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The haplotypes GCA and ACA in ESR1 gene are associated with the susceptibility of recurrent spontaneous abortion (RSA) in Chinese Han
A case-control study and meta-analysis

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Abstract
Estrogen receptor is involved in the pathogenesis of recurrent spontaneous abortion (RSA). The ESR1 and ESR2 genes can mediate nongenomic estrogen responses. This study aimed to assess the genetic association between the ESR1 and ESR2 genes polymorphisms and RSA susceptibility in a Chinese Han population. A total of 258 women who had experienced RSA and 264 unrelated healthy women were recruited. Genotypes of the 6 polymorphisms in the ESR1 (rs9340799, rs2234693, and rs3798759) and ESR2 genes (rs207764, rs4986938, and rs1256049) were analyzed using Snapshot technology. No association was detected between the alleles and genotypes of ESR1 rs9340799, rs2234693, and rs3798759 polymorphims and RSA risk ($P > .05$). Subjects carrying the haplotype of rs9340799A-rs2234693C-rs3798759A had a significantly increased RSA risk in the case group compared with the control group ($P = .0005$, $P_{adj} = .003$, odds ratios [95% CI] = 0.35 [0.19–0.65]). However, subjects carrying the haplotype of rs9340799G-rs2234693C-rs3798759A had a significantly decreased RSA risk in the case group compared with the control group ($P = .0005$, $P_{adj} = .003$, odds ratios [95% CI] = 2.99 [1.57–5.70]). In addition, no association was found between the alleles, genotypes, and haplotypes of ESR2 rs207764, rs4986938, rs1256049 polymorphisms and RSA risk ($P > .05$). In conclusion, the haplotype rs9340799A-rs2234693C-rs3798759A of ESR1 might be a risk factor. And the haplotype rs9340799G-rs2234693C-rs3798759A of ESR1 might be a protective factor for RSA in a Chinese Han population.

Abbreviations: ER = estrogen receptor, HWE = Hardy–Weinberg equilibrium, OR = odds ratios, RSA = recurrent spontaneous abortions.

Keywords: Chinese Han, ESR1, ESR2, polymorphism, recurrent spontaneous abortion

1. Introduction

Recurrent spontaneous abortions (RSAs) are spontaneous abortions that occur 3 or more times in a row with the same partner and affect approximately 1% to 2% of couples.[1,2] Complex etiologies including infection,[3] autoimmune diseases,[4] environmental factors,[5] and chromosomal abnormalities,[6,7] are associated with RSA risk. In addition, 50% affected women with RSA have no specific causes, which is defined as unexplained spontaneous recurrent abortion.[8]

Abnormal expression of estrogen receptor (ER) has been reported in the pathogenesis of unexplained spontaneous recurrent abortion.[9] Estrogen maintains the growth and development of relevant target tissues through the action of ER. When the amount of ER in decidual tissues decreases, the

physiological function of the corresponding hormones will be affected.[10] Two nuclear ER-α and ER-β were shown to mediate the effects of estrogen.[11] ESR gene knockout in animals showed that ESR1 gene (6q25.1), which encodes ER-α, is necessary for the full development of thymus and spleen,[12] while ESR2 gene (14q22–24), which encodes ER-β, is necessary for estrogen-mediated thymic cortical atrophy and thymic cell phenotype transfer.[13]

Numerous polymorphisms in the ESR1 and ESR2 genes have been described. Pineda et al[14] has indicated that the ESR1 rs2234693 (PvuII) and rs9340799 (XbaI) were significantly associated with RSA risk in a Spanish population. Additionally, a haplotype (rs2234693T-rs9340799A) was revealed to be associated with an increased RSA risk.[14] However, there was no genetic association between the ESR1 gene polymorphisms and RSA risk in the Brazilian population.[15] The results of the correlation of ESR1 rs2234693, rs3798759, and rs9340799 and RSA risk in Chinese populations were inconsistent and inconclusive.[16–20] Regarding the ESR2 gene, no association has been identified between polymorphisms, including rs1256049, rs4986938, and rs207764, and RSA risk in Chinese Han,[20,21] Iranian,[22] and Brazilian[15] populations. However, a haplotype consisting of ESR2 rs2077647A-rs4986938G-rs1256049T significantly decreased the risk of RSA in a Chinese Hui population.[19]

As it is of pivotal importance that genetic associations are confirmed in independent cohorts from different countries, we performed this investigation to determine the relationships of ESR1 rs9340799, rs2234693, rs3798759 polymorphisms and ESR2 rs207764, rs4986938, rs1256049 polymorphisms and RSA susceptibility in a Chinese Han population.

2. Materials and methods

2.1. Patient and public involvement

The study protocol was approved by the Ethics Committee of The Changsha Medical University (EC-18-036, 07/10/2018) and was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Informed written consent and written informed consent for genetic analysis were obtained from all participants. Females who had experienced at least three consecutive miscarriages and had no living children were recruited from the first affiliated hospital of Changsha medical university in the present study. Subjects who had preeclampsia, ectopic pregnancy or premature delivery, endocrine abnormalities, infectious diseases, chromosomal abnormalities, or autoimmune diseases were excluded. Ultimately, a total of 258 women (mean age: 27.4 ± 0.7 years) who had experienced RSA were recruited. Among them, 210 subjects had experienced three miscarriages, 31 subjects had experienced 4 miscarriages, 14 subjects had experienced 5 miscarriages, and the remaining 3 women had experienced 6 miscarriages.

In addition, 264 healthy nonpregnant women (mean age: 28.1 ± 1.3) with 2 or more successful pregnancies and live births were selected as the control group according to the age of subjects in the case group. Subjects who had miscarriage, preeclampsia, ectopic pregnancy or premature delivery, endocrine abnormalities, infectious diseases, chromosomal abnormalities, or autoimmune diseases were excluded.

2.2. Genotyping

Six polymorphisms in ESR1 (rs9340799, rs2234693, and rs3798759) and ESR2 (rs2077647, rs4986938, and rs1256049) genes were selected in the present study according to Tang et al[19] Peripheral blood samples were obtained from all subjects and analyzed the distributions of ESR1 and ESR2 genes polymorphisms in a Chinese Han population. Genomic DNA was extracted by standard phenol–chloroform method. Genotyping of the ESR1 and ESR2 polymorphisms was determined by the SNaPshot method. The GeneAmp 9700 polymerase chain reaction thermocycler (Applied Biosystems, Foster City, CA) was used for polymerase chain reaction. The 3730XL genetic analyzer (Applied Biosystems, Foster City, CA) was used for sequencing. GeneScan 3.7 Software (Applied Biosystems, Foster City, CA) was used for data analysis.

2.3. Statistical analysis

Statistical analyses were performed with Shesis software (http://analysis.bio-x.cn/myAnalysis.php). The $\chi 2$ test was used to analyze the the significance of Hardy-Weinberg equilibrium (HWE). A two-sided Fisher exact test was used to assess the distributions of the alleles and genotypes of the ESR1 and ESR2 polymorphisms between patients and controls. Genetic associations between the ESR1 and ESR2 polymorphisms and RSA risk were assessed by corresponding odds ratios (ORs) and 95% confidence intervals (CIs) via a standard logistic regression analysis. The P value was corrected by a stringent Bonferroni correction in multiple comparisons in cases where a significant relationship was found. Haploview 4.2 software was used for linkage disequilibrium analysis. Calculation power was obtained at the 0.05 level of significance, assuming an OR of 1.5 (small effect size) by using the G*Power software (www.gpower.hhu.de). A $P < .05$ indicates a statistical significance.

2.4. Meta-analysis

A meta-analysis was performed by including the data from the present study and studies documented to the associations between ESR1 or/and ESR2 polymorphisms and RSA from PubMed, China National Knowledge Infrastructure , and the Cochrane Library databases. The meta-analysis was performed out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses . The following keywords were used in literature search: Estrogen receptor 1; ESR1; Estrogen receptor 2; ESR2; Recurrent spontaneous abortion; RSA; miscarriage; pregnancy loss; polymorphism; single nucleotide polymorphisms; single nucleotide polymorphisms before February 01, 2022. No limit was applied for language. Studies included in the present meta-analysis should meet the following criteria: case-control design; available genotype frequencies in both case and control groups; the frequency of allele in the control group should be in HWE. Exclusion criteria: repeat studies, abstracts, letters, or reviews. Publishes against to the inclusion criteria.

The Stata software (version 12.0; Stata Corp LP, College Station, TX) and Revman software (version 5.3) were used in the present meta-analysis. Crude OR and 95% CIs were calculated to test the strength of associations between ESR1/2 genes polymorphisms and RSA risks. The statistical significance of the pooled ORs under different genetic models (allelic, recessive,

additive, and dominant models) were determined by Z test and considered significant when $P<.05$. A test of heterogeneity was conducted using Cochran Q test and Higgins I-squared statistic. I^2 value of $>50\%$ indicates heterogeneity among studies. A random effect model was applied if significant heterogeneity was observed ($I^2>50\%$, $P<.05$). Otherwise the fixed effect model was used. Sensitivity analysis was performed to assess the effects of individual study on pooled results and the stability of results. Publication bias was accessed with funnel plots by Begg test and Egger test.

3. Results

The genotype distributions of all the included polymorphisms were in HWE ($P>.05$). Linkage disequilibrium analysis indicated that ESR1 rs9340799 and rs2234693 were in strong linkage (D'>0.80) (Fig. 1A). And, ESR2 rs4986938 and rs1256049 were in weak linkage (D'$=0.56$) (Fig. 1B).

No associations were detected between the alleles of ESR1 (rs9340799, rs2234693, and rs3798759) and ESR2 (rs2077647, rs4986938, and rs1256049) genes polymorphisms and RSA risk ($P>.05$). In addition, the distributions of the additive, dominant, and recessive models of ESR1 (rs9340799, rs2234693, and rs3798759) and ESR2 (rs2077647, rs4986938, and rs1256049) genes polymorphisms in the case group were similar to those in the control group ($P>.05$). The results did not alter significantly, even after Bonferroni correction (Tables 1 and 2).

Haplotypes containing ESR1 (rs9340799, rs2234693, and rs3798759) and ESR2 (rs2077647, rs4986938, and rs1256049) were analyzed separately. The distribution of the haplotype defined by rs9340799G, rs2234693C, and rs3798759A was significantly lower in the case group than that in the control group ($P=.0005$, $P_{adj}=.003$, OR [95% CI] $=0.35[0.19–0.65]$). A significant difference was found for the frequency of the haplotype defined by rs9340799A, rs2234693C, and rs3798759A between the case and control groups ($P=.0005$, $P_{adj}=.003$, OR [95% CI] = 2.99[1.57–5.70]) (Table 3).

As shown in Figure 2, a total of 7 studies were finally retrieved from the databases.[15–18,20–22] Among the included studies, 3 studies reported the association between ESR1 polymorphisms and RSA risk.[16–18] And 3 articles reported the association between ESR2 polymorphisms and RSA risk.[20–22] One study reported the association between both ESR1 and ESR2 polymorphisms and RSA risk.[15] Five studies reported the association between ESR1 rs9340799, rs2234693 and ESR2 rs1256049, rs4986938 polymorphisms, and RSA risk. No publications were detected for the association between the ESR1 rs3798759 and ESR2 rs2077647 polymorphisms and RSA risk. The information such as first author's name, year, ethnicity, number of case and control, genotyping methods, mean ages in the included studies were summarized in Table 4. The combined results indicated that the allelic, dominant, additive, and recessive models of ESR1 rs9340799, rs2234693, and ESR2 rs1256049 were not associated with RSA risk ($P>.05$). While, significantly decreased risk of RSA was detected in subjects who carrying the additive model ($P=.002$, OR [95% CI]$=0.49[0.21, 0.76]$) and recessive model ($P=.0002$, OR [95% CI] $=0.46[0.30, 0.69]$) of ESR2 rs4986938 (Fig. 3 and Table 5).

Significant heterogeneity was observed in all genetic models (except for the recessive model) of ESR1 rs2234693 and ESR1 rs9340799 ($I^2>50\%$). After excluding the study conducted by Pan et al.[16] The significant heterogeneities disappeared ($I^2<50\%$). In addition, significant heterogeneity was observed in the allelic additive and dominant models of ESR2 1256049 polymorphism ($I^2>50\%$). The heterogeneities in these models were contributed mainly by Morandi Alessio et al.[15] Removal of this study from meta-analysis gave $<50\%$ heterogeneities (Table 5).
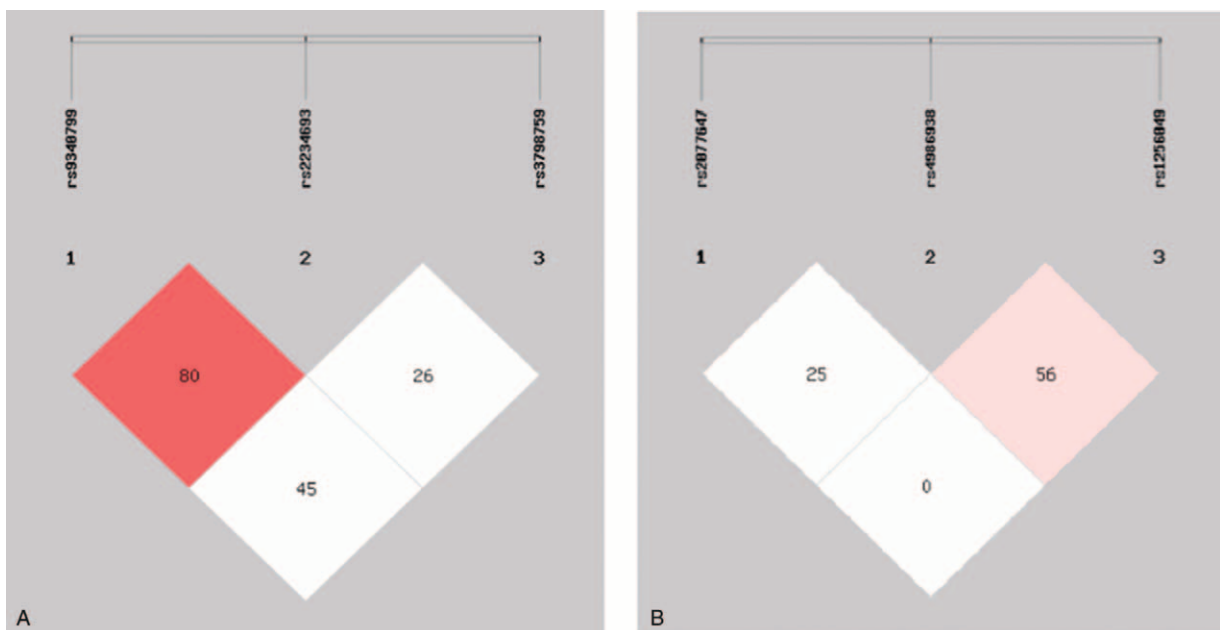

Figure 1. Pairwise linkage disequilibrium of ESR1 and ESR2 single-nucleotide polymorphisms in relation to recurrent spontaneous abortion. The numbers in the squares refer to the pairwise linkage disequilibrium measured as D'. Haplotype blocks were defined using a setting pairwise D'. A, Linkage disequilibrium of ESR1 gene. B, Linkage disequilibrium of ESR2 gene.

Table 1

Distributions of the *ESR1 and ESR2* SNPs in case and control.

Genes	SNP ID	Position	Minor Allele	MAF (frequency) Case	MAF (frequency) Control	P	P (corrected)[a]	OR[b] (95% CI)	Power
ESR1	rs9340799 (A > G)	6:151842246	G	0.203	0.252	.06	–	0.68 (0.41–1.12)	64.8
	rs2234693 (T > C)	6:151842200	C	0.412	0.392	.49	–	1.14 (0.75–1.75)	
	rs3798759 (A > C)	6:151959418	C	0.302	0.304	.94	–	1.24 (0.78–1.96)	
ESR2	rs2077647 (T > G)	14:151807942	G	0.373	0.405	.29	–	1.04 (0.67–1.61)	
	rs4986938 (C > T)	14:64233098	T	0.109	0.147	.07	–	0.91 (0.46–1.79)	
	rs1256049 (C > T)	14:64257333	T	0.364	0.397	.27	–	1.22 (0.79–1.87)	

−= not significant, 95% CI = 95% confidence intervals, MAF = minor allele frequency, OR = odds ratio, SNP = single-nucleotide polymorphism.
[a] The Bonferroni's correction was carried out to correct the P value.
[b] OR and 95% CI are calculated for the minor allele of each SNP.

Table 2

Frequency of *ESR1 and ESR2* genes genotypes in cases and controls.

Ethics	SNPs, rs, (A > B)	Genotypes Case (AA/AB/BB)	Genotypes Control (AA/AB/BB)	OR [95% CI], P (Addmodel) BB/AA	P[a] (adj)	OR [95% CI], P (Dommodel) BB+AB/AA	P[a] (adj)	OR [95% CI], P (Recmodel) BB/AB+BB	P[a] (adj)
ESR1	rs9340799 (A > G)	168/75/15	149/97/18	0.59[0.17–2.01],.40	–	1.40[0.41–4.69],.58	–	0.61[0.33–1.13],.12	
	rs2234693 (T > C)	96/110/51	105/111/48	1.29[0.57–2.94],.53	–	0.78[0.37–1.64],.52	–	1.11[0.61–2.05],.73	
	rs3798759 (A > C)	132/96/30	134/99/31	0.75[0.29–1.90],.54	–	1.20[0.49–2.94],.69	–	0.76[0.42–1.37],.36	
ESR2	rs4986938 (C > T)	208/44/6	195/60/9	0.42[0.08–2.22], –	–	2.53[0.48–13.41], –	–	1.11[0.51–2.42],.79	–
	rs1256049 (C > T)	97/134/27	77/145/42	0.57[0.21–1.55],.27	–	1.62[0.66–3.96],.29	–	0.82[0.43–1.56],.55	–
	rs2077647 (T > G)	109/107/43	97/112/45	0.91[0.41–2.05],.82	–	1.13[0.53–2.38],.76	–	1.01[0.55–1.83], 1.00	

−= not significant, A = minor allele, B = major allele, SNP = single-nucleotide polymorphism.
[a] The Bonfferoni correction was applied to correct the P value.

Sensitivity analysis on the overall risk estimate by excluding 1 study at a time was confirmed. The ORs were not significantly altered (Fig. 4A–D). We performed the Begg and Egger tests to evaluate the publication bias. None of funnel plots explored the evidence of publication bias for ESR1 and ESR2 polymorphims and RSA risk (Fig. 4E–H).

Table 3

Frequencies of haplotypes (>1%) containing SNPs in *ESR1 and ESR2* and association between cases and controls.

Gene	Haplotype[a]	Case (freq.)	Control (freq.)	P[b]	P value (corrected)[c]	OR [95% CI]
ESR1	ACA	0.100	0.243	.0005	.003	0.35[0.19–0.65]
	GCA	0.214	0.085	.0005	.003	2.99[1.57–5.70]
	GCC	0.041	0.054	.59	–	0.76[0.28–2.07]
	GTA	0.385	0.334	.26	–	1.28[0.83–2.00]
ESR2	TTC	0.064	0.060	.88	–	1.07[0.45–2.56]
	TCC	0.352	0.252	.04	.24	1.63[1.02–2.59]
	TCT	0.199	0.288	.05	–	0.61[0.37–1.01]
	GTC	0.020	0.033	.43	–	0.58[0.15–2.56]
	GCC	0.174	0.217	.31	–	0.76[0.45–1.29]
	GCT	0.170	0.130	.29	–	1.37[0.76–2.49]

−= not significant, 95% CI = 95% confidence intervals, OR = odds ratio, SNP = single-nucleotide polymorphism.
[a] The program, Plink, was used to estimate common (frequency > 0.01) haplotypes constructed by three SNPs of ESR1 (rs9340799, rs2234693, rs3798759) and *ESR2 (*rs2077647, rs4986938, rs1256049*).*
[b] Each haplotype was compared with the other haplotypes combined.
[c] The Bonfferoni correction was applied to correct the P value.

4. Discussion

In the present study, we have investigated the association between the ESR1 (rs9340799, rs2234693, and rs3798759) and ESR2 (rs2077647, rs4986938, and rs1256049) gene polymorphisms and the risk of RSA. Haplotype containing ESR1 rs9340799G-rs2234693C-rs3798759A was demonstrated to be a protective factor for RSA, and haplotype containing ESR1 rs9340799A-rs2234693C-rs3798759A was demonstrated to be a risk factor for RSA in Chinese Han population. Additionally, the additive and recessive models of ESR2 rs4986938 were protective factors for RSA based on the results of meta-analysis.

ESR gene polymorphisms are associated with multiple reproductive traits, including endometriosis,[23] premature ovarian failure,[24] spermatogenic defect,[25] and uterine leiomyoma.[26] Nilsson et al[27] have suggested that ESR gene polymorphisms may lead to dysregulation of gene transcription and translation, which may lead to abnormal expression and function of ER. Lehrer et al [28] found that the polymorphisms of the B region of the ESR gene were associated with breast cancer patients with ER+ with a history of recurrent abortion. Later, Berkowitz et al [29] determined that the changes of ESR gene polymorphisms detected by the same method were not statistically correlated with RSA in an expanded sample. Yet, Lehrer et al [28] also suggested that there is a polymorphic site that may change the function of ER in an unknown part of the ESR gene.

The rs9340799 and rs2234693 polymorphisms are located in the enhancers and promoters of the ESR1 gene separately, which
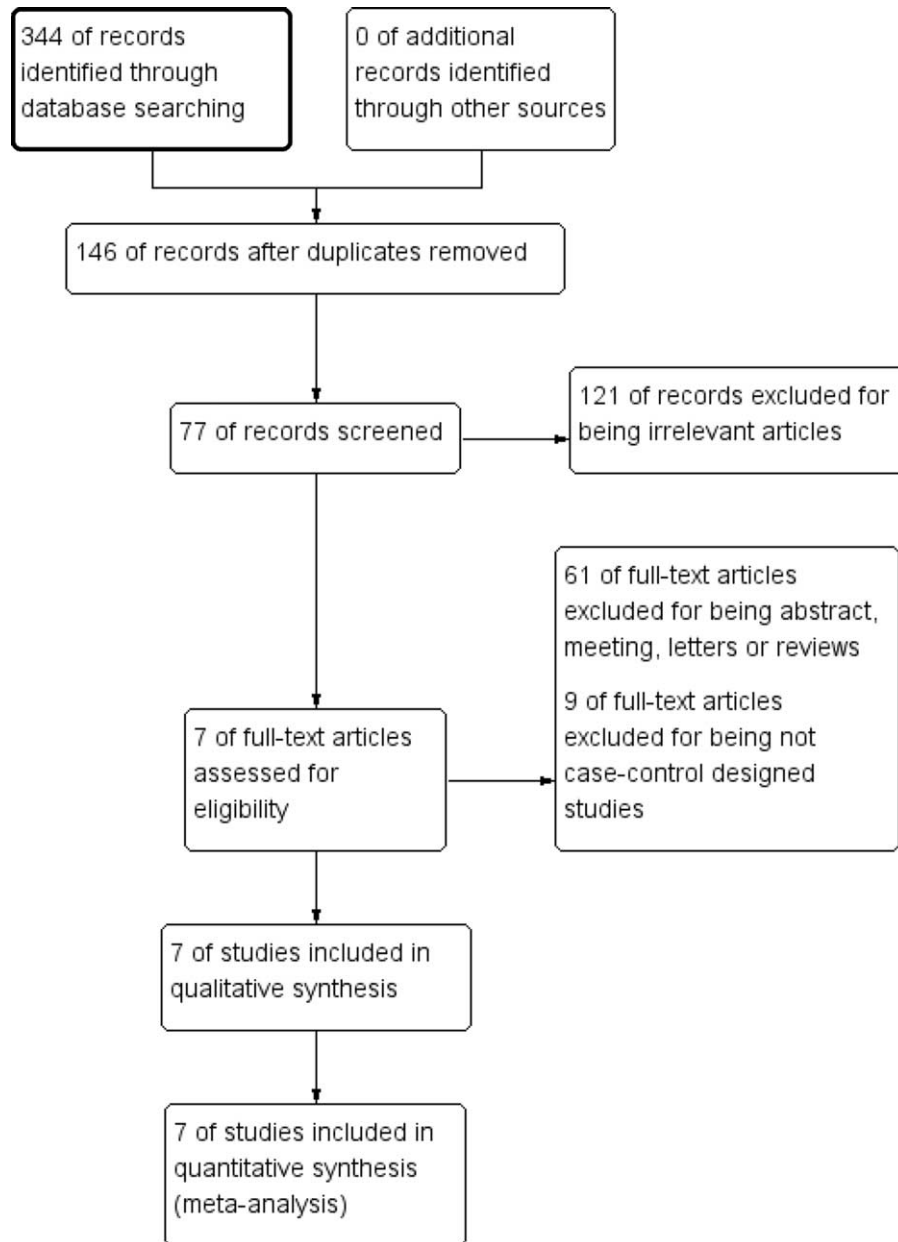
Figure 2. PRISMA flow chart of studies inclusion and exclusion.

Table 4						
Characteristics of included studies.						
First author	**Year**	**Ethnicity**	**Case**	**Control**	**Genotyping methods**	**Age (case/control)**
Guan	2002	Chinese	69	73	PCR-RFLP	29.06 ± 4.46/NA
Liu	2010	Chinese	52	37	PCR-RFLP	28.2 ± 4.7/29.5 ± 7.1
Morandi Alessio	2008	Brazilian	75	75	PCR-RFLP	NA
Pan	2014	Chinese	129	183	PCR-RFLP	NA
Guo	2018	Chinese	85	85	PCR-RFLP	32.54 ± 1. 54/32.4 ± 2. 9
Hu	2012	Chinese	196	182	PCR-RFLP	28.0 ± 4.3/30.1 ± 4.1
Mahdavipour	2016	Iranish	237	102	PCR-RFLP	33.3 ± 0.4/39.2 ± 0.6

NA = not applicable, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism.
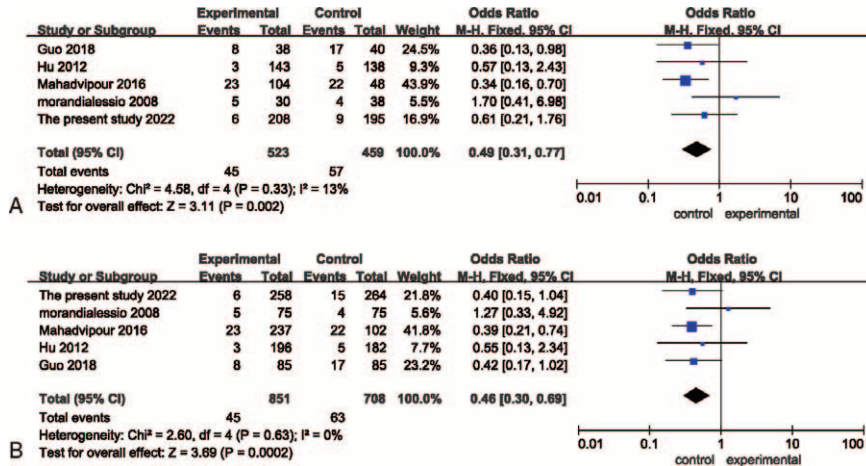
Figure 3. Meta-analysis results of the association between additive and recessive models of ESR2 rs4986938 and RSA. A, Additive model. B, Recessive model.

Table 5

The association between ESR1 and ESR2 polymorphisms and RSA: a meta-analysis.

Polymorphism	Genotype	Number of studies	Test of association			Model	Test of heterogeneity	
			OR	95% CI	P value		P value	I² (%)
ESR1 rs2234693	Allelic model	5	0.93	[0.69, 1.26]	.63	R	.02	65
	Additive model	5	0.77	[0.31, 1.90]	.57	R	.002	76
	Dominant model	5	0.88	[0.53, 1.46]	.61	R	.005	73
	Recessive model	5	1.04	[0.76, 1.41]	.81	F	.31	17
ESR1 rs9340799	Allelic model	5	1.03	[0.66, 1.60]	.89	R	.0004	81
	Additive model	5	1.09	[0.40, 2.89]	.87	R	.0008	79
	Dominant model	5	1.06	[0.63, 1.70]	.89	R	.003	75
	Recessive model	5	1.04	[0.52, 2.11]	.91	R	.03	62
ESR2 rs1256049	Allelic model	5	0.97	[0.65, 1.47]	.90	R	.04	60
	Additive model	5	0.52	[0.20, 1.34]	.18	R	.10	57
	Dominant model	5	1.08	[0.63, 1.85]	.79	R	.02	65
	Recessive model	5	0.68	[0.44, 1.05]	.08	F	.65	0
ESR2 rs4986938	Allelic model	5	0.89	[0.74, 1.06]	.18	F	.26	24
	Additive model	5	0.49	[0.21, 0.76]	.002	F	.33	13
	Dominant model	5	1.01	[0.81, 1.26]	.95	F	.22	30
	Recessive model	5	0.46	[0.30, 0.69]	.0002	F	.63	0

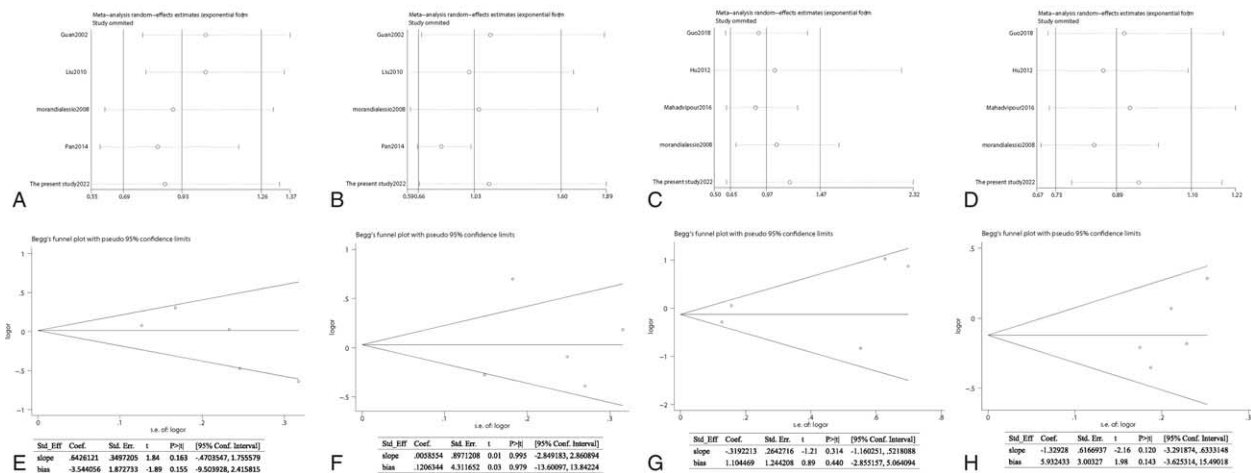CI = confidence interval, F = fixed model, OR = odd ratio, R = random model.

Figure 4. Sensitivity analyse and publication bias of the meta-analysis. A–D, Sensitivity analyses of the ESR1 and ESR2 polymorphims. (A) rs2234693, (B) rs9340799, (C) rs1256049, (D) rs4986938. E–H, Publication bias of literatures for ESR1 and ESR2 polymorphims was tested by Begg funnel plot and Egger test. (E) rs2234693, (F) rs9340799, (G) rs1256049, (H) rs4986938.

are closely related to the transcriptional regulation of the gene.[30] The variants in this region can directly affect the function and quantity of ER and lead to increased susceptibility to diseases. Nilsson et al [31] found that the rs2234693 polymorphism contains a transcription factor Myb-binding site, which can affect Myb-induced transcription activity, thereby affecting ESR1 gene expression. In the present study, we found that the ESR1 rs2234693 was unrelated to RSA in the Chinese Han population, which was similar to the recent results reported by Tang et al [19] in a Ningxia Chinese Han population. However, our results are different from results reported by Pineda et al,[14] Pan et al,[16] Guan et al,[17] and Liu et al.[18] These inconsistent results in previous studies may indicate the important role of gene backgrounds in the pathogenesis of RSA. Interestingly, conflicting results were shown in Chinese Han populations. The complex genetic ethnic specificity in Chinese Han populations might contribute to the difference. In addition, the relatively small sample size in previous studies may influence the results. Therefore, we performed a meta-analysis to precisely evaluate the association between ESR1 polymorphisms and RSA risk. The combined results showed that the ESR1 rs2234693 was not associated with RSA risk, which may reveal that ESR1 rs2234693 polymorphism is not a susceptibility factor for RSA. In previous studies, only 1 study conducted by Pineda et al[14] reported significant association between the ESR1 rs2234693 and RSA risk in a Caucasian population. However, negative results were obtained in other studies in Asian populations. Our results may supported the results that the ESR1 rs2234693 was not a risk factor for RSA in Asian populations. Furthermore, we found that the ESR1 rs9340799 was not associated with RSA risk. This is the same as the results reported by Pineda et al,[14] Pan et al,[16] Guan et al,[17] and Liu et al.[18] The results of meta-analysis also support this conclusion. Thus, we may conclude that the ESR1 rs9340799 polymorphism was not a susceptible factor for RSA.

Haplotype containing ESR1 rs9340799G-rs2234693C-rs3798759A was demonstrated to be a protective factor for RSA, and haplotype containing ESR1 rs9340799A-rs2234693C-rs3798759A was demonstrated to be a risk factor for RSA in the Chinese Han population. In a previous study conducted by Pineda et al,[14] the haplotype containing ESR1 rs9340799A-rs2234693T was implicated to be associated with an increased risk of spontaneous abortion in Spanish ancestry.[14] In addition, Pan et al [16] documented that the haplotype containing ESR1 rs9340799G-rs2234693C was associated with an increased risk of RSA, and the haplotype containing ESR1 rs9340799A-rs2234693T was associated with a lower risk of RSA. Yet, no association was detected between the haplotypes consisting of ESR1 rs9340799, rs2234693, and rs3798759 and RSA risk in the Chinese Han population.[19] Our results were different from those reported by Pineda et al [14] and Pan et al,[16] but were similar with those reported by Tang et al.[19] These inconsistencies may be due to the differences in subject selection criteria and ethnic variation in individual studies.

The essential roles of ER-β in normal ovulation efficiency and in the regulation of follicular growth and oocyte development are well documented.[32,33] Reduced fertility was observed in mice lacking ER-β.[34] The genetic variants of the ESR2 gene and their associations with ovulatory dysfunction, especially those with unknown causes and pregnancy outcomes, have been investigated.[35–36] The distributions of rs1256049 and rs4986938 were 36.4% and 10.9% in the Chinese Han

population, which were similar to those in studies conducted by Hu et al,[20] but were different from those reported by Morandi Alessio et al,[15] Tang et al,[19] Guo and Ali,[21] and Mahdavipour et al.[22] The inconsistencies may be due to the different gene backgrounds in multiple populations. In the present study, negative results were found between the allelic, additive, dominant, and recessive models of ESR2 rs2077647, rs4986938, and rs1256049 polymorphisms and RSA risk in the Chinese Han population, which were similar to those in studies conducted by Morandi Alessio et al,[15] Tang et al,[19] Hu et al,[20] Guo and Ali,[21] and Mahdavipour et al.[22] These results may indicate that the ESR2 rs2077647, rs4986938, and rs1256049 were not the susceptible factors for RSA in the Chinese Han population.

Regarding the relatively small sample sizes in previous studies, we also evaluated the relationship of ESR2 rs4986938 and rs1256049 polymorphisms and RSA risk by a meta-analysis. The results revealed that the additive and recessive model of ESR2 rs4986938 were significantly associated with RSA. However, we noticed that negative results were reported in previous individual study conducted by Morandi Alessio et al,[15] Hu et al,[20] Guo and Ali,[21] and Mahdavipour et al.[22] And the combined results may indicate that the sample size is an important factor for the genetic association study. Although ESR2 rs4986938 and rs1256049 polymorphisms do not lead to the amino acid changes in ER-β protein, it is possible that these 2 polymorphisms are in linkage disequilibrium with other variants that may influence the gene expression or function. Furthermore, an intronic mutation may are responsible for aberrant splice processes. In this study, the ESR2 rs2077647 was not associated with RSA risk. To further confirm these results, a larger number of studies with multiple ethnicity are necessary.

Haplotype analysis has shown that none of the haplotypes consisting of ESR2 rs2077647, rs4986938, and rs1256049 polymorphisms were associated with RSA risk. Notably, Tang et al [19] have suggested that the haplotype containing ESR2 rs2077647A-rs4986938G-rs1256049T was significantly associated with the protective effect on RSA in a Ningxia Chinese Han population. This inconsistency may be due to the complex genetic backgrounds in the Chinese Han populations. To confirm these results, larger numbers of studies with more subjects from multiple ethnicities will be necessary in the future.

There were limitations in the present study. First, the sample sizes included in the studies were relatively small. It is necessary to enroll more subjects to identify the results. Second, the studies included in the meta-analysis were relatively small. There was a lack of data on the association between the ESR1 rs3798759 and ESR2 rs2077647 and RSA risk. Third, both the genetic and environmental factors were determined to play a role in the development of RSA. However, we failed to detect the influence of environmental factors and RSA risk.

In conclusion, our data suggested that haplotype containing ESR1 rs9340799G-rs2234693C-rs3798759A was a protective factor for RSA, while haplotype containing ESR1 rs9340799A-rs2234693C-rs3798759A was a risk factor for RSA. Additionally, the additive and recessive models of ESR2 rs4986938 were protective factors for RSA in the Chinese Han population.

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