, hence ethical approval and informed consent are not required. All included articles have passed ethical approval and informed consent.

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 firstly used to test the heterogeneity of the included literature. It suggested the heterogeneity existed when I^2 was above 50%, and the random effect model was used, and if not the fixed effect model was used instead. Subgroup analysis was carried out when there was a need to find the potential source of the 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.

2.6. Characteristics of the included studies

Overall, a total of twenty four (24) out of one thousand one hundred sixty eight (1168) articles were selected for the final meta-analysis.[14–29] Among the included articles, sixteen (16) studies[9–11,15–26] reported the association between 4VNTR A/B gene mutation and URSA with 2451 cases and 2088 controls. Twelve (12) articles[9–11,22,24,27–29] demonstrated the relationship between G894T and URSA with 2232 cases and 2121 controls

Table 2

Characteristics of studies on the association between G894T 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
			GG	GT	TT	G	T	GG	GT	TT	G	T			
Zammiti et al[9]		Tunisia	350					200					3	0.19	8
	2008		256	83	11	595	105	157	39	4	353	47			
Pereza et al[11]		Gaza	148					149					2	0.05	8
	2010		74	54	20	202	94	65	65	19	195	103			
Oztürk et al[10]		Turkey	115					332					3	0.55	7
	2011		103	12	0	218	12	266	60	6	592	72			
Elgharably et al[22]		Gaza	45					45					3	0.80	9
	2013		26	16	3	68	22	22	19	4	63	27			
Azani et al[24]		Iran	130					110					3	<0.05	7
	2017		81	36	13	198	62	81	25	4	187	33			
Dutra et al[28]		Brazil	145					135					3	0.92	6
	2014		84	53	8	221	69	69	57	9	195	75			
Karvela et al[29]		Greece	126					130					3	0.91	8
	2008		53	57	16	163	89	62	58	10	182	78			
Shin et al[30]		Korea	340					115					3	<0.05	7
	2010		266	60	14	592	88	103	12	0	218	12			
Suryanarayana et al[31]		India	145					99					3	0.77	8
	2006		91	47	7	229	61	69	27	3	165	33			
Parveen et al[32]		India	200					300					2	0.94	6
	2011		155	42	3	352	48	278	22	0	578	22			
Almawi et al[33]		Bahrain	296					305						0.05	7
	2013		154	109	33	417	175	194	95	16	483	127	3		
Luo et al[34]		China	192					201					3	<0.05	6
	2013		118	72	2	308	76	154	41	6	349	53			

involving 4 continents. Nine (9) studies[9,15,20,22,24,29] described the connection between C786T and URSA with 1899 cases and 1294 controls. The baseline characteristics of the studies related to mutation of 4VNTR A/B, G894T, and C786T were respectively shown in Tables 1–3. All of the thirty-seven (37) articles were published before May of 2018. In addition, all the manuscripts were published in English.

3. Results of the overall meta-analysis

3.1. Meta-analysis of 4VNTR A/B polymorphism and URSA risk

Sixteen (16) articles related to 4VNTR and URSA were included. showing that the polymorphism of 4VNTR gene was significantly correlated with the risk of URSA under dominant model (AA + AB vs. BB; OR 1.201, 95%CI 1.045–1.380), heterozygote model

Table 3

Characteristics of studies on the association between C786T 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
			TT	CT	CC	T	C	TT	CT	CC	T	C			
Almawi et al[15]		France	296					305					3	0.54	6
	2013		115	135	46	365	227	140	135	30	415	195			
Zammiti et al[9]		Tunisia	350					200					3	0.19	7
	2008		235	99	16	569	131	138	56	6	332	68			
Shin et al[20]		Korea	340					115					3	0.22	8
	2010		278	61	1	617	63	93	21	1	207	23			
Abulata et al[35]		Egypt	50					50					3	<0.05	6
	2015		12	38	0	62	38	35	15	0	85	15			
Luo et al.[34]		China	142					201					3	<0.05	7
	2013		128	5	9	261	23	153	41	7	347	55			
El-Gharably et al[22]		Gaza	45					42					3	0.88	8
	2013		24	18	3	66	24	39	3	0	42	3			
Azani et al.[24]		Iran	130					110					2	0.26	8
	2017		52	65	13	169	91	66	40	4	172	48			
Dutra et al[36]		Brazil	145					135					2	0.29	7
	2014		71	57	17	199	91	58	58	19	174	96			
Seung Ju Shin et al[37]		Korea	401					136					3	<0.05	6
	2011		278	61	62	617	185	93	21	22	207	65			

Table 4

Meta-analysis of 4VNTR polymorphism and URSA risk.

	I^2 (%)	Model	OR	95%CI		P	Z
AA vs. BB	45.10	FEM	1.415	1.017	1.970	<0.05	2.06
AB vs. BB	12.60	FEM	1.186	1.026	1.371	<0.05	2.31
(AA+AB) vs. BB	27.80	FEM	1.201	1.045	1.380	<0.05	2.58
AA vs. (BB+AB)	43.70	REM	1.135	0.872	1.478	0.306	0.94
A vs. B	38.90	FEM	1.160	1.035	1.301	<0.05	2.55

Table 5

Results of G894T polymorphism and URSA risk.

	I^2 (%)	Model	OR	95%CI		P	Z
TT vs. GG	38.50	FEM	1.585	1.175	2.138	<0.05	3.02
TG vs. GG	73.90	REM	1.193	1.082	1.316	<0.05	3.54
TT+TG vs. GG	77.90	REM	1.210	1.109	1.321	<0.05	4.28
TT vs. TG+GG	26.00	FEM	1.530	1.142	2.052	<0.05	2.85
T vs. G	77.70	REM	1.323	1.179	1.485	<0.05	4.75

(AB vs. BB; OR 1.186, 95%CI 1.026–1.371), homozygote model (AA vs. BB; OR 1.415, 95%CI 1.017–1.970), and additive model (A vs. B; OR 1.160, 95%CI 1.035–1.301). There was no association between URSA and 4VNTR gene under recessive model (AA vs. BB+AB; OR 1.135, 95%CI 0.872, 1.478) (Table 4)

Table 6

Results of G894T mutation and URSA risk of TG vs. GG, T vs. G, TT +TG vs. GG in subgroup analysis.

	I^2 (%)	Model	OR	95%CI		P
TG vs. GG in Asians	44.60	FEM	2.016	1.576	2.579	0.124
TG vs. GG in Caucasians	54.50	REM	1.015	0.846	1.217	<0.05
T vs. G in Asians	59.40	REM	1.947	1.577	2.403	<0.05
T vs. G in Caucasians	73.00	REM	1.104	0.960	1.270	0.164
TT+TG vs. GG in Asians	44.10	FEM	2.097	1.654	2.658	<0.05
TT+TG vs. GG in Caucasians	67.80	REM	1.066	0.897	1.266	0.468

Table 7

Results of G894T mutation and URSA risk of additive model (T vs. G) in subgroup analysis.

	I^2 (%)	Model	OR	95%CI		P
Asia T vs. G	8.30	FEM	1.727	1.372	2.175	<0.05

3.2. Meta-analysis of G894T polymorphism and URSA risk

Twelve (12) articles associated with G894T and URSA were included. The results showed that the polymorphism of G894T was significantly associated with URSA under homozygote model (TT vs. GG; OR 1.585, 95%CI 1.175–2.138) and recessive model (TT vs. TG+GG; OR 1.530, 95%CI 1.142–2.052) (shown

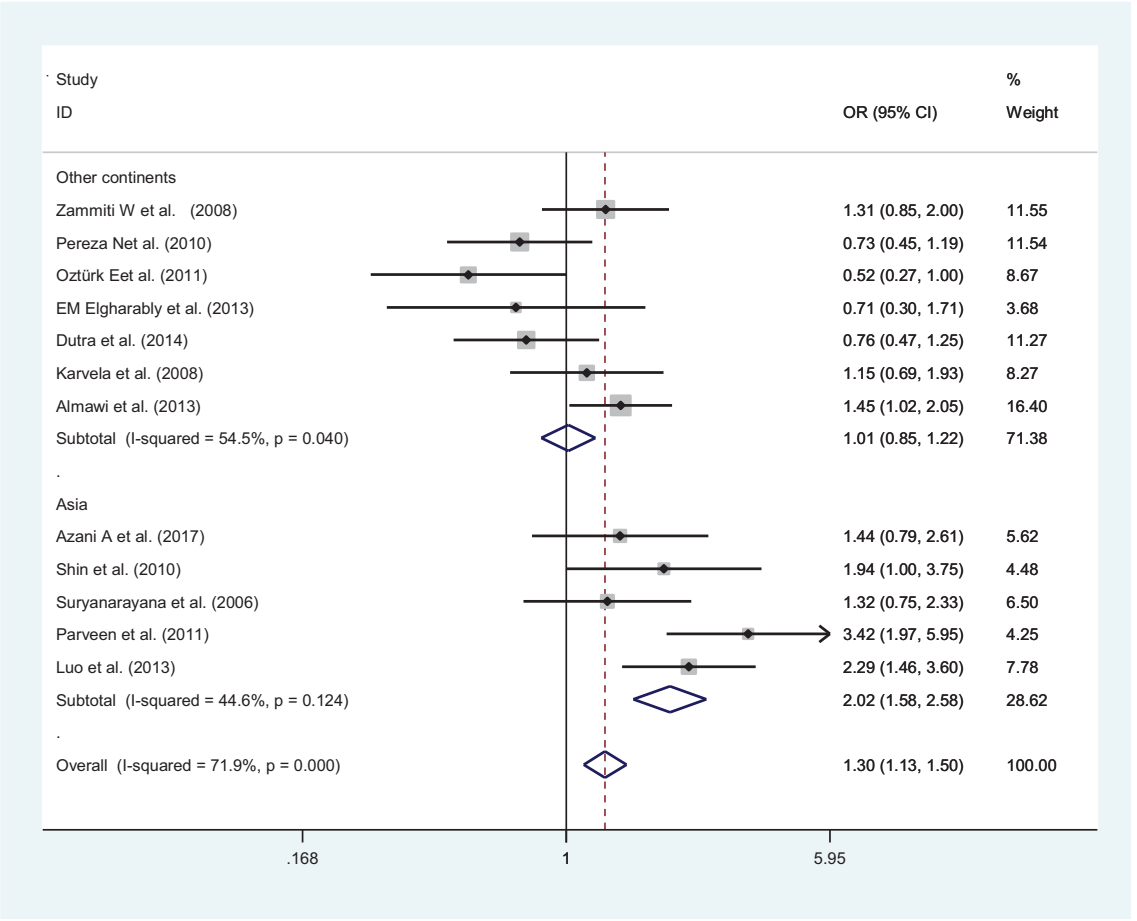


Figure 2. Subgroup analysis of G894T heterozygote model (TG vs. GG) in Asians and Caucasians.

in Table 5). Heterogeneity was found under dominant, heterozygote, and additive models with $I^2 > 50\%$. Subgroup analysis was needed to explore the source of heterogeneity.

3.3. Subgroup analysis

Among the twelve (12) articles included, five (5) and seven (7) studies respectively investigated the association between the eNOS G894T polymorphism and URSA in Asians and Caucasians. And the subgroup analysis was conducted according to the ethnicities (shown in Table 6). Significant association was found in Asians between G894T and URSA risk under dominant model (TT + TG vs. GG) ($P < .05$) but not heterozygote model (TG vs. GG) ($P > .05$). Considering $I^2 > 50\%$ in additive model (T vs. G) of G894T, we removed Luo's research according to the results of the sensitive analysis. The meta-analysis of the remaining articles showed that additive model (T vs. G) of eNOS G894T had significant association with URSA in Asians (shown in Table 7, Figs. 2–5). However the heterogeneity of the subgroup analysis still existed in Caucasians and the result showed that only the heterozygote model (TG vs. GG) was associated with URSA susceptibility.

3.4. Meta-analysis of C786T polymorphism and URSA risk

Nine (9) articles were related to C786T and URSA susceptibility. The results showed that the polymorphism of C786T was significantly correlated with the risk of URSA under the dominant model (TT + CT vs. CC; OR 0.743, 95%CI 0.564–0.980), heterozygote model (CT vs.CC; OR 0.706, 95%CI 0.518–0.962), and homozygote model (TT vs. CC; OR 0.721, 95%CI 0.542–0.959) ($P < .05$) on condition of $I^2 < 50\%$ (shown in Table 8).

3.5. Test for heterogeneity

In the heterogeneity test for the C786T genotypes of each model, I^2 of the recessive model (TT vs. CC + CT) and additive model (T vs. C) were both >50%, indicating that the included studies had heterogeneity. We found that I^2 was still above 50% after the subgroup analysis. This may be raised from the insufficiency of literature and patients were recruited from different countries.

3.6. Publication bias evaluation

In the evaluation process, we found that gene funnel plot analysis showed asymmetry indicating the possibility of publication bias, which could be caused by differences in countries, regions, and races of the people included in the literature. The results were shown in Figures 6–8.

4. Conclusion

As normal pregnancy can lead to significant changes with respect to the hemodynamics in pregnant women, such as increased blood volume and vasodilation, great attention has been paid to

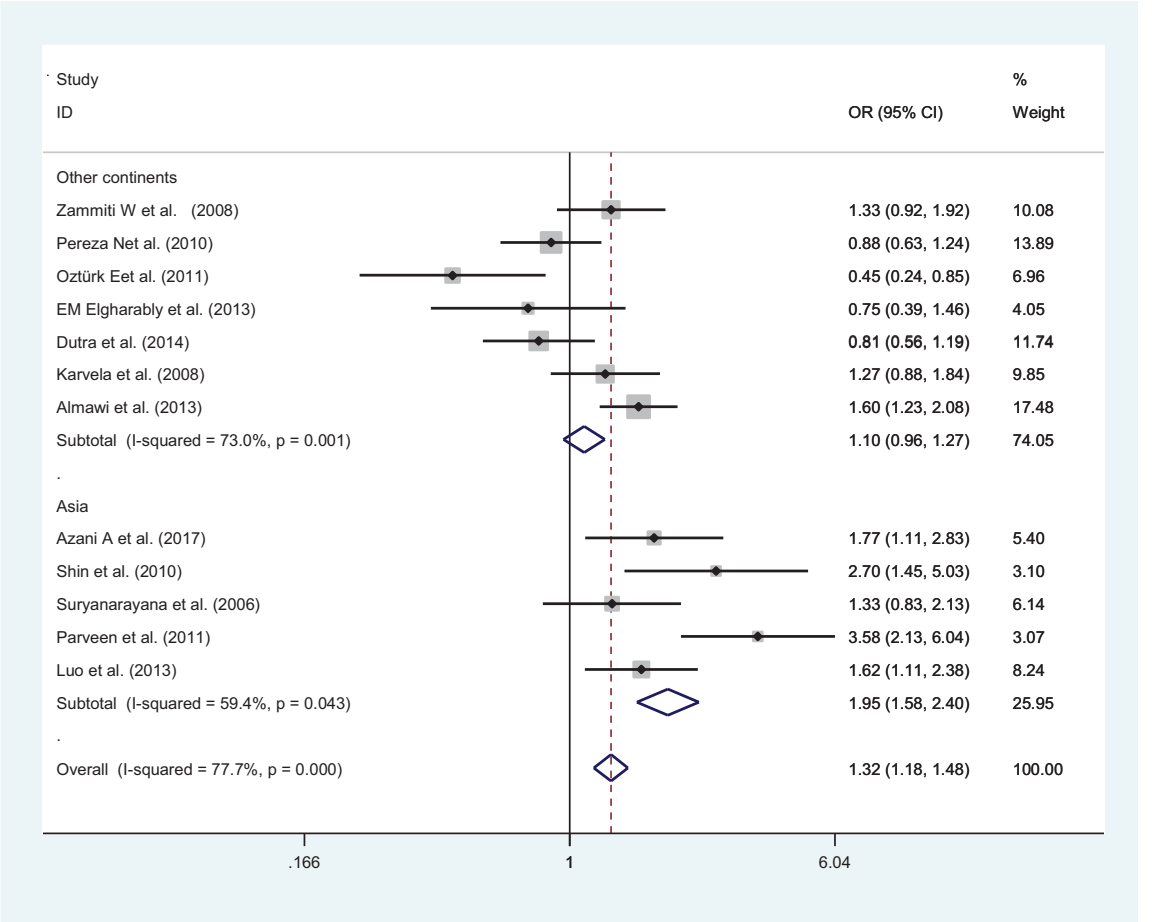


Figure 3. Subgroup analysis of G894T additive model (T vs. G) in Asians and Caucasians.

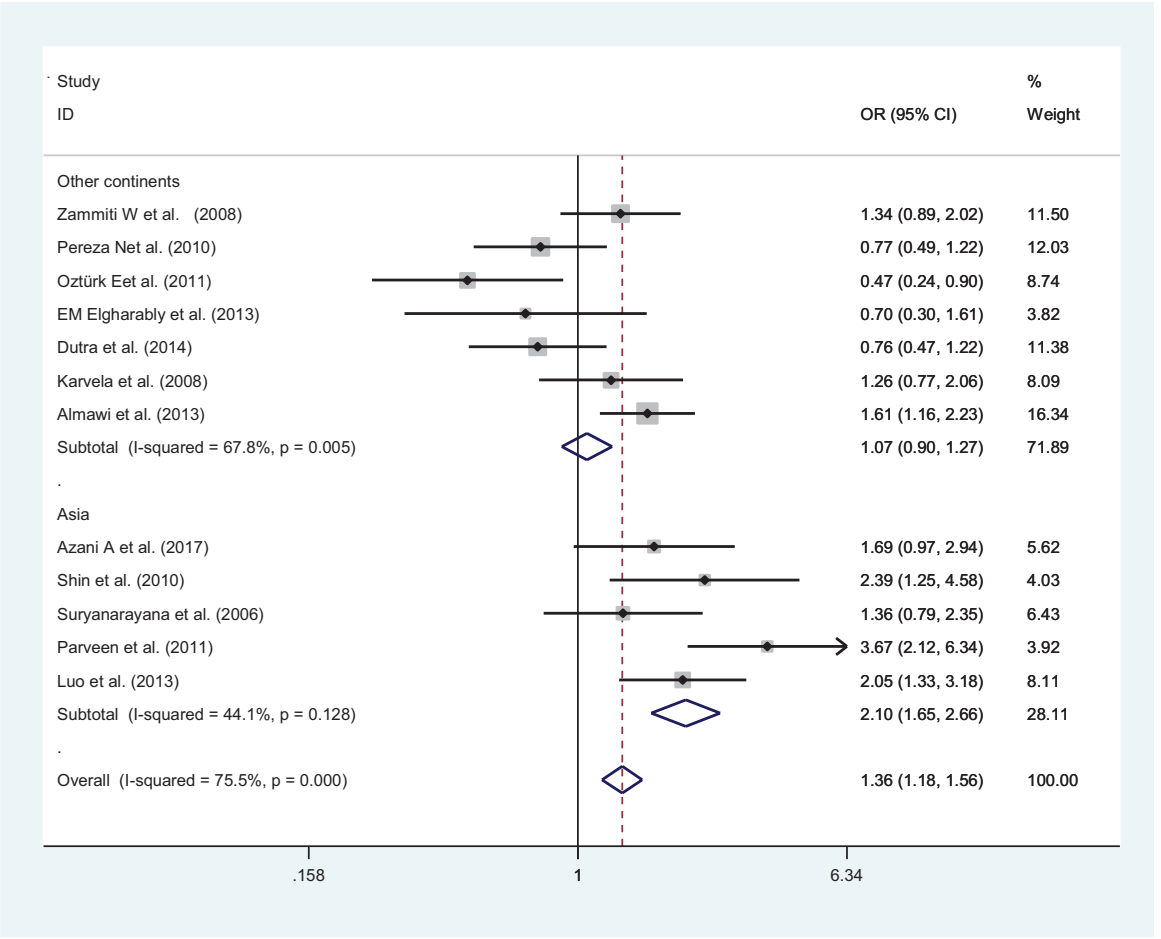


Figure 4. Subgroup analysis of G894T dominant model (TT+TG vs. GG) in Asians and Caucasians.

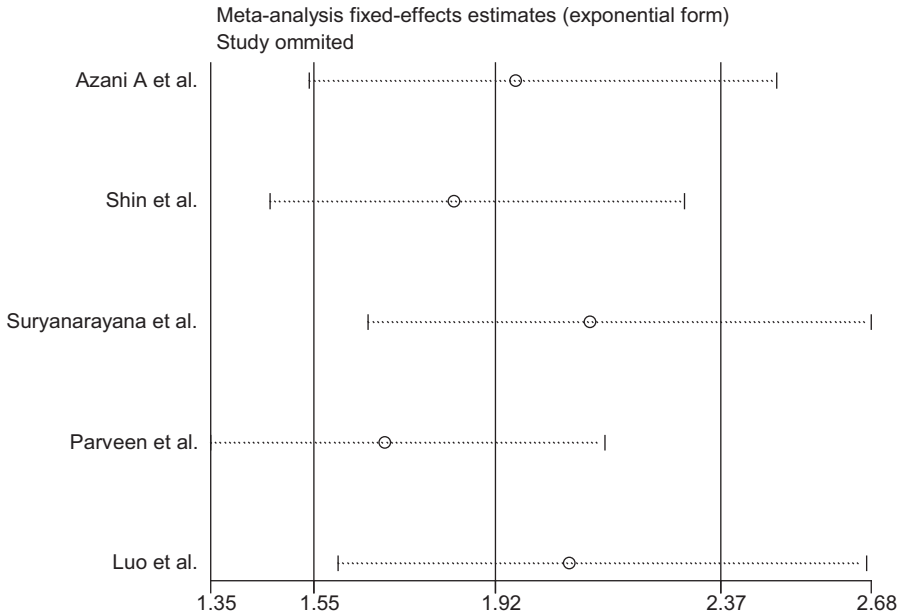
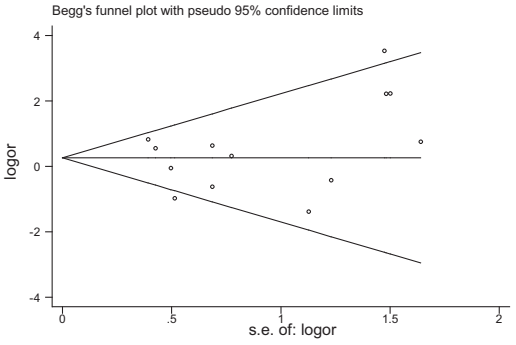


Figure 5. Sensitivity analysis of additive model (T vs. G) in Asians.

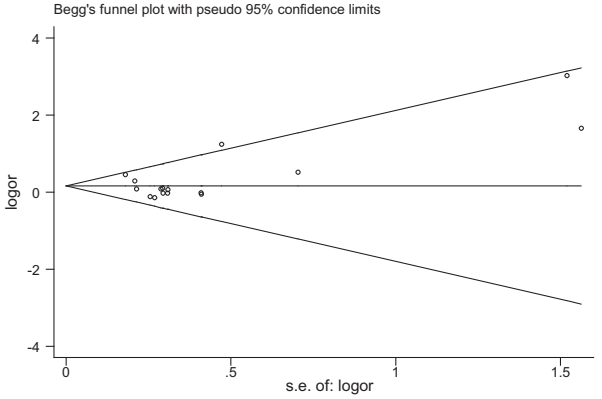
Table 8

Meta-analysis of C786T polymorphism and URSA risk.

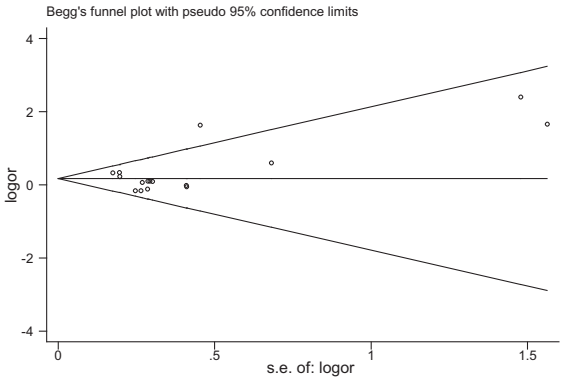
	I^2 (%)	Model	OR	95%CI		P	Z
TT vs. CC	43.30	FEM	0.721	0.542	0.959	<0.05	2.25
CT vs. CC	43.10	FEM	0.706	0.518	0.962	<0.05	2.21
(TT + CT) vs. CC	21.30	FEM	0.743	0.564	0.980	<0.05	2.1
TT vs. (CC + CT)	84.90	REM	0.840	0.718	0.983	<0.05	2.18
T vs. C	80.30	REM	1.027	0.910	1.160	0.664	0.43



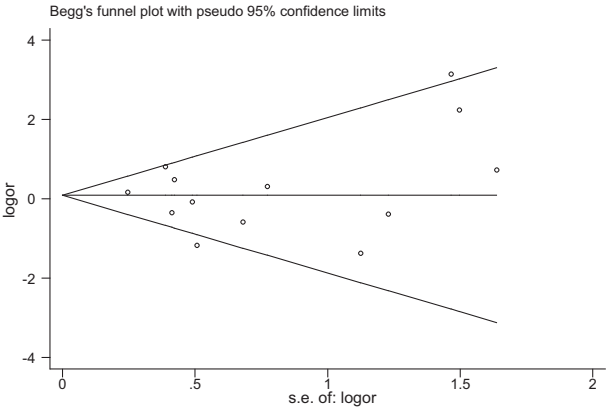
AA vs. BB



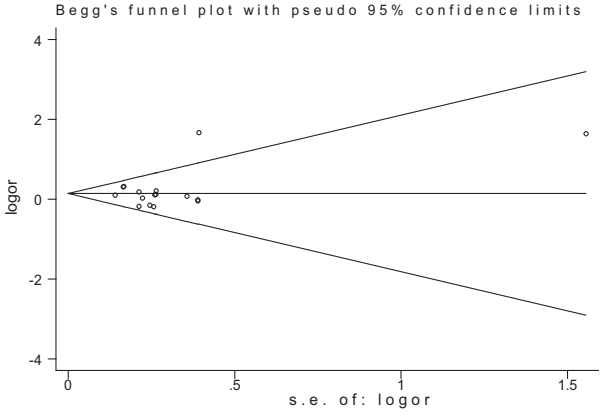
AB vs. BB



(AA + AB) vs. BB



AAvs. (BB+AB)



A vs. B

Figure 6. The publication bias of articles on the relationship between 4VNTR and URSA risk was shown in the funnel figures.

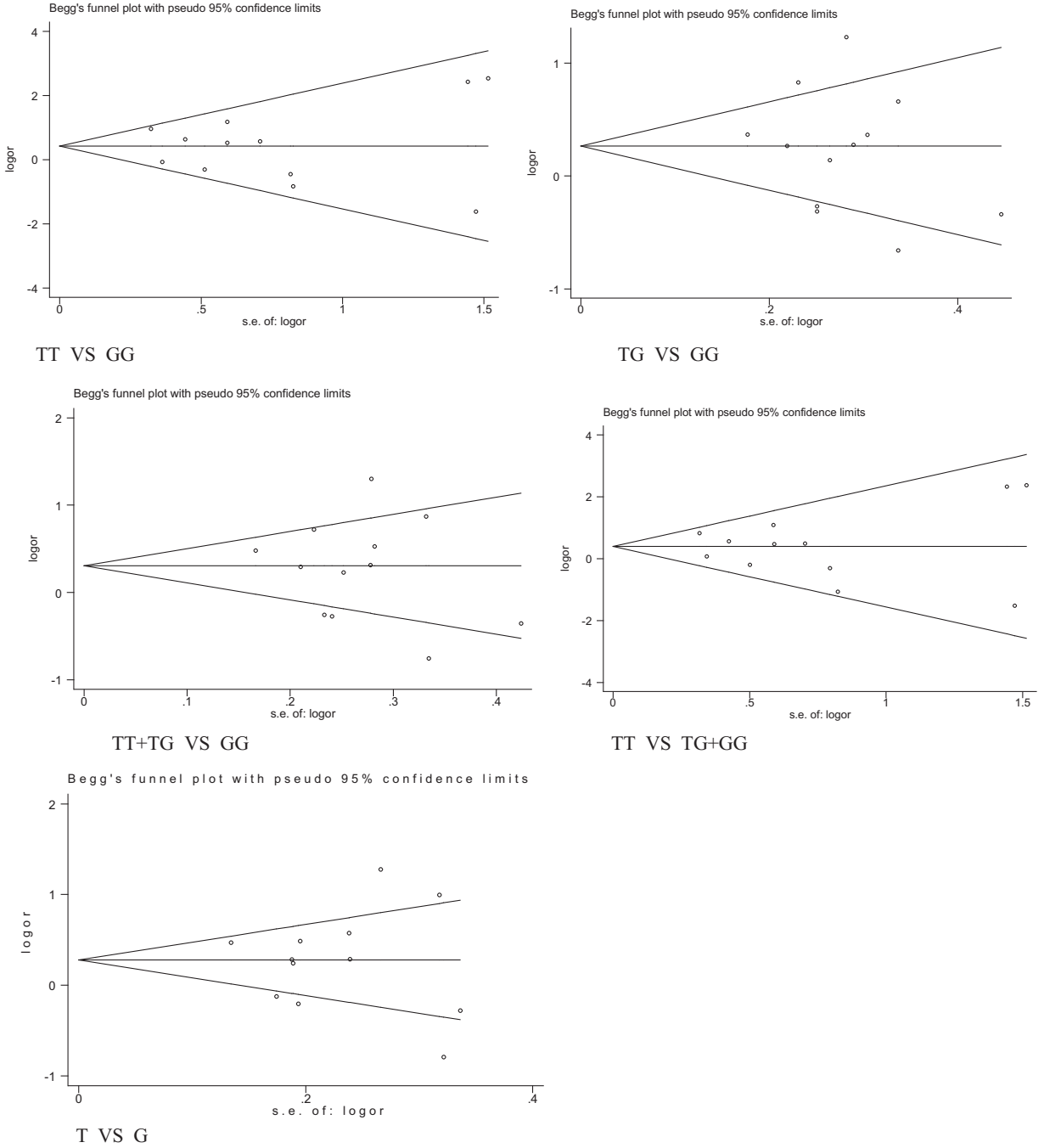


Figure 7. The publication bias of articles on the relationship between G894T and URSA risk was shown in the funnel figure.

NO when considering its role as a vasodilator mediator.[30] Besides, eNOS also involves in ovulation, blastocyst implantation, trophoblast differentiation, trophoblast invasion, all of which are crucial to successful pregnancy.[31] eNOS, a key enzyme in NOS has been shown to be abundantly expressed in trophoblast cells in the early stage of pregnancy. When interstitial trophoblasts invade the maternal spiral arteries of uterus, NO encoded by eNOS acts on artery walls to create a low-resistance and high-caliber uteroplacental unit to accelerate endovascular invasion.[32] Moreover the production of gonadotrophic hormone from placenta is also modulated by the expression of eNOS.[33] Therefore polymorphisms of eNOS which can alter the activity and levels of NO are not surprising to contribute to pregnancy-related vascular disorders, including preeclampsia, early pregnancy loss.[25] The eNOS gene is localized in chromosome 7q35-36,[34] consisted of 26 exons and encoded an mRNA of 4052 nucleotides. Much attention has been concentrated on three functional mutations: intron 4(4a/4b) VNTR, exon 7 (G894T), and promoter C786T. The VNTR in intron 4 consists of five (wild-type B allele) or four (mutant A allele) copies of a 27-bp repeat. It acts as an enhancer/repressor regulating eNOS expression, and the 27-bp repeats are the source of a 27-nt small RNA, which function as a negative feedback regulator. The mutant (A) allele induces lower levels of the 27-nt

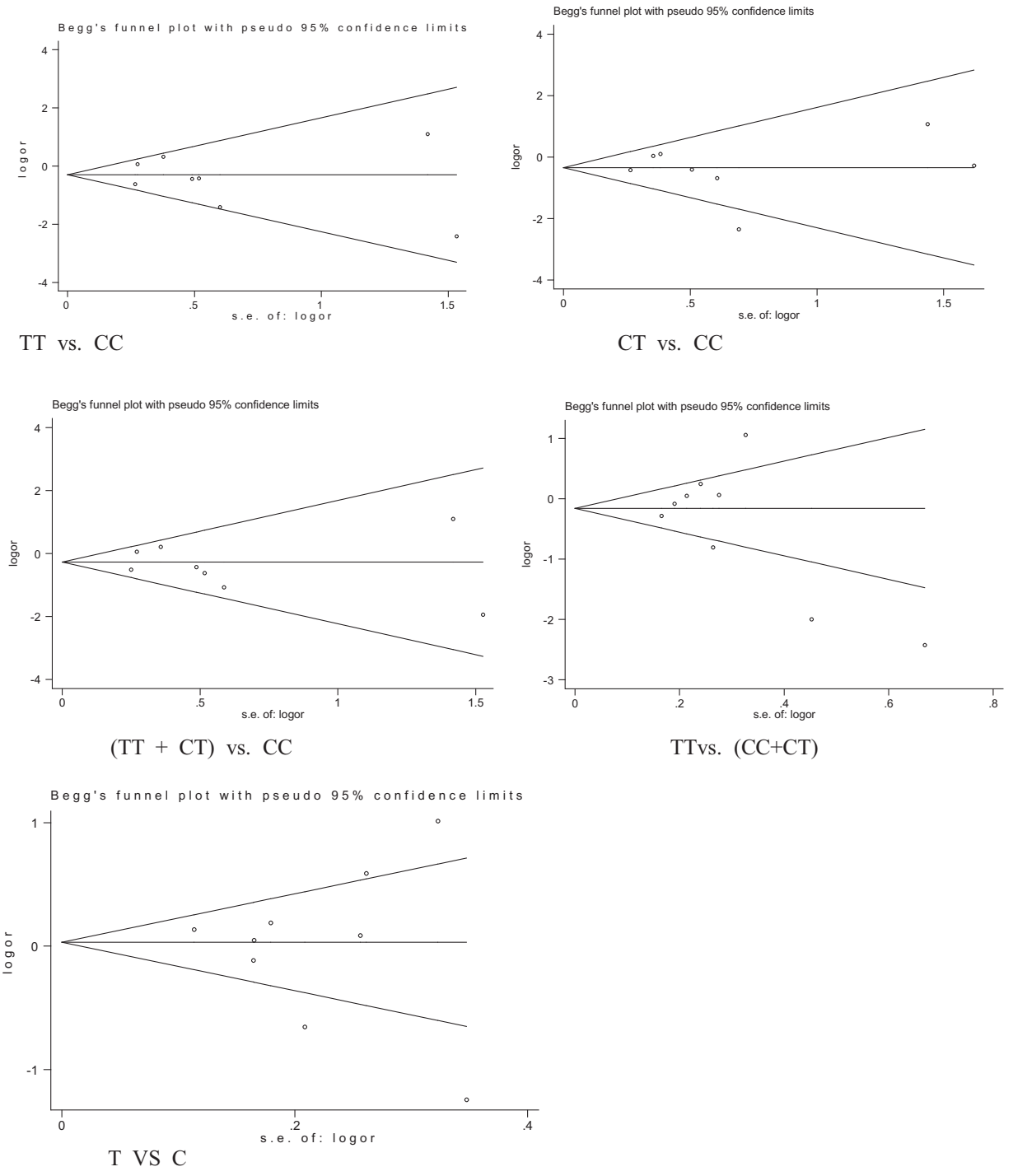


Figure 8. The publication bias of articles on the relationship between C786T and URSA risk was shown in the funnel figure.

small RNA, leading to higher eNOS expression. However, the association between mutation in VNTR and URSA remains inconclusive. Our meta-analysis indicates that the VNTR in intron 4 is associated with URSA under all gene models except for recessive model (AA vs. BB + AB). Cao[13] only observes the relationship between eNOS intron 4 VNTR and URSA under co-dominant (AA vs. BB) and additive (A vs. B) genetic models, which is consistent with our conclusion.

The Glu298Asp missense mutation encoded by exon 7 (G894T) of the eNOS gene[16] is another familiar mutation site that transforms from a guanine (G) to thymine (T) at the nucleotide position 894,[35] leading to a replacement of glutamic acid by aspartic acid at codon 298 (Glu298Asp),[34] which can decrease NO production and impaired maternal-fetal circulation, and finally result in miscarriage. Our meta-analysis confirmed the association between G894T gene and URSA under the homozygote and recessive models ($P < .05$) without heterogeneity ($I^2 <$ 50%). Considering heterogeneity in the remaining gene model, we perform subgroup analysis on ethnicity, and the results show that the dominant and additive models of G894T in Asians and

the heterozygote model in Caucasians are associated with URSA ($P < .05$), which contradicted with Mei-TszSu's[12] conclusion. This may be due to the fact that sample sizes in Mei-TszSu's meta-analysis are relatively small.

The mutation of C786T located in the five flanking region, promoter of eNOS gene, is related to a substitution of thymine (T) to cytosine (C).[36] Our meta-analysis indicates that C786T gene is significantly connected with URSA under dominant, heterozygote, and homozygote model ($P < .05$) without heterogeneity ($I^2 < 50\%$). When considering that I^2 of (TT vs. CC + CT) and (T vs. C) are both >50%, subgroup analysis is carried out, but heterogeneity remained. Although heterogeneity is very common in genetic association meta-analyses we cannot ignore it. This may be raised from the differences in ethnicity, source of control, HWE, or the times of abortion.

The advantage of our meta-analysis is that we have included more literature, studied more gene sites and conducted more in-depth subgroup analysis than the previous meta-analysis. This may help to draw a more scientific and conclusive conclusion. However besides substantial heterogeneity, another limitation in our meta-analysis is the asymmetry in the funnel plots, which suggests that the number of eligible studies included in total is also not enough. 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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