

## Original Article

# Meta-analysis of the association between endometriosis and polymorphisms in ACE and PAI-1

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Received January 13, 2016; Accepted May 6, 2016; Epub June 15, 2016; Published June 30, 2016

**Abstract:** Purpose: This meta-analysis aims to evaluate the associations between endometriosis risk and the polymorphisms of 2350A/G, -240A/T, Alu insertion/deletion (I/D) in angiotensin I converting enzyme (ACE) gene and 4G/5G in plasminogen activator inhibitor-1 (PAI-1) gene. Methods: Studies published until November 2015 were searched in PubMed, EMBASE, ISI Web of Knowledge, China National Knowledge Infrastructure, and Chinese Biomedical Literature Database. The Odds ratio (OR) and 95% confidence interval (CI) were estimated to assess the effect. Heterogeneity testing and sensitivity analysis were also performed. Results: A total of 11 studies with 1486 cases and 1598 controls were identified for final analysis. Three polymorphisms were significantly associated with the risk of endometriosis (ACE 2350A/G: G vs. A: OR = 2.66, 95% CI: 1.29-5.53, P = 0.008; ACE -240A/T: T vs. A: OR = 1.61, 95% CI: 1.04-2.48, P = 0.03; PAI-1 4G/5G: 4G vs. 5G: OR = 2.92, 95% CI: 1.13-7.55, P = 0.027). In the subgroup analysis by ethnicity, none of the three polymorphisms was associated with the risk of endometriosis among Caucasian populations. No association was observed between ACE I/D polymorphism and endometriosis. Conclusions: ACE 2350A/G, ACE -240A/T, and PAI-1 4G/5G polymorphisms may contribute to endometriosis risk. Further large-scale and well-designed studies should be conducted to validate these associations.

**Keywords:** Endometriosis, meta-analysis, angiotensin i converting enzyme, plasminogen activator inhibitor-1, polymorphism

## Introduction

Endometriosis is characterized by the presence of endometrial tissue outside the uterine cavity, mainly on the pelvic peritoneum, ovaries, and rectovaginal septum. Despite the high prevalence of endometriosis, its etiology and pathophysiology remain unclear [1]. Several theories have been proposed to explain the pathogenesis of this disease, among which Sampson's retrograde menstruation theory is the most widely accepted. This theory suggests that endometrial cells implant into the peritoneum or pelvic organs via reflux through the fallopian tubes during menstruation [2]. However, retrograde spill of menstrual blood has been found in 76% of women undergoing laparoscopy during menstruation, whereas only a small percentage of women contract this specific disorder [3]. This phenomenon indicates that some other factors may play a significant role in

the pathogenesis of endometriosis. Given that the endometrial debris needs to implant and grow in other tissues, the roles of tissue-remodeling-related processes, such as angiogenesis and fibroblast proliferation, in the adhesion, invasion, and regression of endometriotic lesions have been stressed in recent studies [4, 5].

The renin-angiotensin system (RAS) is widely known to regulate blood pressure and fluid balance [6]. Local RAS also play a role in blood vessel formation in various tissues, such as the endometrium and ovary [7]. Angiotensin I converting enzyme (ACE), which catalyzes the conversion of angiotensin I to angiotensin II, is a key component of RAS. Several single nucleotide polymorphisms (SNPs) of ACE polymorphisms have been investigated in terms of their potential associations with various illnesses, especially cardiovascular diseases and cancer

[8, 9]. The three most studied sites related to endometriosis are 2350 A/G (rs4343), -240 A/T (rs4291), and 287-bp Alu insertion/deletion (I/D) (rs1799752). To date, the results of these three variants in the pathogenesis of endometriosis remain controversial and no meta-analysis has been published.

Plasminogen activator inhibitor-1 (PAI-1), as an important element of fibrin formation and degradation, can inhibit the fibrinolytic activity of tissue-type plasminogen activator, produce active plasmin, and eventually degrade fibrin [10]. Evidence has also shown that abnormal fibrinolytic activity can make endometrial fragments adhere to and invade the surrounding tissue easily [11]. The guanosine (G) I/D polymorphism located upstream of the start codon of PAI-1, which is named -675 4G/5G (rs-1799768), is an important SNP that can influence PAI-1 biosynthesis [12]. Several studies that focused on the relationship between endometriosis and -675 4G/5G polymorphism have been published; however, the outcomes are inconsistent.

The relatively small sample size and the inadequate statistical power of a single research indicate that the results of association between polymorphisms of ACE 2350A/G, ACE-240A/T, ACE I/D, and PAI-1-675 4G/5G and endometriosis susceptibility may not strong and reliable. Considering the potential influences on these four polymorphisms and endometriosis risk, we performed a meta-analysis of 11 detailed articles to further evaluate the association of ACE 2350 A/G, -240 A/T, I/D, and PAI-1 4G/5G with endometriosis susceptibility.

### Materials and methods

#### *Literature search*

We systematically searched for relevant papers published before November 2015 in PubMed, Embase, ISI Web of Knowledge, CBM (Chinese Biomedical Literature Database), and CNKI (China National Knowledge Infrastructure) databases. No restrictions on language, population, or sample size were set in this meta-analysis. The search strategies were based on the combinations of the following keywords: (“angiotensin converting enzyme” or “ACE” or “plasminogen activator inhibitor” or “PAI” or “serpin peptidase inhibitor clade E” or “SER-

PINE1”) and (“polymorphism” or “variant” or “mutation”) and “endometriosis”. Additional relevant literatures were obtained from the reference lists of the relevant articles.

#### *Inclusion and exclusion criteria*

Studies were included in this meta-analysis based on the following criteria: (1) full-text articles; (2) original case-control or cohort study evaluating at least one of the ACE 2350A/G, ACE -240A/T, ACE I/D, and PAI-1 4G/5G linked with the risk of endometriosis; (3) sufficient data to estimate an odds ratio (OR) and 95% confidence interval (CI); (4) Chinese articles were published in Chinese core periodicals; and (5) no overlapping data. For the studies with the same authors, only those with the largest sample sizes or the most recent publication dates were selected to avoid overlapping patients and controls. Studies were excluded on the basis of the following criteria: (1) meta-analysis, letter, review, or editorial article; (2) study with no control case; (3) studies based on incomplete or sufficient data, such as deficiency of specific genotype distribution in cases and controls.

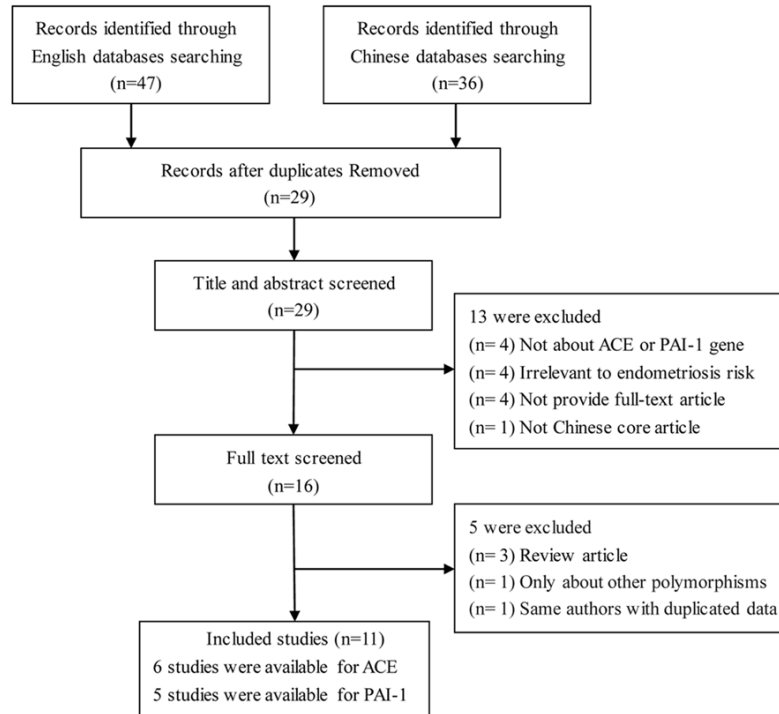
#### *Data extraction*

Two investigators (Luyang Zhao and Chenglei Gu) independently extracted useful data from each study. Discrepancies were resolved through discussion. The following information was collected from each of the included studies: name of the first author, year of publication, country of origin, ethnicity of descent, number of subjects, genotype distribution in cases and controls, source of control, genotype method, outcomes, and probability (*p*) value for the Hardy-Weinberg equilibrium (HWE) test in controls. The authorship and sample sources were also checked to identify the existence of multiple publications from the same study.

#### *Quality evaluation of the included studies*

Newcastle-Ottawa Scale (NOS) was used to assess the quality of the included studies by two investigators above mentioned. The NOS quality score system ranges from zero to nine stars [13]. The study with scores of seven stars or greater was categorized as “high quality”; otherwise, the study was categorized as “low quality”. Discrepancies were resolved through discussion.

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**Figure 1.** Flow diagram of the study search and selection process.

## Statistical analysis

HWE was assessed in the control group by using  $\chi^2$  test for goodness of fit, with  $P < 0.05$  considered as a deviation. OR and 95% CI were used to assess the strengths of the associations between polymorphisms of ACE and PAI-1 and the risk of endometriosis. Five models, namely allele model, dominant model, recessive model, homozygous model, and heterozygous model, were evaluated. The statistical significance ( $P < 0.05$ ) of the pooled ORs was determined by Z-test. Heterogeneity was assessed by Q-test and quantified by  $I^2$  test. When heterogeneity existed ( $P < 0.05$  or  $I^2 > 50\%$ ), the random effects model (DerSimonian Laird method) was used; otherwise, the fixed effects model (Mantel-Haenszel method) was employed. Subgroup analyses stratified with HWE in controls (yes or no), ethnicity (Asian or Caucasian), and NOS quality score (high or low) were also evaluated in ACE 2350A/G, ACE -240A/T, and PAI-1 4G/5G polymorphisms. Sensitivity analysis was performed to assess the quality and stability of the results. All analyses were conducted by Stata 12.0 (Stata Corporation, College Station, TX, USA).

## Results

### Characteristics of the included studies

**Figure 1** presents a detailed flowchart of the inclusion and exclusion process. A total of 83 citations were identified in the initial search. After removing the duplicates, 54 articles were excluded. After screening the title and abstract, 14 articles were eliminated, and the remaining 15 articles were screened in full texts. Three articles were then excluded for reviews [5, 14, 15], and one article for only report other polymorphisms [16]. Finally, 11 studies [17-27] that include 1486 cases and 1598 controls were obtained in this meta-analysis.

The publication year of the included studies ranged from 2005 to 2014. The surname of the first author, publication year, country, ethnicity, sample size, frequencies of various genotypes in patients and controls, minor allele frequency, control source, genotype method, HWE in controls, primary outcome, and NOS quality score for each study are listed in **Table 1**.

### Quantitative data synthesis

Four studies [17, 23, 26, 27] with 619 cases and 567 controls assessed the association between ACE 2350A/G polymorphism and endometriosis susceptibility. The results indicated that ACE 2350A/G polymorphism is associated with endometriosis risk under all genetic models (allele model: OR = 2.66, 95% CI: 1.29-5.53,  $P = 0.008$ ; dominant model: OR = 3.24, 95% CI: 1.31-7.99,  $P = 0.011$ ; recessive model: OR = 1.53, 95% CI: 1.03-2.28,  $P = 0.034$ ; homozygous model: OR = 1.91, 95% CI: 1.22-2.98,  $P = 0.004$ ; and heterozygous model: OR = 3.19, 95% CI: 1.23-8.27,  $P = 0.017$ ; **Figure 2**). After excluding the study in which the genotype distributions in the control groups deviated from HWE, association was still observed

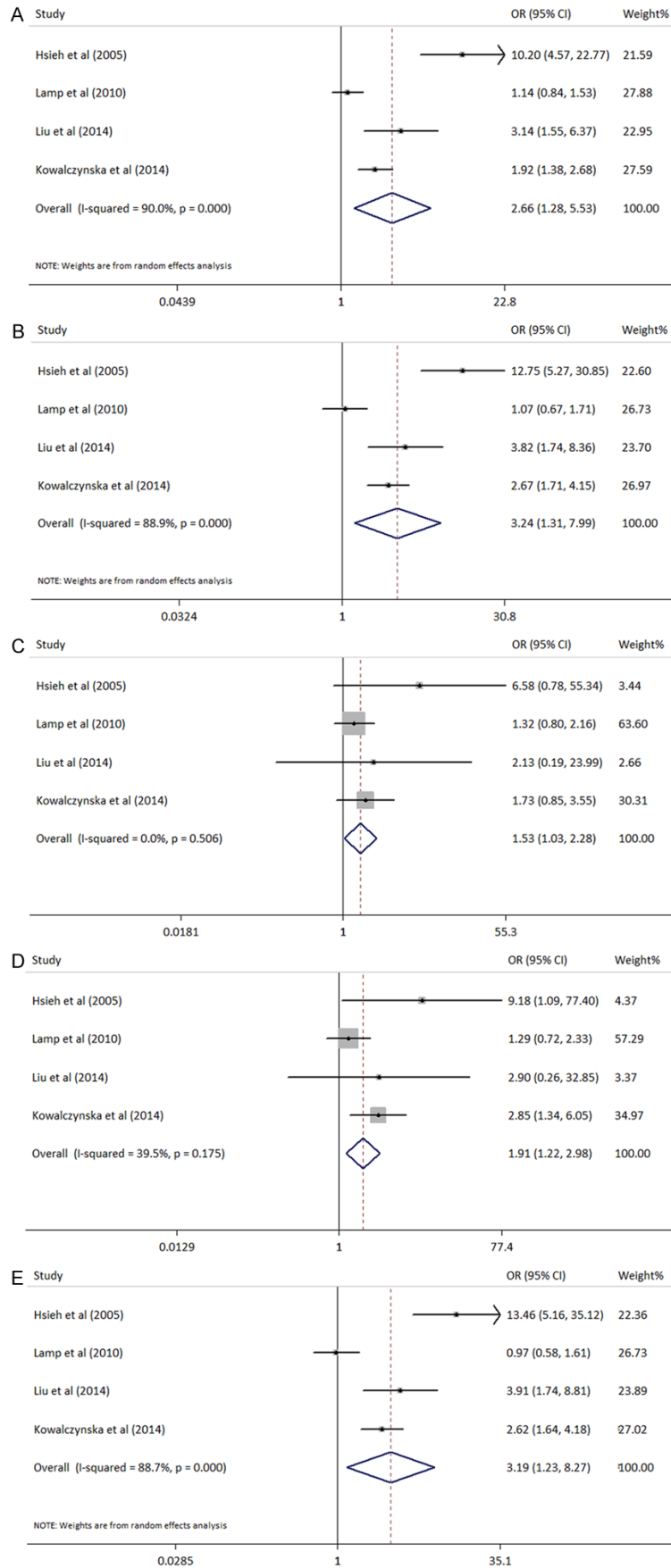
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**Table 1.** The main characteristics and quality of all eligible studies

No	Author	Year	Country	Ethnicity	Mutation site	Case Group					Control Group					Control Source	Genotype method	HWE	Outcome	NOS
						Total	1/1	1/2	2/2	MAF	Total	1/1	1/2	2/2	MAF					
1	Hsieh	2005	Taiwan	Asian	ACE 2350 A/G	150	100	44	6	0.19	159	153	5	1	0.02	NR	RFLP-PCR	No	S	6
1	Hsieh	2005	Taiwan	Asian	ACE -240 A/T	150	65	69	16	0.34	159	100	57	2	0.19	NR	RFLP-PCR	No	S	6
2	Bedaiwy	2006	Canada	Caucasian	PAI-1 4G/5G	75	2	21	52	0.17	43	24	14	5	0.28	HB	PCR	Yes	S	8
3	Hsieh	2007	Taiwan	Asian	ACE I/D	125	63	30	32	0.38	128	13	38	77	0.25	PB	RFLP-PCR	No	S	5
4	Liu	2008	China	Asian	ACE -240 A/T	78	32	34	12	0.37	82	46	32	4	0.24	HB	RFLP-PCR	Yes	S	8
5	Ramon	2008	Spain	Caucasian	PAI-1 4G/5G	170	40	98	32	0.48	219	65	108	46	0.46	HB	AS-PCR	Yes	NS	7
6	Gentilini	2009	Italy	Caucasian	PAI-1 4G/5G	204	59	108	37	0.45	329	75	165	89	0.48	PB	PCR	Yes	NS	4
7	Lamp	2010	Estonia	Caucasian	ACE 2350 A/G	150	41	69	40	0.50	199	57	99	43	0.46	PB	RFLP-PCR	Yes	NS	7
7	Lamp	2010	Estonia	Caucasian	ACE -240 A/T	150	63	69	18	0.35	199	83	102	14	0.38	PB	RFLP-PCR	Yes	NS	7
8	Qian	2010	China	Asian	PAI-1 4G/5G	75	2	21	52	0.17	82	46	26	10	0.28	HB	PCR-RFLP	Yes	S	8
9	Goncalves-filho	2011	Brazil	Brazilian*	PAI-1 4G/5G	140	34	52	54	0.43	148	62	50	36	0.41	HB	PCR-RFLP	No	S	7
10	Liu	2014	China	Asian	ACE 2350 A/G	78	49	27	2	0.20	82	71	10	1	0.07	HB	RFLP-PCR	Yes	S	8
11	Kowalczyńska	2014	Poland	Caucasian	ACE 2350 A/G	241	76	131	34	0.41	127	70	46	11	0.27	HB	RFLP-PCR	Yes	S	4
11	Kowalczyńska	2014	Poland	Caucasian	ACE I/D	241	61	118	62	0.5	127	44	55	28	0.39	HB	PCR	Yes	NS	4

1, wild-type allele; 2, mutant-type allele; MAF, minor allele frequency; NR, not recorded; HB, hospital-based; PB, population-based; PCR, polymerase chain reaction; AS-PCR, allele-specific PCR; HWE, Hardy-Weinberg equilibrium; S, significant; NS, not significant; NOS, Newcastle-Ottawa Scale. \*Term Brazilian represented Brazilian population.

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**Figure 2.** Forest plot of ORs for the association between ACE 2350A/G polymorphism and susceptibility to endometriosis. A. Allele model. B. Dominant model. C. Recessive model. D. Homozygous model. E. Heterozygous model.

in the allele model (OR = 1.77, 95% CI: 1.06-2.95, P = 0.030), dominant model (OR = 2.13, 95% CI: 1.31-7.99, P = 0.044) and homozygous model (OR = 1.86, 95% CI: 1.02-3.40, P = 0.042). In the subgroup analyses, association was only observed among Asian group in the allele model (OR = 5.57, 95% CI: 1.73-17.91, P = 0.004), dominant model (OR = 6.86, 95% CI: 2.08-22.61, P = 0.002), homozygous model (OR = 5.56, 95% CI: 1.12-27.58, P = 0.036) and heterozygous model (OR = 7.07, 95% CI: 2.08-24.06, P = 0.002). In addition, correlation was only found in the low NOS score group under the dominant model (OR = 5.56, 95% CI: 1.17-26.37, P = 0.031), homozygous model (OR = 3.30, 95% CI: 1.54-7.09, P = 0.002), and heterozygous model (OR = 5.63, 95% CI: 1.11-28.7, P = 0.038). All these results are listed in **Table 2**.

Three studies [17, 20, 23] that include 378 endometriosis patients and 281 controls analyzed the association between ACE -240A/T polymorphism and endometriosis. The association was statistically significant under the allele model (OR = 1.61, 95% CI: 1.04-2.48, P = 0.032, **Figure 3A**), recessive model (OR = 3.29, 95% CI: 1.31-8.24, P = 0.011, **Figure 3B**), and homozygous model (OR = 3.90, 95% CI:

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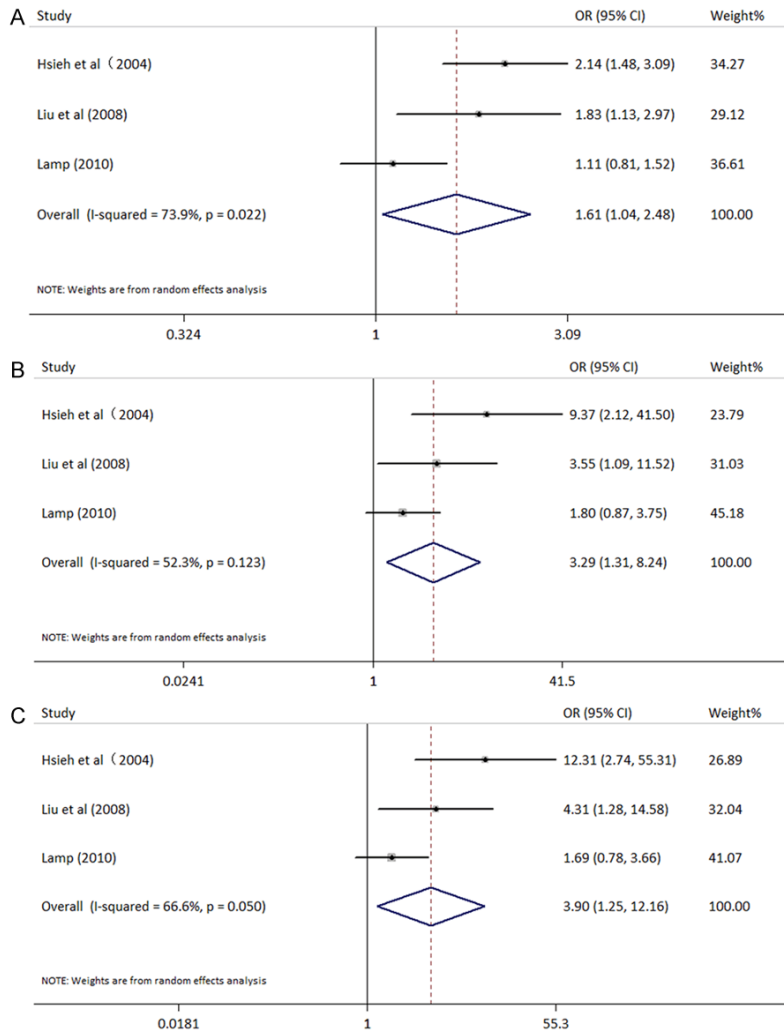
**Table 2.** Main results of the pooled data in the meta-analysis of ACE polymorphisms

Variant	n	Allele Model (2 vs. 1)			Dominant model (2/2+1/2 vs. 1/1)			Recessive model (2/2 vs. 1/2 +1/1)			Homozygous model (2/2 vs. 1/1)			Heterozygous model (1/2 vs. 1/1)		
		OR [95% CI]	P <sub>OR</sub>	I <sup>2</sup> (%)	OR [95% CI]	P <sub>OR</sub>	I <sup>2</sup> (%)	OR [95% CI]	P <sub>OR</sub>	I <sup>2</sup> (%)	OR [95% CI]	P <sub>OR</sub>	I <sup>2</sup> (%)	OR [95% CI]	P <sub>OR</sub>	I <sup>2</sup> (%)
ACE 2350A/G		2.66 [1.29, 5.53]	0.008	90.0	3.24 [1.31, 7.99]	0.011	88.9	1.53 [1.03, 2.28]	0.034	0.0	1.91 [1.22, 2.98]	0.004	39.5	3.19 [1.23, 8.27]	0.017	88.7
<i>HWE in control</i>																
Yes	3	1.77 [1.06, 2.95]	0.030	79.1	2.13 [1.31, 7.99]	0.044	81.8	1.46 [0.97, 2.18]	0.067	0.0	1.86 [1.02, 3.40]	0.042	28.2	2.07 [0.93, 4.63]	0.075	82.9
No	1	10.20 [4.57, 22.78]	0.000	-	12.75 [5.27, 30.85]	0.000	-	6.58 [0.78, 55.34]	0.083	-	9.18 [1.09, 77.40]	0.042	-	13.46 [5.16, 35.12]	0.000	-
<i>Ethnicity</i>																
Caucasian	2	1.47 [0.88, 2.46]	0.143	81.2	1.69 [0.69, 4.15]	0.250	87.0	1.44 [0.96, 2.17]	0.079	0.0	1.85 [0.86, 4.00]	0.118	61.9	1.60 [0.60, 4.25]	0.344	87.6
Asian	2	5.57 [1.73, 17.91]	0.004	79.0	6.86 [2.08, 22.61]	0.002	75.5	4.03 [0.81, 19.91]	0.088	0.0	5.56 [1.12, 27.58]	0.036	0.0	7.07 [2.08, 24.06]	0.002	73.8
<i>Quality score</i>																
High	2	1.79 [0.67, 4.84]	0.249	93.1	1.94 [0.56, 6.76]	0.298	86.6	1.35 [0.83, 2.18]	0.231	0.0	1.35 [0.76, 2.40]	0.301	0.0	1.88 [0.48, 7.36]	0.367	87.8
Low	2	4.25 [0.81, 22.29]	0.087	85.2	5.56 [1.17, 26.37]	0.031	90.0	2.29 [0.79, 6.70]	0.129	27.1	3.30 [1.54, 7.09]	0.002	3.8	5.63 [1.11, 28.70]	0.038	89.3
ACE -240A/T	3	1.61 [1.04, 2.48]	0.032	73.9	1.57 [0.92, 2.67]	0.097	70.8	3.29 [1.31, 8.24]	0.011	52.3	3.90 [1.25, 12.16]	0.019	66.6	1.34 [0.83, 2.18]	0.230	61.6
<i>HWE in control</i>																
Yes	2	1.38 [0.85, 2.25]	0.197	65.7	1.29 [0.71, 2.35]	0.408	60.8	2.17 [1.17, 4.06]	0.014	0.0	2.39 [0.99, 5.78]	0.054	38.2	1.10 [0.66, 1.85]	0.712	42.8
No	1	2.14 [1.48, 3.09]	0.000	-	2.22 [1.41, 3.50]	0.001	-	9.37 [2.12, 41.50]	0.003	-	12.31 [2.74, 55.31]	0.001	-	1.86 [1.16, 2.98]	0.009	-
<i>Ethnicity</i>																
Caucasian	1	1.11 [0.81, 1.52]	0.518	-	0.99 [0.64, 1.52]	0.956	-	1.80 [0.87, 3.75]	0.115	-	1.69 [0.78, 3.66]	0.181	-	0.89 [0.57, 1.40]	0.614	-
Asian	2	2.02 [1.51, 2.71]	0.000	0.0	2.08 [1.44, 3.00]	0.000	0.0	5.18 [2.02, 13.25]	0.001	3.2	6.63 [2.39, 18.43]	0.000	13.3	1.74 [1.19, 2.56]	0.004	0.0
<i>Quality score</i>																
High	2	1.38 [0.85, 2.25]	0.197	65.7	1.29 [0.71, 2.35]	0.408	60.8	2.17 [1.17, 4.06]	0.014	0.0	2.39 [0.99, 5.78]	0.054	38.2	1.10 [0.66, 1.85]	0.712	42.8
Low	1	2.14 [1.48, 3.09]	0.000	-	2.22 [1.41, 3.50]	0.001	-	9.37 [2.12, 41.50]	0.003	-	12.31 [2.74, 55.31]	0.001	-	1.86 [1.16, 2.98]	0.009	-
ACE I/D	2	1.95 [0.31, 12.16]	0.474	98.2	0.54 [0.10, 2.88]	0.468	95.2	0.78 [0.42, 1.43]	0.414	65.8	0.37 [0.02, 6.57]	0.501	97.3	0.51 [0.06, 4.66]	0.552	95.7

P<sub>OR</sub>\* P-values of Odds Ratio; HWE, Hardy-Weinberg equilibrium; 1, wild-type allele; 2, mutant-type allele.



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**Figure 3.** Forest plot of ORs for the association between ACE -240A/T polymorphism and susceptibility to endometriosis. A. Allele model. B. Recessive model. C. Homozygous model.

1.25-12.16,  $P = 0.019$ , **Figure 3C**). After excluding one study that deviated from HWE, association was only observed in the recessive model (OR = 2.17, 95% CI: 1.17-4.06,  $P = 0.014$ ). In the subgroup analyses, correlation was only observed among Asian populations (all  $P < 0.01$ ). All these results are shown in **Table 2**.

Two studies [19, 27] that include 366 endometriosis patients and 255 controls investigated the relationship between ACE I/D polymorphism and endometriosis susceptibility. No associations were observed among the five genetic models (**Table 2**).

Five studies [18, 21, 22, 24, 25] with 644 cases and 777 controls reported the potential

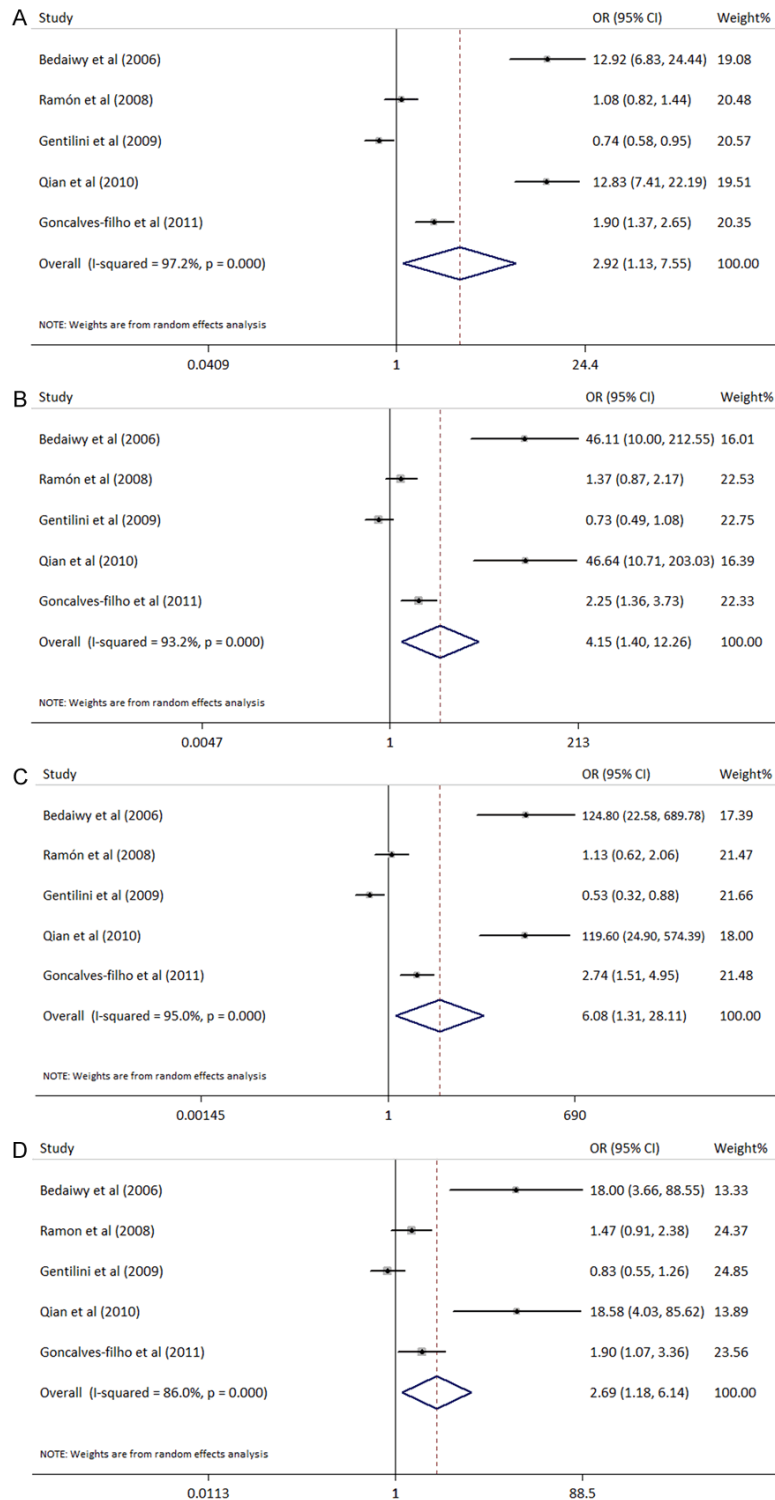
association between the PAI-1 4G/5G polymorphism and endometriosis. Association was observed under the allele model (OR = 2.92, 95% CI: 1.13-7.55,  $P = 0.027$ , **Figure 4A**), dominant model (OR = 4.15, 95% CI: 1.40-12.26,  $P = 0.010$ , **Figure 4B**), homozygous model (OR = 6.08, 95% CI: 1.32-28.11,  $P = 0.021$ , **Figure 4C**), and heterozygous model (OR = 2.69, 95% CI: 1.18-6.14,  $P = 0.018$ , **Figure 4D**). After excluding the studies in which the genotype distributions in the control groups deviated from HWE, the same associations were observed in the dominant model (OR = 5.48, 95% CI: 1.21-24.76,  $P = 0.027$ ) and heterozygous model (OR = 3.35, 95% CI: 1.09-10.35,  $P = 0.036$ ). Subgroup analysis of ethnicity showed significant association in Asian and Brazilian populations (all  $P < 0.05$ ). When stratified by NOS, association was observed in both high quality and low quality groups (all  $P < 0.05$ ). These results are displayed in **Table 3**.

### Heterogeneity and sensitivity analysis

For ACE 2350 A/G polymorphism, heterogeneity was observed in the allele model, dominant model and heterozygous model. For ACE -240 A/T, ACE I/D and PAI-1 4G/5G polymorphisms, heterogeneity was observed in all genetic models. To identify the source of heterogeneity, we conducted subgroup analyses according to HWE in controls, ethnicity, and quality score. However, none of them could completely explain the heterogeneity.

In the sensitivity analysis, we sequentially removed the eligible studies to assess the influence of each individual study on the pooled ORs. The results showed that the pooled ORs

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**Figure 4.** Forest plot of ORs for the association between PAI-1 4G/5G polymorphism and susceptibility to endometriosis. A. Allele model. B. Dominant model. C. Homozygous model. D. Heterozygous model.

were not qualitatively changed when any single study was omitted under ACE 2350 A/G, ACE-

very few reports have focused on the relationship between endometriosis and SNPs. After a

240 A/T and PAI-1 4G/5G polymorphisms, indicating that the analysis was reliable and robust.

### Discussion

The ACE gene, which is located on chromosome 17q-23, contains some common gene polymorphisms that related with many ordinary diseases, such as Alzheimer's disease [28], hypertension [29], and vasculitis [30]. Among nearly 20 common polymorphisms of ACE, 2350 A/G, -240 A/T and I/D are the three most studied polymorphisms, which can influence ACE serum and plasma levels. Previous studies indicated that ACE 2350\*G and 240\*T allele may be associated with an increased ACE concentration [9, 31], and DD homozygotes in ACE I/D polymorphism presented approximately twice the ACE levels of II homozygotes [32]. The exact mechanism by which these three SNPs affect the ACE concentration remains unclear. Some investigators suggested that certain genotypes may influence the 3D structure of the transcript and/or its enzymatic activity [33, 34]. Basically, high ACE levels can enhance angiogenesis, and ACE inhibitors will inhibit tumor growth [35]. Considering the similarity to tumors and the specific RAS system in the endometrium, some investigators assumed that altered ACE polymorphisms may be related to the development of endometriosis. To date,



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**Table 3.** Main results of the pooled data in the meta-analysis PAI-1 4G/5G polymorphism

Variant	n	Allele Model (4G vs. 5G)			Dominant model (4G/4G+4G/5G vs. 5G/5G)			Recessive model (4G/4G vs. 4G/5G +5G/5G)			Homozygous model (4G/4G vs. 5G/5G)			Heterozygous model (4G/5G vs. 5G/5G)		
		OR [95% CI]	P <sub>OR</sub>	I <sup>2</sup> (%)	OR [95% CI]	P <sub>OR</sub>	I <sup>2</sup> (%)	OR [95% CI]	P <sub>OR</sub>	I <sup>2</sup> (%)	OR [95% CI]	P <sub>OR</sub>	I <sup>2</sup> (%)	OR [95% CI]	P <sub>OR</sub>	I <sup>2</sup> (%)
Overall	5	2.92 [1.13, 7.56]	0.027	97.2	4.15 [1.40, 12.26]	0.010	93.2	2.90 [0.91, 9.24]	0.072	94.8	6.08 [1.32, 28.11]	0.021	95.0	2.69 [1.18, 6.14]	0.018	86.0
HWE in control																
Yes	4	3.30 [0.93, 11.70]	0.065	97.8	5.48 [1.21, 24.76]	0.027	94.7	3.29 [0.68, 15.97]	0.139	96.0	8.27 [0.95, 72.04]	0.057	96.1	3.35 [1.09, 10.35]	0.036	89.1
No	1	1.90 [1.37, 2.65]	0.000	-	2.25 [1.36, 3.73]	0.002	-	1.95 [1.18, 3.24]	0.010	-	2.74 [1.51, 4.95]	0.001	-	1.90 [1.07, 3.36]	0.028	-
Ethnicity																
Caucasian	3	2.07 [0.66, 6.50]	0.211	97.0	2.65 [0.71, 9.88]	0.148	93.1	1.88 [0.456, 7.77]	0.383	94.1	3.28 [0.49, 22.02]	0.222	94.8	1.97 [0.72, 5.38]	0.187	86.6
Asian	1	12.83 [7.41, 22.19]	0.000	-	46.64 [10.71, 203.03]	0.000	-	16.28 [7.14, 37.10]	0.000	-	119.60 [24.90, 574.39]	0.000	-	18.58 [4.03, 85.62]	0.000	-
Brazilian*	1	1.90 [1.37, 2.65]	0.000	-	2.25 [1.36, 3.73]	0.002	-	1.95 [1.18, 3.24]	0.010	-	2.74 [1.51, 4.95]	0.001	-	1.90 [1.07, 3.36]	0.028	-
Quality score																
High	4	4.18 [1.33, 13.2]	0.015	96.8	7.26 [1.86, 28.41]	0.004	92.2	4.42 [1.13, 17.31]	0.033	94.1	12.32 [1.88, 80.73]	0.009	94.2	4.12 [1.49, 11.38]	0.01	82.8
Low	1	0.74 [0.58, 0.95]	0.017	-	0.73 [0.49, 1.08]	0.114	-	0.60 [0.39, 0.92]	0.019	-	0.53 [0.32, 0.88]	0.015	-	0.83 [0.55, 1.27]	0.389	-

P<sub>OR</sub>, P-values of Odds Ratio; HWE, Hardy-Weinberg equilibrium. \*Term Brazilian represented Brazilian population.

comprehensive search, only six articles with 672 cases and 777 controls are included. The results indicated that ACE 2350 A/G polymorphism is associated with the pathogenesis of endometriosis up to 3.2-fold risk (dominant model). The ACE -240 A/T polymorphism also showed a significant association with endometriosis, particularly in TT genotype, which can increase 2.9-fold endometriosis risk compared with AA genotype. The OR values of both polymorphisms decreased when confined with HWE in controls; however, *P*-values still showed significant associations. Women with ACE 2350\*G allele presented a 77% increased risk of endometriosis compared with those exhibiting ACE 2350\*A allele. Compared with the -240\*AA genotype, the -240\*TT genotype increased risk of endometriosis by 1.1-fold. Subgroup analysis by ethnicity revealed that the ACE 2350 A/G and -240 A/T polymorphisms are associated with the increased risk of endometriosis among Asian populations only and not in Caucasian populations. Since only one published articles focused on Caucasian, a small-study bias may have arisen from the results in this regard. Moreover, ACE I/D polymorphism did not show a positive result. However, this evidence is relatively weak because only two articles meet the inclusion criteria.

The PAI-1 gene, which is located on human chromosome 7q21.3-q22, consists of nine exons and eight introns [36]. Genetic polymorphisms in the PAI-1 gene influence the level of PAI-1 expression [37]. A single G nucleotide I/D in PAI-1 gene, named 4G/5G, is the most-studied polymorphism of the PAI-1 gene. The 4G allele is more transcriptionally active than the 5G allele because the latter contains an additional binding site for a repressor [38]. Therefore, 4G/4G genotype carrier has higher concentration of PAI-1 than people carrying 5G. Overexpression of PAI-1 may result in the impairment of the fibrinolytic system and prone to endometriosis. Five case-control studies with 644 cases and 821 controls, which were launched to determine the association between the PAI-1 4G/5G polymorphism and endometriosis risk, were retrieved in this meta-analysis. Overall, the results indicate a significant association between the PAI-1 4G/5G polymorphism and increased risk of endometriosis under all genetic models. The homozygote 4G/4G confers six-fold risk on the susceptibility to endo-

metriosis compared with the homozygote 5G/5G. Subgroup analysis by ethnicity, determined significantly increased risks among Asians and Brazilians but not in Caucasians.

Our meta-analysis results are consistent with the presumption of previous research. The present findings also suggest that the studied polymorphisms may be used as effective susceptibility markers for endometriosis. However, some limitations should be considered in interpreting such results. First, the most predominant limitation in this meta-analysis is the obvious heterogeneity in the overall analysis. Although the potential sources, including HWE, ethnicity, and NOS quality score, have been evaluated separately, these sources cannot completely explain the heterogeneity. Another possible explanation suggests that endometriosis is a complex disease influenced by the environment and heredity; some factors such as menstrual cycle, toxic-exposure history, and lifestyle may also affect the pathogenesis of this disease [39]. In addition, endometriosis can be classified into four stages by revised classification of the American Fertility Society (r-AFS) score, which can reflect the severity and progression of endometriosis. Different stages of endometriosis can display various features, and diverse genetic alterations. Moreover, endometriosis can be classified into peritoneal, ovarian, deeply infiltrating, and other different types; each of these types should be considered a separate entity to consider the possibility of varied pathogenesis [40]. All these differences, which lack detailed information or cannot be quantified, can partially contribute to heterogeneity. Second, studies that focused on the four selected SNPs and endometriosis are limited and the sample sizes are relatively small, indicating that small trials bias may occurred. Third, our meta-analysis did not focus on the genetic linkages and haplotypes of endometriosis due to insufficient information. Fourth, some selection biases may have been caused by the inclusion of only English and Chinese articles, as well as only Asian, Caucasian, and Brazilian subjects. Other ethnicities, particularly African populations, should also be considered. Therefore, further large-scale and well-designed studies should be conducted to validate these associations to achieve a comprehensive understanding of their possible roles in endometriosis.

To date, several genome-wide association (GWA) studies that focused on patients with endometriosis have been published. Many susceptible polymorphisms in several related genes, such as NFE2L3, CDKN2BAS, ID4, and WNT4, have been revealed [41-44]. To our best knowledge, we believe that no SNP association between ACE gene and PAI-1 gene and endometriosis was revealed. Thus, future elaborate GWA studies should be expected to reveal the association between the polymorphisms in ACE and PAI-1 and endometriosis risk.

In conclusion, this meta-analysis is the first to investigate the association between endometriosis and polymorphisms of ACE and PAI-1. The results presented evidence for the association of ACE 2350 A/G and ACE-240 A/T polymorphisms with endometriosis risk, particularly in Asian population. The findings also indicated that PAI-1 4G/5G polymorphism may play important roles in the pathogenesis of endometriosis, particularly in Asian and Brazilian populations. Further larger-cohorts studies with respect to disease stages, environmental exposure, endometriosis type and other factors that may alter the heterogeneity should be conducted to validate these associations. Such investigations may finally lead to a comprehensive understanding of their possible roles in the endometriosis development.

### Acknowledgements

This study was funded by the National Natural Science Foundation of China (grant number: 81571411).

### Disclosure of conflict of interest

None.

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