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Association of the Vascular Endothelial Growth Factor Gene Polymorphisms (–460C/T, +405G/C and +936T/C) with Endometriosis: A Meta-Analysis

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Summary

Published data on the association between the vascular endothelial growth factor (*VEGF*) gene –460C/T (rs833061), +405G/C (rs2010963), +936T/C (rs3025039) polymorphisms and endometriosis risk are inconclusive. Eleven eligible case-control studies including 2690 cases and 2803 controls were included in this meta-analysis through searching the databases of PubMed and CBMdisc (up to August 1, 2011). In the overall analysis, no significant association between the –460C/T and +405G/C polymorphisms and risk of endometriosis was observed. However, significant associations were observed between endometriosis risk and *VEGF* +936T polymorphism with summarized odds ratio of 1.19 (95%CI, 1.02–1.37), 1.18 (95%CI, 1.03–1.37), 1.15 (95%CI, 1.01–1.30) for CT versus CC genotype, dominant mode (CT/TT vs. CC) and allele comparison (T vs. C), respectively. Furthermore, stratified analysis showed that significantly strong association between +936T/C polymorphism and endometriosis was present only in stage III–IV (OR = 1.32 for dominant mode; OR = 1.30 for T vs. C), but not in stage I–II. However, no significantly increased risk of endometriosis was found in any of the genetic models in Asians or in Caucasians. This meta-analysis supports that *VEGF* +936T/C polymorphism is capable of causing endometriosis susceptibility.

Keywords: Vascular endothelial growth factor, polymorphism, endometriosis, meta-analysis

Introduction

Endometriosis is a common gynecological disease defined by the presence of ectopic endometrial glands and stroma, which is associated with both pelvic pain and infertility. Up to 10% of women of reproductive age may be affected. It is a multifactorial and polygenic disease in which angiogenesis may be implicated (Shifren et al., 1996; Donnez et al., 1998; McLaren, 2000; Tan et al., 2002; Gilabert-Estelles et al., 2007). Endometrial angiogenesis is promoted by numerous inducers and growth factors, including vascular endothelial growth factor (VEGF). VEGF increases vascular permeability and induces endothelial cell proliferation, migration, differentiation and capillary formation (Ferrara, 2004). Several studies have investigated and observed that *VEGF* mRNA and protein levels were significantly higher in women with

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endometriosis (Donnez et al., 1998; Di Carlo et al., 2009), which supported a key role for VEGF in the pathological angiogenesis in endometriosis (Shifren et al., 1996; Kupker et al., 1998; Fasciani et al., 2000; Tan et al., 2002; Khan et al., 2003; Matalliotakis et al., 2003; Gilabert-Estelles et al., 2007). After the attachment of endometrial cells, high VEGF levels could provoke an increase in the subperitoneal vascular network and facilitate implantation and viability of endometrial cells (Donnez et al., 1998). Moreover, it has been demonstrated that anti-human VEGF antibody could effectively interfere with the maintenance and growth of endometriosis by inhibiting angiogenesis in a nude mouse model (Nap et al., 2004).

The human *VEGF* gene is located on Chromosome 6p21.3 (Vincenti et al., 1996) and consists of eight exons exhibiting alternate splicing which results in a family of proteins (Tischer et al., 1991). Several transcription factor-binding sites are found in the *VEGF* 5′-untranslated region (5′-UTR) and variation within the region increases the transcriptional activity (Fukumura et al., 1998; Lambrechts et al., 2003). Three functional *VEGF* polymorphisms in 5′ or 3′-UTR [–460C/T (rs833061), +405G/C (rs2010963) and +936T/C (rs3025039)] have been found to be associated with variation in VEGF protein production (Renner et al., 2000; Watson et al., 2000) and have been related to several diseases in which angiogenesis is involved (Krippl et al., 2003; Lin et al., 2003; Lee et al., 2005). Up to now, a number of molecular epidemiological studies have been conducted to examine the association between *VEGF* –460C/T, +405G/C, +936T/C polymorphisms and risk of endometriosis in diverse populations (Hsieh et al., 2004; Bhanoori et al., 2005; Kim et al., 2005; Ikuhashi et al., 2007; Gentilini et al., 2008; Kim et al., 2008; Zhao et al., 2008; Cosin et al., 2009; Liu et al., 2009; Attar et al., 2010; Lamp et al., 2010; Altinkaya et al., 2011), however, there is no conclusive result available. This discrepancy exists partially because of the possible small effect of the polymorphism on endometriosis risk and the relatively small sample size in each of the published studies. Therefore we performed this meta-analysis to derive a more precise estimation of these associations. The goal of this study is to pool together data from multiple investigations, to analyze the heterogeneity among different investigations and to derive a more precise estimation of these associations.

Materials and Methods

Identification of Relevant Studies and Eligibility Criteria

The databases of PubMed and CBMdisc (Chinese Biomedical Literature Database) were retrieved up to August 1, 2011. The following key words were used: "(vascular endothelial growth factor or *VEGF*) and (polymorphism or polymorphisms) and endometriosis". Additional studies were identified by a hand search of references of original or review articles on this topic. Studies included in our meta-analysis had to meet all of the following criteria: (i) studied in humans; (ii) using a case-control study design; and (iii) had detailed genotype frequency of cases and controls or could be calculated from the article text. If more than one article was published using the same case series, the study with the largest sample size or providing more detail information was selected. In this study, data for meta-analysis were available from 11 studies, including 2690 cases and 2803 controls.

Data Extraction

Information was carefully extracted from all eligible publications independently by two investigators (Shaohua Xu and Wei Wu) according to the pre-specified selection criteria. Disagreement was resolved by discussion with co-authors. The following information from each study was extracted for analysis: first author's name, year of publication, country of origin, ethnicity, total number of cases and controls, distribution of genotypes and Hardy-Weinberg equilibrium (HWE). Different ethnicity was categorized as Asian and Caucasian.

Statistical Analysis

The risk of endometriosis associated with the three polymorphisms of the *VEGF* gene was estimated for each study by odds ratio (OR), together with its 95% confidence interval (CI), respectively. Heterogeneity assumption was checked by the χ^2-based Q-test and was regarded to indicate significance for $P < 0.05$ (Lau et al., 1997). A fixed-effect model using the Mantel-Haenszel method and a random-effects model using the DerSimonian and Laird method were used to combine values from studies. These two models provide similar results when heterogeneity between studies is absent; otherwise, the random-effects model is more appropriate (Mantel & Haenszel, 1959; DerSimonian & Laird, 1986). We first estimated the risks of the heterozygote and variant homozygote compared with the wild-type homozygote, respectively, and then evaluated the risks of the combined variant homozygote and heterozygote versus the wild-type homozygote, and the variant homozygote versus the combined heterozygote and wild-type homozygote, assuming dominant and recessive effects of the variant allele, respectively. Meta regression was used to illustrate potential reasons of between-study heterogeneity. Egger's test and inverted funnel plots were utilized to provide a diagnosis of publication bias (linear regression

asymmetry test) (Egger et al., 1997). All analyses were performed using Stata version 9.2 software (Stata, College Station, TX). All statistical evaluations were made assuming a two-sided test with a significance level of 0.05, unless stated otherwise.

Results

Study Characteristics

Through the literature search and selection based on inclusion criteria, 11 articles were identified by reviewing potentially relevant articles (Hsieh et al., 2004; Bhanoori et al., 2005; Kim et al., 2005; Ikuhashi et al., 2007; Zhao et al., 2008; Cosin et al., 2009; Liu et al., 2009; Attar et al., 2010; Lamp et al., 2010; Altinkaya et al., 2011). The characteristics of the selected studies are listed in Table 1. Among the included articles, the distribution of genotypes in the controls was consistent with the HWE for all selected studies, except for two studies for –460C/T (Hsieh et al., 2004; Attar et al., 2010), and one study for +405G/C (Kim et al., 2005). Publication dates ranged from 2004 to 2011. The number of cases included in the studies varied from 52 to 958, with a mean of 245, and the number of controls varied from 60 to 959, with a mean of 255.

Meta-Analysis Results

The evaluation of the association between *VEGF* –460C/T, +405G/C, +936T/C polymorphisms and endometriosis risk are presented in Tables 2, 3 and 4. In the overall analysis, we found no evidence for association between endometriosis and the *VEGF* polymorphism –460C/T or +405G/C in any of the genetic models (Tables 2, 3, Figs S1, S2). However, a significantly elevated association between the +936T/C polymorphism and endometriosis was found in three genetic models (CT vs. CC: OR = 1.19, 95% CI, 1.02–1.37, P = 0.391 for the heterogeneity test; CT/TT vs. CC: OR = 1.18, 95% CI, 1.03–1.37, P = 0.244 for the heterogeneity test; T vs. C: OR = 1.15, 95% CI, 1.01–1.30, P = 0.174 for the heterogeneity test; Table 4, Fig. S3).

The association between the three polymorphisms and endometriosis was further stratified by ethnicity. When tested individually in each ethnic group, there is no association (Tables 2–4). Additionally, we stratified disease stage of the case group. The stratified analysis showed that the +936T/C polymorphism was associated with a significant increase in the risk of stage III–IV endometriosis in dominant model and T versus C allele genetic models (dominant model, CT/TT vs. CC: OR, 1.32; 95% CI, 1.04–1.66; P = 0.103 for the heterogeneity test and T vs. C: OR, 1.30; 95% CI, 1.06–1.58; P = 0.082 for the heterogeneity test).

Publication Bias

Begg's funnel plot and Egger's test were performed to assess the publication bias of the literature. The shape of the funnel plot did not reveal any evidence of obvious asymmetry (Fig. S4). Then, the Egger's test was used to provide statistical evidence of funnel plot symmetry. The results still did not suggest any evidence of publication bias (–460C/T: P = 0.60 for TC vs. TT and dominant model, P = 0.23 for CC vs. TT, P = 0.07 with recessive model and P = 0.47 for C vs. T; +405G/C: P = 0.27 for CG vs. CC and dominant model, P = 0.39 for GG vs. CC, P = 0.90 with recessive model and P = 0.47 for T vs. C; +936T/C: P = 0.46 for CT vs. CC, TT vs. CC, dominant model and recessive model and P = 0.81 for T vs. C).

Discussion

The present meta-analysis, including 2690 cases and 2803 controls, explored the association between the *VEGF* –460C/T, +405G/C, +936T/C polymorphisms and endometriosis risk. In the overall analysis, no significant association was observed between the –460C/T and +405G/C polymorphisms and risk of developing endometriosis. By contrast, a significant association was found between the +936T/C polymorphism and the risk of developing endometriosis in CT versus CC, CT/TT versus CC and T versus C genetic models. Stratified analysis showed that significantly strong association between +936T/C polymorphism and endometriosis was present only in stage III–IV (OR = 1.32 for dominant mode; OR = 1.30 for T vs. C allele), but not in stage I–II disease. However, no significantly increased risk of endometriosis was found in any of the genetic models in Asian or in Caucasian subjects. Our results indicate that the *VEGF* +936T allele is a genetic risk factor for developing endometriosis, especially for stage III–IV disease.

VEGF is an endothelial cell-specific angiogenic protein that appears to play an important role in a variety of oestrogen target tissues in regulating endometrial angiogenesis at a local level (Girling & Rogers, 2005). *VEGF* gene is a promising candidate gene that appears to play a key role in the pathogenesis of endometriosis, as increased *VEGF* mRNA expression and elevated protein levels in peritoneal fluid and serum of endometriosis patients have been reported (Donnez et al., 1998; Fasciani et al., 2000; Mahnke et al., 2000; McLaren, 2000; Gilabert-Estelles et al., 2007; Di Carlo et al., 2009). Polymorphisms in *VEGF* have been reported to have an effect on the regulation of gene expression that results in altered levels of

Table 1 Main Characteristics of All Studies Included in the Meta-Analysis.

First author	Year	Country	Ethnicity	Case	Control	Genotypes												HWE[1]
						Stage I–II			Stage III– IV			Case all			Control			
–460C/T (rs833061)						**TT**	**TC**	**CC**	**TT**	**TC**	**CC**	**TT**	**TC**	**CC**	**TT**	**TC**	**CC**	
Hsieh YY	2004	China	Asian	122	131	0	0	0	68	54	0	68	54	0	48	83	0	0.00
Bhanoori M	2005	India	Asian	215	210	0	0	0	56	112	47	56	112	47	56	112	42	0.30
Kim SH	2005	Korea	Asian	215	289[2]	0	0	0	113	83	19	113	83	19	157[2]	110[2]	22[2]	0.66
Ikuhashi Y	2007	Japan	Asian	147	181	10	12	2	62	55	6	72	67	8	80	84	17	0.45
Zhao ZZ	2008	Austria	Caucasian	958	959	…[3]	…[3]	…[3]	…[3]	…[3]	…[3]	227	502	224	218	495	234	0.16
Cosín R	2009	Spain	Caucasian	186	180	4	14	1	46	83	38	50	97	39	48	86	46	0.55
Liu Q	2009	China	Asian	344	360	0	0	0	201	130	13	201	130	13	229	114	17	0.56
Attar R	2010	Turkey	Caucasian	52	60	6	2	3	21	12	8	27	14	11	30	11	19	0.00
Altinkaya SO	2011	Turkey	Caucasian	98	94	20	2	0	72	4	0	92	6	0	92	2	0	0.92
All				2337	2464	40	30	6	639	533	131	906	1065	361	958	1097	397	
+405G/C (rs2010963)						**CC**	**CG**	**GG**	**CC**	**CG**	**GG**	**CC**	**CG**	**GG**	**CC**	**CG**	**GG**	
Bhanoori M	2005	India	Asian	215	210	0	0	0	4	71	140	4	71	140	18	79	113	0.43
Kim SH	2005	Korea	Asian	215	289[2]	0	0	0	50	89	76	50	89	76	36[2]	157[2]	96[2]	0.02
Ikuhashi Y	2007	Japan	Asian	147	181	3	11	10	19	65	38	22	76	48	31	94	56	0.43
Gentilini D	2008	Italy	Caucasian	203	140	…[3]	…[3]	…[3]	…[3]	…[3]	…[3]	28	106	69	14	59	67	0.85
Zhao ZZ	2008	Austria	Caucasian	958	959	…[3]	…[3]	…[3]	…[3]	…[3]	…[3]	85	422	442	74	413	459	0.15
Cosín R	2009	Spain	Caucasian	186	180	3	11	5	15	80	72	18	91	77	16	80	84	0.62
Attar R	2010	Turkey	Caucasian	52	60	7	2	2	22	14	5	29	16	7	21	30	9	0.75
Altinkaya SO	2011	Turkey	Caucasian	98	94	7	11	4	18	46	12	25	57	16	84	10	0	0.59
All				2074	2113	20	35	21	147	365	324	261	928	875	294	922	884	
+936T/C (rs3025039)						**CC**	**CT**	**TT**	**CC**	**CT**	**TT**	**CC**	**CT**	**TT**	**CC**	**CT**	**TT**	
Ikuhashi Y	2007	Japan	Asian	147	181	16	8	0	64	48	11	80	56	11	118	53	10	0.22
Zhao ZZ	2008	Austria	Caucasian	958	959	…[3]	…[3]	…[3]	…[3]	…[3]	…[3]	674	264	19	691	233	21	0.80
Cosín R	2009	Spain	Caucasian	186	180	13	6	0	119	43	5	132	49	5	146	33	1	0.55
Liu Q	2009	China	Asian	344	360	0	0	0	234	100	10	234	100	10	248	103	9	0.66
Lamp M	2010	Estonia	Caucasian	150	199	…[3]	…[3]	…[3]	…[3]	…[3]	…[3]	104	43	3	137	56	6	0.92
All				1785	1879	29	14	0	417	191	26	1224	512	48	1340	478	47	

[1]Hardy-Weinberg equilibrium (HWE) was calculated by goodness-of-fit χ^2-test.
[2]The sum of fertile women and patients without endometriosis.
[3] . . . : An absence of data for that study.

Table 2 Main results for the *VEGF* –460C/T Polymorphism in the Meta-Analysis.

	Studies	TC vs. TT				CC vs. TT				TC/CC vs. TT (Dominant model				CC vs. TT/TC (Recessive model				C vs. T			
		OR (95% CI)	*P*	P_h^1	P_h^2	OR (95% CI)	*P*	P_h^1	P_h^2	OR (95% CI)	*P*	P_h^1	P_h^2	OR (95% CI)	*P*	P_h^1	P_h^2	OR (95% CI)	*P*	P_h^1	P_h^2
Total	9	1.00 (0.88–1.14)	0.989	0.068		0.91 (0.75–1.10)	0.310	0.759		0.97 (0.86–1.10)	0.671	0.086		0.91 (0.77–1.07)	0.243	0.635		0.96 (0.88–1.05)	0.361	0.192	
Ethnicity																					
Asian	5	0.92 (0.67–1.26)[3]	0.598	0.017	0.740	0.96 (0.69–1.35)	0.827	0.466	0.653	0.91 (0.67–1.24)[3]	0.549	0.018	1.000	0.97 (0.71–1.32)	0.827	0.462	0.636	0.98 (0.86–1.11)	0.718	0.076	0.737
Caucasian	4	1.03 (0.84–1.25)	0.790	0.499		0.88 (0.70–1.11)	0.281	0.728		0.98 (0.81–1.18)	0.805	0.596		0.89 (0.74–1.07)	0.218	0.471		0.95 (0.85–1.06)	0.371	0.458	
Disease stage[4]																					
Stage I-II	4	1.46 (0.79–2.71)	0.226	0.561		0.65 (0.25–1.72)	0.388	0.634		1.22 (0.68–2.18)	0.499	0.556		0.49 (0.20–1.21)	0.124	0.342		0.93 (0.62–1.39)	0.710	0.399	
Stage III–IV	8	0.98 (0.75–1.26)[3]	0.824	0.047		0.90 (0.68–1.19)	0.284	0.579		0.97 (0.83–1.14)	0.516	0.060		0.89 (0.69–1.15)	0.238	0.491		0.96 (0.85–1.08)	0.319	0.141	

Test for heterogeneity [1]in groups and [2]between groups. [3]Random-effects model was used when the *P*-value for heterogeneity test was ≤ 0.05, otherwise the fixed-effect model was used. [4]Only studies that provided specific data on subgroups of cases are included; no heterogeneity test between groups could be conducted because the same control groups were used for comparison in several of the included studies. CI, confidence interval; OR, odds ratio.

Table 3 Main results for the *VEGF* +405G/C polymorphism in the meta-analysis.

	Studies	CG vs. CC				GG vs. CC				CG/GG vs. CC (Dominant model)				GG vs. CC/CG (Recessive model)				G vs. C			
		OR (95% CI)	*P*	P_h^1	P_h^2	OR (95% CI)	*P*	P_h^1	P_h^2	OR (95% CI)	*P*	P_h^1	P_h^2	OR (95% CI)	*P*	P_h^1	P_h^2	OR (95% CI)	*P*	P_h^1	P_h^2
Total	8	1.30 (0.62–2.72)[3]	0.487	<0.001	0.067	1.04 (0.61–1.78)[3]	0.873	<0.001		1.35 (0.63–2.87)[3]	0.442	<0.001		1.00 (0.76–1.30)[3]	0.974	0.004		1.19 (0.83–1.70)[3]	0.341	<0.001	
Ethnicity																					
Asian	3	1.12 (0.36–3.53)[3]	0.843	<0.001	0.067	1.42 (0.47–4.33)[3]	0.538	0.001	0.488	1.26 (0.41–3.91)[3]	0.687	<0.001	0.205	1.26 (1.00–1.59)	0.054	0.312	0.009	1.14 (0.76–1.70)[3]	0.522	0.004	0.188
Caucasian	5	1.41 (0.48–4.17)[3]	0.530	<0.001		0.89 (0.44–1.78)[3]	0.734	0.005		1.40 (0.45–4.37)[3]	0.568	<0.001		0.83 (0.57–1.23)[3]	0.358	0.022		1.28 (0.70–2.32)[3]	0.426	<0.001	
Disease stage[4]																					
Stage I–II	4	1.31 (0.22–7.78)[3]	0.766	<0.001		1.77 (0.29–10.93)[3]	0.540	0.006		1.47 (0.23–9.56)[3]	0.690	<0.001		1.53 (0.42–5.57)[3]	0.523	0.017		1.55 (0.40–5.95)[3]	0.523	<0.001	
Stage III–IV	6	1.59 (0.50–5.07)[3]	0.432	<0.001		1.47 (0.61–3.54)[3]	0.387	<0.001		1.73 (0.54–5.61)[3]	0.359	<0.001		1.15 (0.82–1.62)[3]	0.423	0.049		1.44 (0.83–2.51)[3]	0.199	<0.001	

Test for heterogeneity [1]in groups and [2]between groups. [3]Random-effects model was used when the *P*-value for heterogeneity test was ≤ 0.05, otherwise the fixed-effect model was used. [4]Only studies that provided specific data on subgroups of cases are included; no heterogeneity test between groups could be conducted because the same control groups were used for comparison in several of the included studies. CI, confidence interval; OR, odds ratio.

Table 4 Main results for the *VEGF* +936T/C polymorphism in the meta-analysis.

	Studies	CT vs. CC				TT vs. CC				CT/TT vs. CC (Dominant model)				TT vs. CC/CT (Recessive model)				T vs. C			
		OR (95% CI)	*P*	P_h^1	P_h^2	OR (95% CI)	*P*	P_h^1	P_h^2	OR (95% CI)	*P*	P^1	P_h^2	OR (95% CI)	*P*	P_h^1	P_h^2	OR (95% CI)	*P*	P_h^1	P_h^2
Total	5	1.19 (1.02–1.38)	0.023	0.391		1.17 (0.78–1.77)	0.456	0.455		1.18 (1.03–1.37)	0.020	0.244		1.10 (0.73–1.66)	0.637	0.542		1.15 (1.01–1.30)	0.029	0.174	
Ethnicity																					
Asian	2	1.18 (0.90–1.54)	0.231	0.156	0.955	1.38 (0.73–2.63)	0.322	0.626	0.445	1.20 (0.92–1.55)	0.176	0.142	0.929	1.27 (0.67–2.41)	0.456	0.794	0.504	1.17 (0.94–1.46)	0.157	0.181	0.821
Caucasian	3	1.19 (1.00–1.42)	0.053	0.350		1.04 (0.61–1.78)	0.886	0.243		1.18 (0.99–1.40)	0.059	0.193		1.00 (0.58–1.70)	0.990	0.275		1.14 (0.98–1.33)	0.094	0.105	
Disease stage[3]																					
Stage I–II	2	1.42 (0.72–2.82)	0.313	0.388		0.65 (0.08–5.12)	0.686	0.262		1.26 (0.64–2.49)	0.503	0.284		0.62 (0.08–4.83)	0.648	0.290		1.06 (0.58–1.95)	0.849	0.209	
Stage III–IV	3	1.27 (1.00–1.62)	0.050	0.172		1.80 (0.98–3.30)	0.057	0.346		1.32 (1.04–1.66)	0.020	0.103		1.63 (0.90–2.97)	0.108	0.417		1.30 (1.06–1.59)	0.011	0.082	

Test for heterogeneity [1]in groups and [2]between groups. [3]Only studies that provided specific data on subgroups of cases are included; no heterogeneity test between groups could be conducted because the same control groups were used for comparison in several of the included studies. CI, confidence interval; OR, odds ratio.

VEGF, and may therefore contribute to the pathogenesis of endometriosis (Watson et al., 2000; Cosin et al., 2009).

The +936C/T polymorphism locates in the 3′-UTR of the *VEGF* gene (Jin et al., 2005) and the +936T allele has been shown to correlate with lower VEGF plasma levels (Renner et al., 2000; Krippl et al., 2003). Several potential mechanisms have been suggested: (i) the +936C>T transition in the 3′-UTR may lead to the loss of a potential binding site for activator protein 4 (AP-4) (Renner et al., 2000), which is a helix-loop-helix transcription factor, enhancing expression of several viral and cellular genes by binding to specific enhancer sites (Mermod et al., 1988; Hu et al., 1990); (ii) this polymorphism may be in linkage disequilibrium with another unknown polymorphism elsewhere; and (iii) the C>T transition may lead to a change of the binding miRNAs. In order to explore the probable mechanisms, the variant +936C>T in the 3′-UTR was analyzed for putative binding miRNAs through MicroSNiPer software (http://cbdb.nimh.nih.gov/microsniper/) (Barenboim et al., 2010). As shown in Figure S5, +936C>T variation creates a binding site for miR-4475, and deletes a binding site for miR-1973. So, we infer that the genetic variant could regulate *VEGF* gene expression by the creation/deletion of miRNAs and in turn affect the susceptibility of endometriosis. Whether this indeed is the case requires further investigation.

A recent meta-analysis on this polymorphism included six studies and concluded no overall association between these three polymorphisms and endometriosis (Zhao et al., 2008). Several observations in the study, such as no association of *VEGF* −460C/T, +405G/C with endometriosis, are similar to ours. However, the association between *VEGF* +936T/C polymorphism and endometriosis was different from ours. Cumulative analysis clearly states less likelihood of finding no overall association as reported by Zhao et al. Inclusion of few recent studies in our meta-analysis could be responsible for the differences in the overall inference.

Some limitations of this meta-analysis should be acknowledged. First, in the ethnic subgroup analyses, the number of each subgroup was relatively small, not having enough statistical power to explore the real association. Second, our results were based on unadjusted estimates, while a more precise analysis should be conducted if all individual data were available, which would allow for the adjustment by other co-variants including age, body mass index, smoking status, drinking status and other lifestyle factors. Third, publication bias, which can occur when studies with null or unexpected results are not published, is of concern. Therefore, we can't exclude the effect of potential publication bias on this meta-analysis.

In conclusion, this meta-analysis suggests that the *VEGF* +936T polymorphism is capable of causing endometriosis susceptibility. It is necessary to conduct large sample studies

using standardized unbiased genotyping methods, homogeneous patients with endometriosis and well-matched controls. Additionally, concerning endometriosis with multifactorial etiology, more studies or complete case-control studies, especially stratified by different ethnic background, environmental exposure or other risk factors, should be performed to clarify possible roles of *VEGF* polymorphism in the pathogenesis of endometriosis in the future.

Author Contributions

Study design: SX WW. Execution: SX WW HS JL. Analysis: WW XL CL. Manuscript drafting: SX WW HS. Critical discussion: YX SW BDM KM XW.

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Conflict of Interest

The authors declare no conflict of interest.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Figure S1 Forest plot of the *VEGF* -406C/T polymorphism and endometriosis risk.

Figure S2 Forest plot of the *VEGF* +405G/C polymorphism and endometriosis risk.

Figure S3 Forest plot of the *VEGF* +936T/C polymorphism and endometriosis risk.

Figure S4 Funnel plot analysis to detect publication bias.

Figure S5 A predict effect of the variant +936C>T on the binding miRNA.

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