

Association of Estrogen Receptor 1 Genetic Polymorphisms with Recurrent Spontaneous Abortion Risk

Xun-Qiang Yin^{1,2}, Hong-Mei Ju³, Qiang Guo², Lin Zhao², Xiao-Xiao Zhu², Ran Wei², Zhen Zhang², Yun-Hong Zhang^{1,2}, Bin Wang⁴, Xia Li²

¹School of Medicine and Life Sciences, University of Jinan-Shandong Academy of Medical Sciences, Jinan, Shandong 250062, China

²Laboratory for Molecular Immunology, Institute of Basic Medicine, Shandong Academy of Medical Sciences, Jinan, Shandong 250062, China

³Department of Gynaecology, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, Shandong 250011, China

⁴Department of Peripheral Vascular Disease, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, Shandong 250011, China

Abstract

Background: Estrogen is one of the most important reproductive steroidal hormones and plays a critical role in the maintenance of pregnancy, and its function is mediated by estrogen receptor 1 (*ESR1*). The polymorphisms of *ESR1* were involved in recurrent spontaneous abortion (RSA); however, the association between *ESR1* polymorphisms and RSA remains controversial. The present meta-analysis was aimed to clarify the association between *ESR1 PvuII* (-397C/T, rs2234693) and *XbaI* (-351A/G, rs9340799) polymorphisms and the risk of RSA.

Methods: All the included articles were retrieved from PubMed, Embase, Cochrane Library, China National Knowledge Infrastructure, and Wanfang Med Online Database up to January 3, 2018. Data were processed in the Stata 12.0 software. The odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated using fixed-effects models (FEM)/random-effects models (REM).

Results: Seven case-control studies with 836 cases and 1164 controls were included in the study. Generally, the *ESR1* polymorphisms were not associated with RSA in any of the genetic analysis models. However, it was found that as rs9340799 polymorphism was related to increased risk of RSA in non-Asian group in the homozygous genetic model (OR = 2.40, 95% CI = 1.05–5.50, *P* = 0.039). Moreover, in Asian group, rs9340799 polymorphism was found to be related to decreased RSA risk in both the heterozygous model (OR = 0.53, 95% CI = 0.33–0.85, *P* = 0.009) and the dominant genetic model (OR = 0.55, 95% CI = 0.30–0.98, *P* = 0.042).

Conclusions: Generally, there was no significant association between the polymorphisms of *ESR1* and the risk of RSA. However, subgroup analysis indicated that *ESR1* rs9340799 polymorphism was related to increased RSA risk in the non-Asian group while associated with decreased RSA risk in Asian group.

Key words: Estrogen Receptor 1; Meta-Analysis; Polymorphism; Recurrent Spontaneous Abortion; Risk

INTRODUCTION

Recurrent spontaneous abortion (RSA), defined as two or more consecutive miscarriages before 20 weeks of gestation,^[1] is a pregnancy complication occurring in approximately 3% of fertile couples.^[2,3] Although various etiological factors are considered risk factors of RSA, such as anatomic abnormalities of the genital tract, uterine structural abnormalities, parental chromosome abnormalities, endocrine and/or metabolic disorders, autoimmune abnormalities, and environmental factors, the causative factors in approximately 50% of RSA patients still have not been identified.^[4,5] Therefore, there is an urgent need to identify risk factors for the prevention and treatment of RSA. Estrogen is one of the most important reproductive steroidal hormones associated with placental and fetal development,

and it plays a critical role in the maintenance of pregnancy by modulating pregnancy-related hormone production and uteroplacental blood flow.^[6] Estrogen deficiency was reported to be associated with a high risk of subsequent miscarriage.^[7]

The function of estrogen is mediated by the estrogen receptor (ESR), a member of the nuclear receptor

Address for correspondence: Dr. Xia Li,

Laboratory for Molecular Immunology, Institute of Basic Medicine,
Shandong Academy of Medical Sciences, Jinan, Shandong 250062, China
E-Mail: 786735868@qq.com

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superfamily of transcription factors; there are two ESRs, ESR-alpha and ESR-beta, which are encoded by *ESR1* and *ESR2*, respectively.^[8] It was found that the expression of ESR1 in uterus of mice is much higher than ESR2, indicating the more important role of *ESR1* in RSA.^[9] Moreover, studies have demonstrated that female *ESR2* knockout mice are subfertile and primarily lack efficient ovulatory function; however, female *ESR1* knockout mice are infertile because of anovulation and insensitivity to estrogen, indicating the essential role of *ESR1* in successful pregnancy.^[10,11] *ESR1* (140 kb), located on chromosome 6q25.1, is a common component of the *ESR* complex in the tissue of pregnant females.^[12,13] Two single nucleotide polymorphisms (SNPs) – rs2234693 (-397C>T, defined by the restriction enzyme *PvuII*) and rs9340799 (-351A>G, defined by the restriction enzyme *XbaI*) – in intron 1 of *ESR1* were demonstrated to be associated with RSA,^[14–18] indicating the potential role of *ESR1* genetic polymorphisms in RSA, and these polymorphisms may be taken as promising biomarkers for evaluating the risk of RSA. However, the existing evidence remains controversial because of the inconsistency of findings among studies.^[19–21] Although a 2011 study reported no significant association between *ESR1* polymorphisms and RSA, the community is still unable to reach a consensus. For example, some studies demonstrated that both the rs2234693 and rs9340799 polymorphisms of *ESR1* are associated with increased RSA risk,^[14,15] while other studies reported that only one of the two polymorphisms – either rs2234693 or rs9340799 – affects RSA risk.^[17] Moreover, some studies indicated that neither the rs2234693 nor the rs9340799 polymorphism of *ESR1* is associated with RSA risk.^[19,20] Considering the heterogeneous approaches and limited sample size of earlier studies, we designed this meta-analysis to further elucidate the association between *ESR1* polymorphisms and RSA risk with larger sample sizes and more detailed analysis.

METHODS

Search strategy

A comprehensive literature search was performed in PubMed, Embase, Cochrane Library, China National Knowledge Infrastructure, and Wanfang Med Online Database up to January 3, 2018, by two independent researchers (Xun-Qiang Yin and Qiang Guo) under the supervision of the other two authors (Xia Li and Hong-Mei Ju) without language restrictions. The following keywords and MeSH terms were applied: (“Estrogen receptor 1” OR “ESR1” OR “ER1” OR “Estrogen receptor alpha” OR “ESR alpha” OR “ER alpha”) AND (“recurrent embryo loss” OR “recurrent pregnancy wastage” OR “recurrent pregnancy loss” OR “recurrent spontaneous abortion” OR “recurrent miscarriage” OR “recurrent abortion” OR “recurrent fetal loss” OR “habitual abortion”) AND (“polymorphism” OR “gene” OR “genetic” OR “variant” OR “variation” OR “mutation” OR “SNP” OR “allele”). In addition, the reference lists of the retrieved articles were searched manually.

Inclusion and exclusion criteria

The eligible studies were performed from February 2002 to February 2014 and identified using the following inclusion criteria: (1) the design of the original article was a cohort study or a case-control study; (2) the study evaluated the association between *ESR1* polymorphisms and the risk of RSA; (3) RSA was defined as the occurrence of two or more miscarriages in the first two trimesters of pregnancy, and the controls were women with at least one successful pregnancy without a history of complicated pregnancies, miscarriages, still births, small for gestational age fetuses, preeclampsia, ectopic pregnancy, or preterm delivery; (4) the genotype distributions in both the cases and the controls were available to calculate the odds ratios (ORs) and their corresponding 95% confidence intervals (95% CIs); and (5) for duplicates, only the study with more complete data and a more extensive interval of enrollment was included. Studies were excluded if: (1) they were letters, abstracts, meta-analyses, or review articles; (2) they did not include healthy control population and publish without raw data; (3) the genotype frequencies were unavailable; (4) *ESR1* polymorphisms were not detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP); (5) they did not conform to the criteria for RSA; or (6) they were duplicates of previous publications. The selection of the studies was performed by two investigators independently, according to the inclusion and exclusion criteria, by screening the title, abstract, and full text. Any disagreement was checked and resolved by discussion among the authors.

Data extraction

The bibliographic search and data extraction were conducted independently by two investigators from all eligible publications according to the above inclusion criteria and exclusion criteria. The following information was gathered from all identified studies according to a standardized data collection form: author name, publication year, study country, ethnicity, diagnostic criteria for RSA, genotype method, genotype frequencies in cases and controls, and the *P* value of Hardy–Weinberg equilibrium (HWE) in controls. Each ethnic descent was classified as Asian or non-Asian. Any disagreement was resolved by discussion among the authors.

Quality assessment of included studies

A quality assessment was independently performed for all of the included studies by two authors (Ran Wei and Zhen Zhang) using the Newcastle–Ottawa Scale (NOS),^[22] and any disagreement was resolved by discussion and consensus. The NOS comprised the following three parameters of quality: selection, comparability, and exposure. Studies with scores of 8 points or higher were considered to be of higher quality.

Statistical analysis

The OR and CI were calculated to evaluate the relation between the *ESR1* rs2234693 and rs9340799 gene polymorphisms and RSA risk. The significance of pooled OR was determined by a Z-test $\alpha = 0.05$, it is the size of a

test. The *OR* was calculated by the codominant, homozygous, heterogeneous, dominant, and recessive models based on the genotype frequencies in cases and controls. These models are the five common statistical methods for the analysis of genetic polymorphisms, and the principle of these models is to repeat the comparison of various genotypes to reduce error probability.^[23] The suspicion of heterogeneity based on the selected studies was investigated by the Chi-square-based *Q*-test and inconsistency index (*I*²) test.^[24] When the *P* value of the *Q*-test was <0.05 or *I*² was more than 50%, the degree of heterogeneity was considered significant, and the random-effects model (REM)^[25] was used; otherwise, the fixed-effects model (FEM)^[26] was selected. To consider potential geographic variation, we performed subgroup analysis stratified by geographic location. Sensitivity analysis was performed by sequential omission of individual studies. Potential publication bias was explored using Begg's funnel plot and Egger's linear regression test.^[27] If publication bias was identified, the nonparametric "trim and fill" method was used to adjust for the bias. HWE in controls was examined using a Chi-square goodness-of-fit test. All analyses were performed with Stata software (version 12.0; Stata Corporation, College Station, Texas, USA). A two-sided *P* value was considered statistically significant if it was <0.05.

RESULTS

Studies included in the meta-analysis

The detailed study selection process is summarized in Figure 1. Based on the searching strategy, 198 records were retrieved. According to the exclusion criteria, 177 articles were excluded after the titles and abstracts were reviewed, and 14 articles were excluded for not being case-control studies or not fitting the study criteria. In the final meta-analysis, seven articles involving a total of 836 cases and 1164 controls were identified from the electronic databases according to

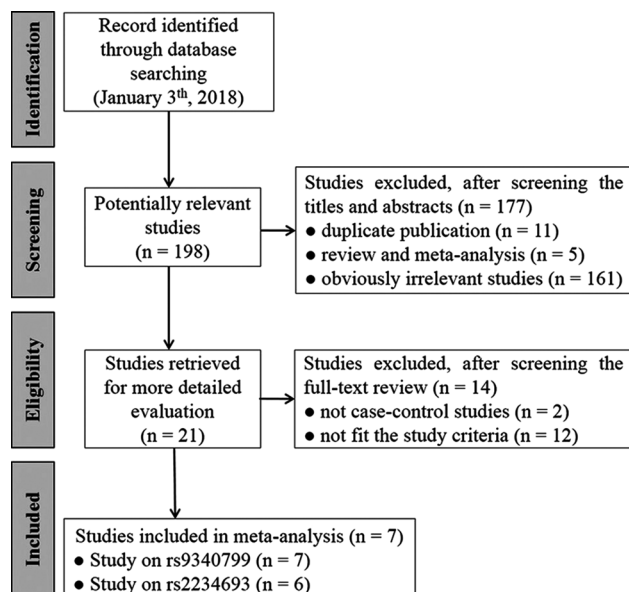


Figure 1: Flow chart of literature search and study selection.

the inclusion and exclusion criteria.^[15-21] The characteristics of the identified studies are presented in Table 1. These case-control studies were published between 2002 and 2014. Among them, three studies were conducted in China, one in Iran, one in Ukraine, one in Spain, and one in Brazil. *ESR1* polymorphisms including *PvuII* (rs2234693, -397C/T) and *XbaI* (rs9340799, -351A/G) were reported in these studies. DNA samples used for identifying *ESR1* polymorphisms were extracted from blood in all included studies. The methods used for genotyping were PCR-RFLP. Genotype distributions among the controls of most studies were consistent with HWE. In addition, all seven studies were assessed for quality according to the NOS, and all studies scored 8 stars, suggesting higher quality.

Meta-analysis of the association between the estrogen receptor 1 polymorphisms and recurrent spontaneous abortion

According to the *I*² and *P* values of the two SNPs, heterogeneity existed in the codominant (*I*² = 62.6%) model, the homozygous (*I*² = 55.9%) model, the heterozygous (*I*² = 62.3%) model, and the dominant (*I*² = 67.1%) model of rs2234693, as well as the codominant (*I*² = 78.6%) model, the homozygous (*I*² = 70.4%) model, the dominant (*I*² = 64.3%) model, and the recessive (*I*² = 70.7%) model of rs9340799. Thus, a REM was used to analyze studies on the codominant, homozygous, heterozygous, and dominant models of rs2234693 and the codominant, homozygous, dominant, and recessive models of rs9340799. Meanwhile, a FEM was used to analyze studies on the recessive model of rs2234693 and the heterozygous model of rs9340799. The main results of this meta-analysis are described in Table 2. Overall, there was no significant association between the polymorphisms of *ESR1* (rs2234693 and rs9340799) and the risk of RSA in any of the five genetic models. Subgroup analysis was performed to further identify the effects of heterogeneity on the results. It was showed that the rs2234693 polymorphism was not associated with the risk of RSA in both Asian and non-Asian groups under any of the five genetic models. As for rs9340799 polymorphism, significant association was found in non-Asian group in the homozygous genetic model [*OR* = 2.40, 95% *CI* = 1.05–5.50, *P* = 0.039; Figure 2a], indicating that rs9340799 polymorphism might increase the risk of RSA in non-Asians. Moreover, significant association between the rs9340799 polymorphism and RSA risk was identified in Asian group in both the heterozygous model [*OR* = 0.53, 95% *CI* = 0.33–0.85, *P* = 0.009; Figure 2b] and the dominant genetic model [*OR* = 0.55, 95% *CI* = 0.30–0.98, *P* = 0.042; Figure 2c], suggesting that rs9340799 polymorphism may play opposite roles in RSA in Asians and non-Asians. The main results of the subgroup analysis were displayed in Table 3.

Sensitivity analysis

Sensitivity analysis was conducted by sequential omission of individual studies to evaluate the stability of the overall results. This sensitivity analysis suggested that the results concerning the -397C/T (rs2234693) gene polymorphism and

Table 1: Main characteristics of the studies included in the meta-analysis

Gene polymorphisms	References	Year	Country	Ethnicity	Pregnancy loss (times)	Method	Genotype*		P†	Quality score‡
							Case	Control		
rs2234693	Pan <i>et al.</i> ^[15]	2014	China	Chinese	Two or more	PCR-RFLP	21/66/42	20/87/76	0.506	8
	Mahdavi pour <i>et al.</i> ^[19]	2014	Iran	Iranian	Three or more	PCR-RFLP	41/126/74	10/58/36	0.053	8
	Kucherenko <i>et al.</i> ^[17]	2013	Ukraine	Ukrainian	Two or more	PCR-RFLP	18/37/20	21/53/32	0.910	8
	Hu <i>et al.</i> ^[20]	2012	China	Chinese	Two or more	PCR-RFLP	27/90/79	30/76/76	0.605	8
	Pineda <i>et al.</i> ^[16]	2010	Spain	Spanish	Two or more	PCR-RFLP	6/21/21	100/225/115	0.616	8
	Aléssio <i>et al.</i> ^[21]	2008	Brazil	Caucasian and African Brazilian	Three or more	PCR-RFLP	9/48/18	14/37/24	0.969	8
rs9340799	Guan <i>et al.</i> ^[18]	2002	China	Chinese	Two or more	PCR-RFLP	9/28/32	10/45/18	0.034	8
	Pan <i>et al.</i> ^[15]	2014	China	Chinese	Two or more	PCR-RFLP	63/41/25	119/50/14	0.012	8
	Mahdavi pour <i>et al.</i> ^[19]	2014	Iran	Iranian	Three or more	PCR-RFLP	90/121/33	39/59/6	0.007	8
	Hu <i>et al.</i> ^[20]	2012	China	Chinese	Two or more	PCR-RFLP	129/58/9	109/65/8	0.302	8
	Pineda <i>et al.</i> ^[16]	2010	Spain	Spanish	Two or more	PCR-RFLP	24/19/4	153/221/67	0.379	8
	Aléssio <i>et al.</i> ^[21]	2008	Brazil	Caucasian and African Brazilian	Three or more	PCR-RFLP	33/38/4	35/31/9	0.600	8
	Guan <i>et al.</i> ^[18]	2002	China	Chinese	Two or more	PCR-RFLP	41/24/4	33/35/5	0.288	8

*Genotype for *ESR1* rs2234693, CC/CT/TT; *ESR1* rs9340799, AA/AG/GG; †P value for Hardy-Weinberg equilibrium in control group; ‡Assessed by the Newcastle-Ottawa Scale for the assessment of case-control studies. PCR: Polymerase chain reaction; RFLP: Restriction fragment length polymorphism; *ESR1*: Estrogen receptor 1.

Table 2: Meta-analysis results for the *ESR1* polymorphisms and RSA risk

Gene polymorphisms	Inherited model	Heterogeneity-test		Analysis model	Pooled OR (95% CI)	P
		P for Q-test	I ² (%)			
rs2234693	Codominant (C vs. T)	0.013	62.6	REM	0.98 (0.77–1.24)	0.839
	Homozygous (CC vs. TT)	0.034	55.9	REM	0.99 (0.62–1.59)	0.969
	Heterozygous (CT vs. TT)	0.014	62.3	REM	0.95 (0.66–1.36)	0.777
	Dominant (CT + CC vs. TT)	0.006	67.1	REM	0.95 (0.65–1.37)	0.773
	Recessive (CC vs. TT + CT)	0.137	38.2	FEM	1.04 (0.79–1.37)	0.793
rs9340799	Codominant (A vs. G)	0.000	78.6	REM	1.03 (0.72–1.48)	0.883
	Homozygous (AA vs. GG)	0.005	70.4	REM	0.94 (0.43–2.04)	0.877
	Heterozygous (AG vs. GG)	0.104	45.2	FEM	0.73 (0.48–1.10)	0.134
	Dominant (AG + AA vs. GG)	0.016	64.3	REM	0.87 (0.44–1.71)	0.679
	Recessive (AA vs. AG + GG)	0.004	70.7	REM	1.10 (0.74–1.64)	0.647

ESR1: Estrogen receptor 1; RSA: Recurrent spontaneous abortion; REM: Random-effects model; FEM: Fixed-effects model; OR: Odds ratio; CI: Confidence interval; P value of overall effect.

RSA risk was stable and robust [Figure 3], and the association between the -351A/G (rs9340799) gene polymorphism and RSA risk was also stable and robust [Figure 4].

Publication bias

Funnel plots and Egger's linear regression tests were performed to assess the publication bias of the included studies. For rs2234693, the shapes of the funnel plots of the five genetic models seemed symmetrical, suggesting that there was no significant publication bias. Meanwhile, Egger's test also did not show strong, statistically significant publication bias in any of the five models (codominant genetic model [C vs. T], $t = -1.59$, $P = 0.173$; homozygous genetic model [CC vs. TT], $t = -0.99$, $P = 0.366$; heterozygous genetic model [CT vs. TT], $t = -1.02$, $P = 0.355$; dominant genetic model [CT + CC vs. TT], $t = -1.03$, $P = 0.351$; and recessive genetic model [CC vs. TT + CT], $t = -0.73$, $P = 0.496$). As for rs9340799, the shapes of the funnel plots and the results of Egger's test suggested that there was some

significant publication bias (codominant genetic model [A vs. G], $t = 1.52$, $P = 0.203$; homozygous genetic model [AA vs. GG], $t = 3.26$, $P = 0.031$; heterozygous genetic model [AG vs. GG], $t = 2.22$, $P = 0.090$; dominant genetic model [AG + AA vs. GG], $t = 3.07$, $P = 0.037$; recessive genetic model [AA vs. AG + GG], $t = 0.89$, $P = 0.425$) [Figure 5]. Therefore, we applied trim-and-fill analysis to the homozygous genetic model (AA vs. GG) and the dominant genetic models (AG + AA vs. GG) [Figure 6]. The data showed that there was no significant difference in pooled OR before and after the adjustment, indicating that the results were reliable and stable.

DISCUSSION

Along with progesterone, estrogen is one of the two most important female sex steroid hormones, both of which play crucial roles in the progression of the preimplantation phase and the maintenance of pregnancy.^[6] Estrogen

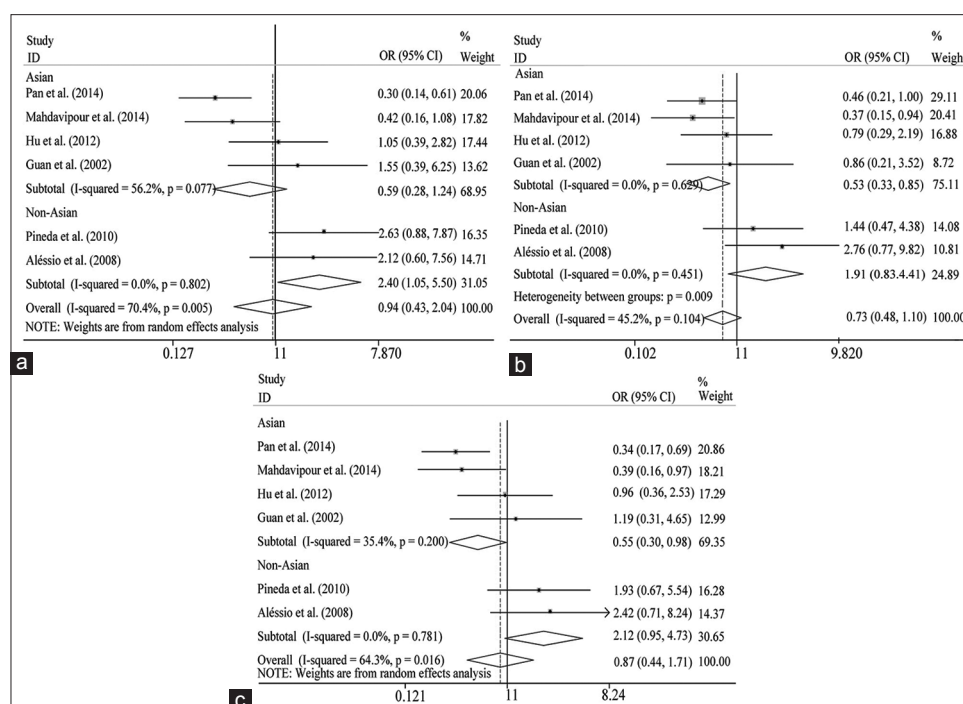


Figure 2: Forest plots of subgroup analysis for the associations between rs9340799 polymorphism and RSA risk. (a) The homozygous genetic model. (b) The heterozygous genetic model. (c) The dominant genetic model.

Table 3: Results of subgroup analysis for the *ESR1* polymorphisms and RSA risk

Gene polymorphisms	Inherited model	Subgroup	Heterogeneity-test		Analysis model	Pooled OR (95% CI)	P
			P for Q-test	I ² (%)			
rs2234693	Codominant (C vs. T)	Asian	0.042	63.4	REM	1.05 (0.78–1.40)	0.764
	Codominant (C vs. T)	Non-Asian	0.045	67.7	REM	0.88 (0.56–1.37)	0.567
	Homozygous (CC vs. TT)	Asian	0.081	55.5	REM	1.19 (0.67–2.13)	0.552
	Homozygous (CC vs. TT)	Non-Asian	0.083	59.9	REM	0.74 (0.31–1.74)	0.489
	Heterozygous (CT vs. TT)	Asian	0.023	68.6	REM	0.93 (0.58–1.50)	0.778
	Heterozygous (CT vs. TT)	Non-Asian	0.044	67.9	REM	0.98 (0.48–1.98)	0.949
	Dominant (CT + CC vs. TT)	Asian	0.018	70.4	REM	0.98 (0.61–1.55)	0.915
	Dominant (CT + CC vs. TT)	Non-Asian	0.025	72.9	REM	0.91 (0.44–1.90)	0.811
	Recessive (CC vs. TT + CT)	Asian	0.222	31.7	FEM	1.20 (0.85–1.70)	0.292
	Recessive (CC vs. TT + CT)	Non-Asian	0.193	39.2	FEM	0.79 (0.49–1.26)	0.319
rs9340799	Codominant (A vs. G)	Asian	0.001	81.7	REM	0.91 (0.58–1.42)	0.665
	Codominant (A vs. G)	Non-Asian	0.220	33.4	REM	1.36 (0.90–2.06)	0.142
	Homozygous (AA vs. GG)	Asian	0.077	56.2	REM	0.59 (0.28–1.24)	0.167
	Homozygous (AA vs. GG)	Non-Asian	0.802	0.0	REM	2.40 (1.05–5.50)	0.039
	Heterozygous (AG vs. GG)	Asian	0.629	0.0	FEM	0.53 (0.33–0.85)	0.009
	Heterozygous (AG vs. GG)	Non-Asian	0.451	0.0	FEM	1.91 (0.83–4.41)	0.130
	Dominant (AA + AG vs. GG)	Asian	0.200	35.4	REM	0.55 (0.30–0.98)	0.042
	Dominant (AA + AG vs. GG)	Non-Asian	0.781	0.0	REM	2.12 (0.95–4.73)	0.065
	Recessive (AA vs. AG + GG)	Asian	0.006	75.6	REM	1.01 (0.61–1.66)	0.973
	Recessive (AA vs. AG + GG)	Non-Asian	0.082	66.9	REM	1.34 (0.62–2.88)	0.456

ESR1: Estrogen receptor 1; RSA: Recurrent spontaneous abortion; REM: Random-effects model; FEM: Fixed-effects model; OR: Odds ratio; CI: Confidence interval; P value of overall effect.

exerts its function by binding to ESR1 and ESR2, which are members of the nuclear receptor protein superfamily of transcription factors.^[28] Infertility has been observed in *ESR1* knockout female mice, indicating that the function of estrogen is carried out by its receptor ESR1, and an extensive attention has been focused on elucidating the

association between *ESR1* polymorphisms and the risk of RSA. Due to inconsistent opinions on the role of *ESR1* polymorphisms in RSA risk, the present meta-analysis was performed to further elucidate the association between the effects of *ESR1* polymorphisms and the risk of RSA.

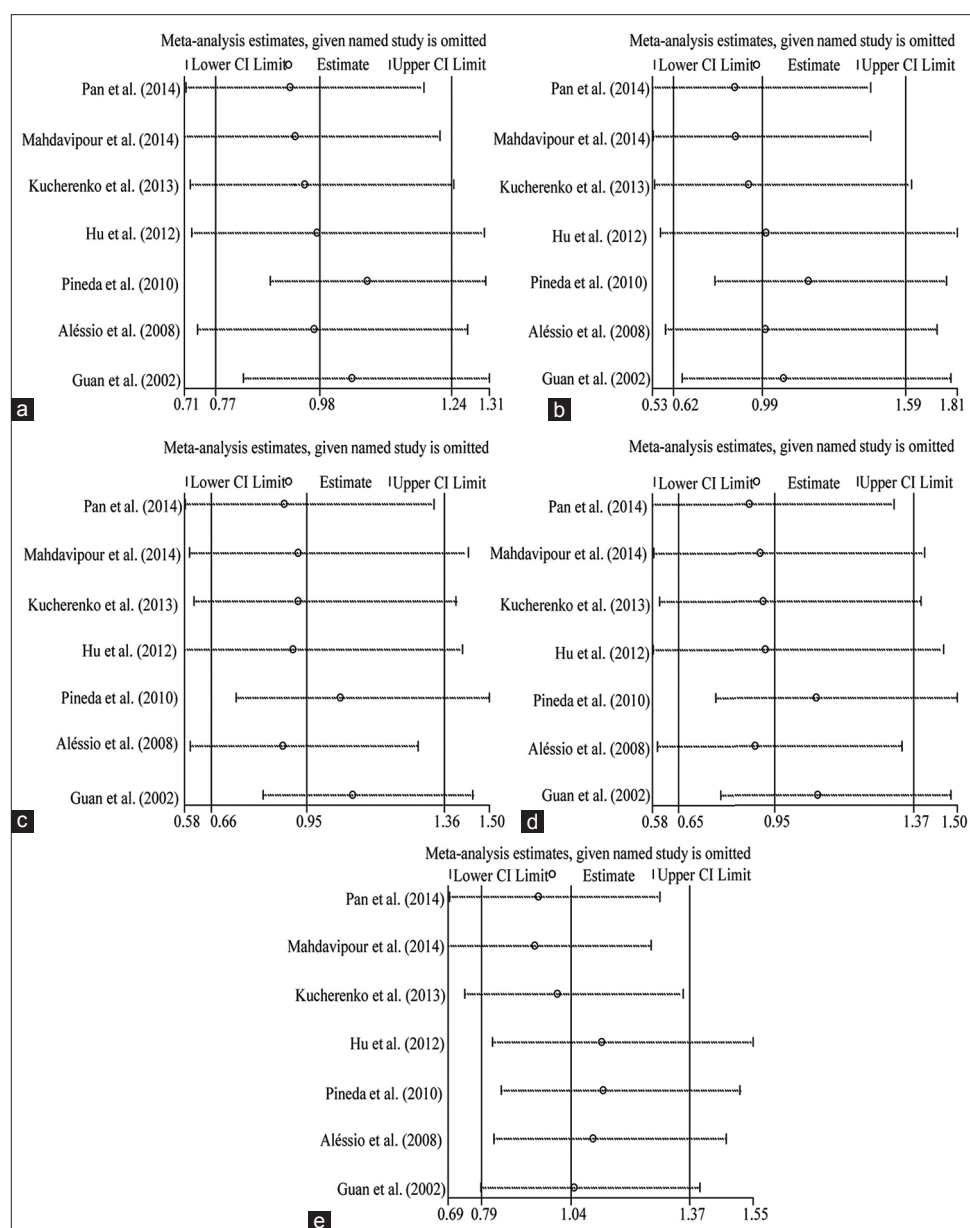


Figure 3: Sensitivity analysis for the associations between the rs2234693 polymorphism in the ESR1 gene and RSA risk. (a) The codominant genetic model. (b) The homozygous genetic model. (c) The heterozygous genetic model. (d) The dominant genetic model. (e) The recessive genetic model.

In the present meta-analysis, the association between the polymorphisms of *ESR1* (rs2234693 and rs9340799) and RSA risk was examined based on seven studies with a total of 836 RSA patients and 1164 healthy controls.^[15-21] Our results showed that, generally, the polymorphisms of *PvuII* (rs2234693) and the *XbaI* (rs9340799) in *ESR1* were not related to RSA risk. In the subgroup analysis, there was no significant association between the *PvuII* (rs2234693) polymorphism of *ESR1* and the risk of RSA in both Asian and non-Asian patients. However, subgroup analysis indicated that *XbaI* rs9340799 (-351A/G) polymorphism was related to increased RSA risk in non-Asian group but decreased RSA risk in Asian group. A possible explanation for this phenomenon is that the linkage disequilibrium patterns in alleles may differ between ethnic population, and RSA

polymorphisms may have opposite roles in RSA for Asians and non-Asians. However, it is also possible that some other factors may interfere with the analysis results. For example, the sample size and information were limited in the included studies, and it is difficult to determine the origin of the patients. Moreover, there was a substantial difference between the numbers of Asian and non-Asian RSA patients. Concerning the *XbaI* (rs9340799) polymorphism of *ESR1*, there were 638 Asian cases in the six relevant publications, while the number of non-Asian cases was 122. Therefore, the variation in quantity between the Asian and non-Asian subgroups may affect the results. Nevertheless, the genotype distribution of the controls generally did not deviate from HWE. Although there was publication bias according to Begg's funnel plot and Egger's linear regression test, the

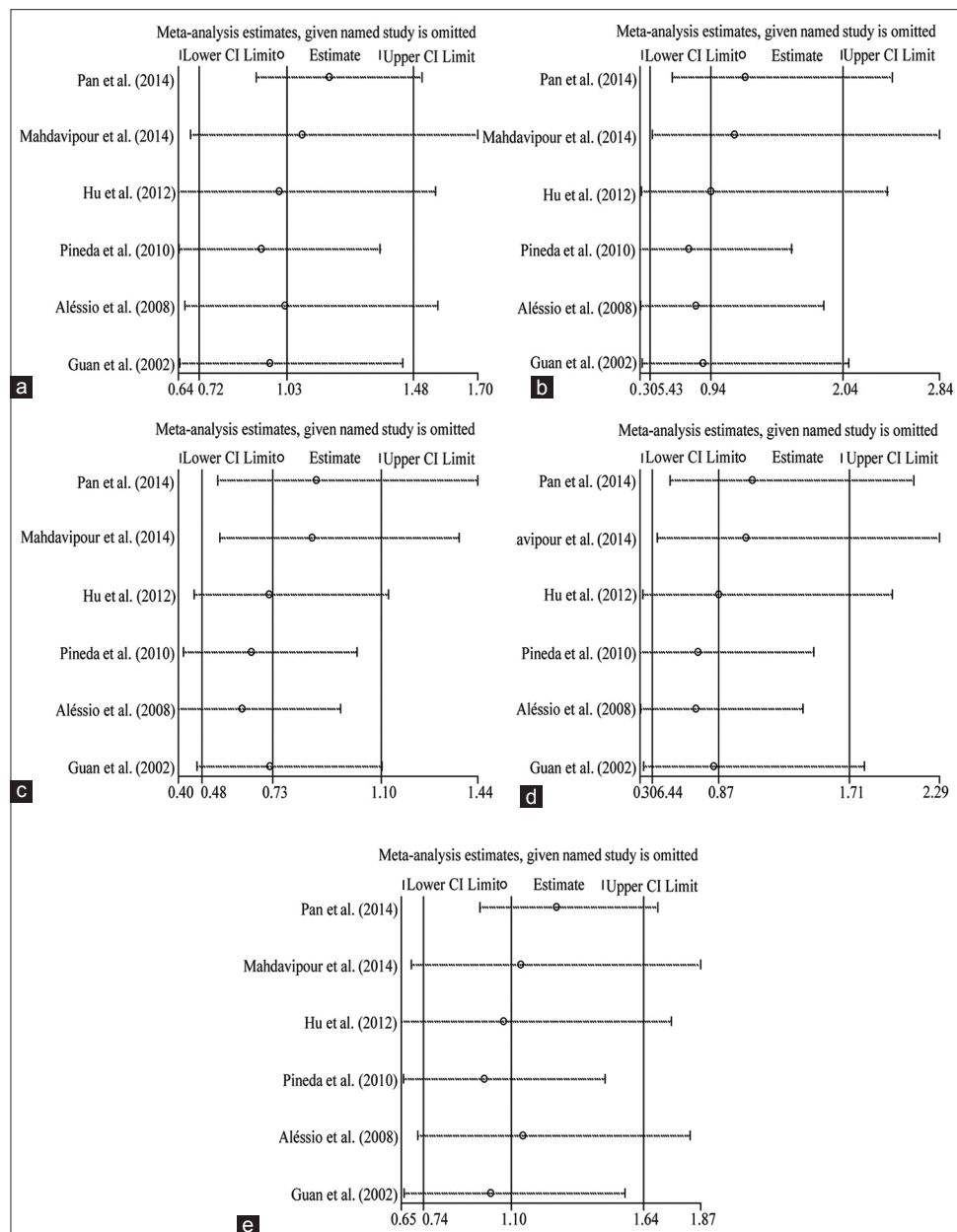


Figure 4: Sensitivity analysis for the associations between the rs9340799 polymorphism in the ESR1 gene and RSA risk. (a) The codominant genetic model. (b) The homozygous genetic model. (c) The heterozygous genetic model. (d) The dominant genetic model. (e) The recessive genetic model.

P value did not show any significant difference before and after “trim and fill” analysis, and the NOS score also indicated that the included studies were credible.

In summary, our findings revealed that different polymorphisms may have a different relationship with the risk of RSA in different ethnic groups. Generally, the *ESR1* rs2234693 (-397C/T) and rs9340799 (-351A/G) polymorphisms might not be associated with RSA risk. However, *ESR1* rs9340799 (-351A/G) polymorphism may play opposite roles in RSA in different ethnic populations. As for Asians, *ESR1* rs9340799 (-351A/G) polymorphism may decrease the risk of RSA; while for non-Asians, *ESR1* rs9340799 (-351A/G) polymorphism may increase the risk of RSA. Our findings contribute to a better understanding

of genetic polymorphisms of *ESR1* in RSA and pinpoint a novel biomarker and potential therapeutic target for RSA patients. Meanwhile, we are aware of several limitations of this study. First, the sample size of each individual study included in the current meta-analysis was relatively small, and the information on the patients was not adequate to perform more thorough subgroup studies based on factors such as age, the frequency of pregnancy and abortion, and environmental exposure to evaluate the heterogeneity among the included studies. Moreover, even though geographical information could be obtained from the included studies, ethnic origin of the RSA patients could not be acquired from the included studies. Therefore, additional well-designed studies with large sample sizes are required to further

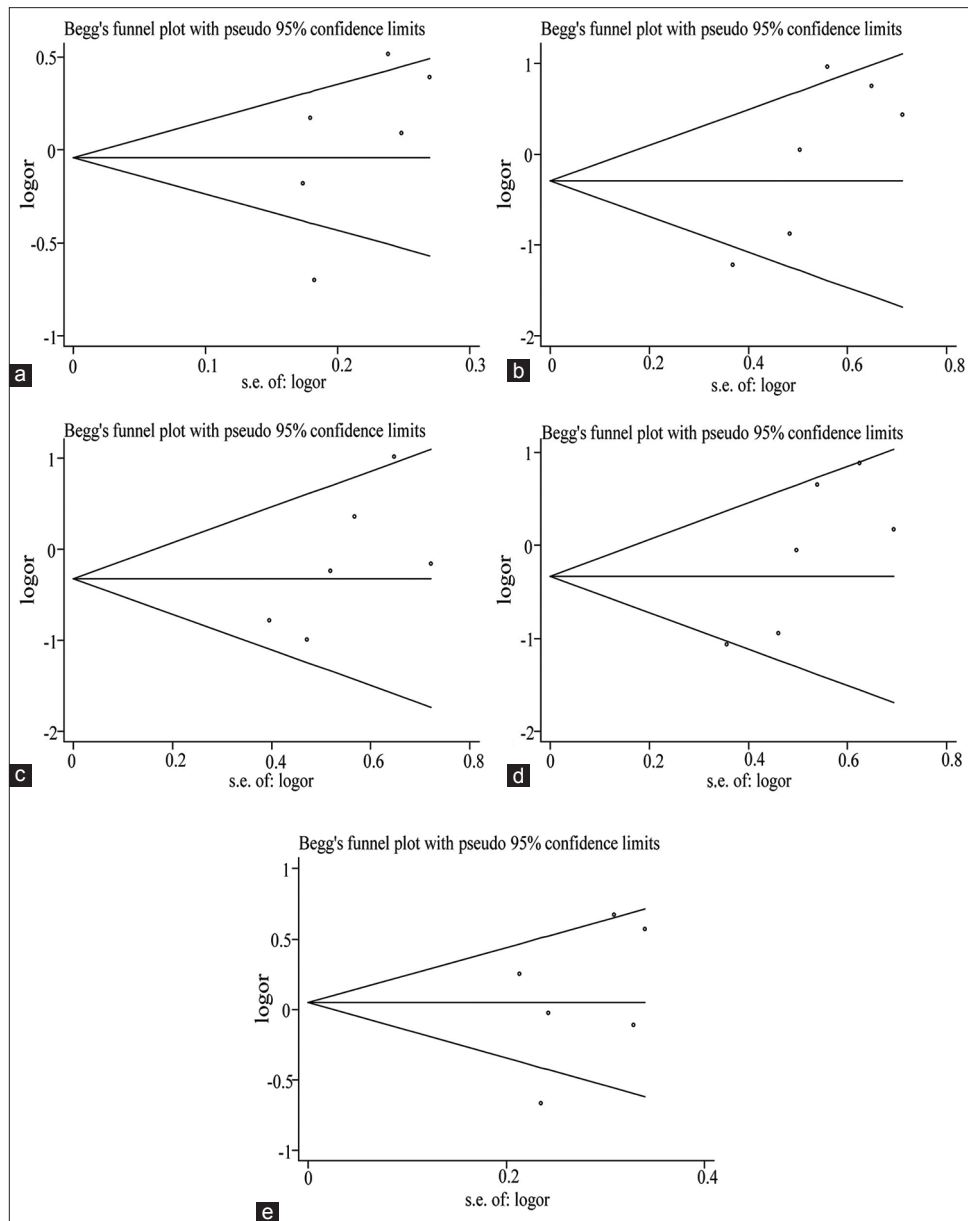


Figure 5: Funnel plots for the associations between the rs9340799 polymorphism in the ESR1 gene and RSA risk. (a) The codominant genetic model. (b) The homozygous genetic model. (c) The heterozygous genetic model. (d) The dominant genetic model. (e) The recessive genetic model.

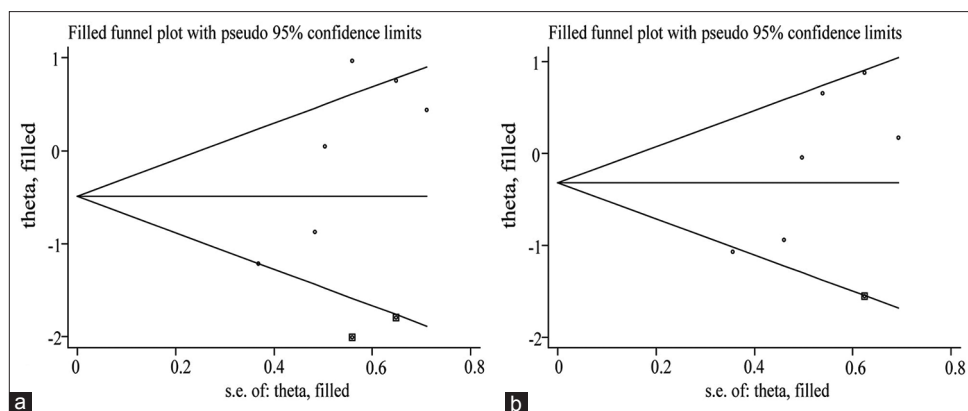


Figure 6: Funnel plots of trim and fill analysis for the associations between the rs9340799 polymorphism in the ESR1 gene and RSA risk. (a) The homozygous genetic model. (b) The dominant genetic model.

elucidate the association between *ESR1* polymorphisms and the risk of RSA.

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Conflicts of interest

There are no conflicts of interest.

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雌激素受体1基因多态性与复发性自然流产风险的Meta分析

摘要

背景：雌激素是一种重要的生殖甾体激素，在妊娠建立与维持中具有重要作用，其生物学效应主要是由雌激素受体1(*ESR1*)介导的。研究证实*ESR1*基因多态性与复发性自然流产(RSA)有关，但两者间关系仍存在争议。本研究旨在通过Meta分析阐明*ESR1 PvuII*(-397 C/T, rs2234693)和*XbaI*(-351 A/G, rs9340799)多态性与RSA风险的相关性，以期进一步阐释*ESR1*基因多态性在RSA发病中的作用，为进一步探索RSA发病机制及临床诊断与治疗提供科学依据。

方法：本研究纳入的文章为截止到2018年1月3日检索自Pubmed、Embase、Cochrane Library、中国知网和万方医学在线数据库的文献，运用STATA 12.0软件进行数据处理，并采用固定效应(FEM)/随机效应模型(REM)计算比值比(ORs)和95%可信区间(95% CIs)。

结果：共纳入7篇病例对照研究，包括836例复发性自然流产病例和1164名正常生育妇女对照。Meta分析结果表明，对于任何一种遗传分析模型来说，*ESR1*多态性与RSA发病风险均不相关。然而，对不同地域进行亚组分析发现，对于rs9340799多态性来说，在非亚洲组中纯合遗传模型(OR = 2.40, 95% CI = 1.05-5.50, $P = 0.039$)显示与RSA风险增加显著相关;此外，在亚洲组中杂合遗传模型(OR = 0.53, 95% CI = 0.33-0.85, $P = 0.009$)和显性遗传模型(OR = 0.55, 95% CI = 0.30-0.98, $P = 0.042$)与RSA风险降低相关。

结论：总的来说，*ESR1*基因多态性与RSA风险无明显相关。然而，亚组分析表明*ESR1* rs9340799多态性与非亚洲人RSA风险增加相关，而与亚洲人RSA风险降低相关，提示种族遗传差异可能是影响*ESR1*基因多态性与RSA风险的因素，但仍需扩大样本进一步证实。