The association between vascular endothelial growth factor (VEGF) +405G>C genetic polymorphism and endometriosis

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Abstract

The vascular endothelial growth factor (VEGF) is one of the most important candidate genes for the development of endometriosis, and VEGF genetic polymorphisms might be potentially associated with endometriosis risk. However, the results still remain controversial. The objective of this study aimed to perform a comprehensive meta-analysis to explore a better understanding of the effects of VEGF +405G>C genetic polymorphism on the risk of endometriosis. A total of eleven eligible studies were eventually identified in this meta-analysis, including 2829 endometriosis cases and 2947 controls. In the overall analysis, no significant association between the VEGF +405G>C genetic polymorphism and the risk of endometriosis was detected in all genetic models (for homozygote comparison [CC versus vs. GG]: OR = 1.21, 95% CI 0.67–2.19, $P = 0.537$; for heterozygote comparison [CG vs. GG]: OR = 1.16, 95% CI 0.86–1.56, $P = 0.348$; for dominant comparison CC/CG vs. GG: OR = 1.10, 95% CI 0.93–1.30, $P = 0.263$; for recessive comparison [CC vs. CG/GG]: OR = 1.03, 95% CI 0.73–1.47, $P = 0.857$; allele comparison [C vs. G]: OR = 0.99, 95% CI 0.70–1.40, $P = 0.962$). In the subgroup analysis by ethnicities, there was no significant association between VEGF +405G>C genetic polymorphism and endometriosis risk in Asians and/or Caucasians under all genetic models (all P -values >0.05). No publication bias was observed in this study. This meta-analysis supports that the VEGF +405G>C genetic polymorphism is not significant associated with the risk of endometriosis.

Keywords: Endometriosis, VEGF gene, genetic polymorphism, meta-analysis

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Introduction

Endometriosis, a common and chronic gynecological disorder, is defined as growth of hormonally responsive and endometrial tissue outside of the uterine cavity which causes diverse diseases and signs such as infertility, pelvic pain and dysmenorrhea.^{1–7} The prevalence of endometriosis has been reported to be approximately 2–18% among women who seek tubal ligations, and about 5–50% of women with infertility.^{3,8} Endometriosis is a multi-factorial disease which causes by both environmental and genetic factors.^{3,4} However, the pathogenesis of endometriosis is still poorly understood. In recent years, several published articles indicated that the vascular endothelial growth factor (VEGF) is one of the most important candidate genes for the development of endometriosis, and genetic polymorphisms in VEGF gene might be potentially associated with the risk of endometriosis.^{3–7,9–20} The +405G>C genetic polymorphism in 5'-untranslated region of VEGF gene (rs2010963), one of the most studied genetic variants resulting from G to C mutations, has been reported to affect on the regulation of VEGF gene expression and contributed to the risk of

endometriosis in diverse populations.^{3–7,9–17} However, findings from these published articles were conflicting but not conclusive. Therefore, in this study, we conducted a meta-analysis of all available published articles to explore a better understanding of the effects of VEGF +405G>C genetic polymorphism on the risk of endometriosis.

Materials and methods

Publication search strategy

To identify all eligible studies on the potential association between the VEGF +405G>C genetic variant and endometriosis risk, the database of Pubmed, Elsevier Science Direct, Wiley Online Library, Excerpta Medica Database (EMBASE) and Chinese National Knowledge Infrastructure (CNKI) were retrieved up to 6 August 2014. The following search terms were used: “endometriosis”, “VEGF” OR “vascular endothelial growth factor”, “+405G>C” OR “rs2010963” and “genetic variant” OR “genetic polymorphism”. Publication searching was performed without limitation on publication date and publishing language. All references

of included studies were reviewed to find additional eligible literatures.

Inclusion and exclusion criteria

The enrolled eligible literatures in the present meta-analysis had to meet all of the following inclusion criteria: (1) studied in human; (2) the study using a case-control design; (3) the study evaluating the association between the *VEGF* +405G>C genetic polymorphism and endometriosis risk; and (4) the study offering enough data for estimating the odds ratios (ORs) and 95% confidence intervals (CIs). The exclusion criteria are as follow: (1) duplicated of previous publications; (2) no control population; (3) incomplete data; (4) abstract, review, meta-analyses, and editorial articles.

Data extraction

All the publication literatures and detailed data were extracted independently by two investigators (Fang Fang and Lili Gong). The disagreements were resolved by additional assessment from all authors. The following information were collected from each eligible article: the first author's name, year of publication, country, ethnicities, study design, number of cases and controls, numbers of allele and genotype, and genotyping methods. Different ethnicities were categorized as Caucasian and Asian.

Statistical analysis

All statistical analyses were analyzed by the STATA software 11.0 (STATA Corporation, College Station, TX, USA). *P*-values less than 0.05 were settled as statistically significant level. The risk of endometriosis associated with the +405G>C genetic polymorphism in the *VEGF* gene was evaluated for each enrolled study by the pooled ORs, together with their 95% CIs. Five genetic models of the pooled ORs were analyzed: the homozygote comparison (CC vs. GG), heterozygote comparison (CG vs. GG), dominant model (CC/CG vs. GG), and recessive model (CC vs. CG/GG), allele comparison (C vs. G), respectively. The Z-test was utilized to evaluate the significance of

pooled ORs. The chi-square-based Q-test^{21,22} and I² index²³ were used to check the heterogeneity assumption. *P* < 0.10 and/or I² index > 50% for Q-test indicated significant heterogeneity among the studies,²⁴ and the random-effects model (the DerSimonian and Laird method) was then used to calculate the pooled ORs.²⁵ Otherwise, the fixed effect model (the Mantel-Haenszel method) was conducted.²⁶ The subgroup analyses were assessed by different ethnicities. The Egger's linear regression and Begg's funnel plot methods were used to detect the publication bias.^{27,28}

Results

General characteristics of eligible studies

Through the article search and selection according to the inclusion and exclusion criteria, 11 eligible studies with 2829 endometriosis cases and 2947 controls were finally included in the present meta-analysis.^{3-7,9-14} The general characteristics of included eligible studies in the meta-analysis are presented in Table 1. Among the enrolled literatures, there were three studies of Asians decent^{3,5,9} and eight studies of Caucasians decent.^{4,6,7,10-14} These subjects were carried out in Korean, Japanese, Indian, Spanish, Turkish, Austrians, Italians, and Iranians. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and mass-assay methods were utilized to analyze the *VEGF* +405G>C genetic polymorphisms genotypes in these enrolled articles.

Meta-analysis

The evaluation of the potential association strength between *VEGF* +405G>C genetic polymorphism and the risk of endometriosis is summarized in Table 2. In the overall analysis, there were no evidence for significant association between *VEGF* +405G>C genetic polymorphism and the risk of endometriosis in any of the genetic models (for homozygote comparison [CC vs. GG]: OR = 1.21, 95% CI 0.67-2.19, *Z* = 0.62, *P* = 0.537, Figure 1; for heterozygote comparison [CG vs. GG]: OR = 1.16, 95% CI 0.86-1.56, *Z* = 0.94, *P* = 0.348; for dominant comparison [CC/CG vs.

Table 1 The general characteristics of eligible studies included in the meta-analysis

First author	Year	Country	Ethnicity	Genotyping methods	No. (cases/controls)	Case (%)			Control (%)		
						GG	CG	CC	GG	CG	CC
Bhanoori	2005	India	Asian	PCR-RFLP	215/210	140	71	4	113	79	18
Kim	2005	Korea	Asian	PCR-RFLP	215/219	76	89	50	74	116	29
Ikuhashi	2007	Japan	Asian	PCR-RFLP	146/181	48	76	22	56	94	31
Gentilini	2008	Italy	Caucasian	PCR-RFLP	203/140	69	106	28	67	59	14
Zhao	2008	Austria	Caucasian	MassAssay	949/946	442	422	85	459	413	74
Cosin	2009	Spain	Caucasian	PCR-RFLP	186/180	77	91	18	84	80	16
Attar	2010	Turkey	Caucasian	PCR-RFLP	52/60	7	16	29	9	30	21
Toktam	2010	Iran	Caucasian	PCR-RFLP	150/144	48	80	22	83	48	13
Altinkaya	2011	Turkey	Caucasian	PCR-RFLP	98/94	16	57	25	0	10	84
Emamifar	2012	Iran	Caucasian	PCR-RFLP	480/600	97	230	153	258	276	66
Saliminejad	2013	Iran	Caucasian	PCR-RFLP	135/173	55	57	23	60	86	27

PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism.

Table 2 The meta-analysis of VEGF +405G>C genetic variant and endometriosis risk

Comparisons	Population	N	Test of association			Model	Test of Heterogeneity		
			OR (95% CI)	Z	P-value		χ^2	P-value	I ² (%)
CC vs. GG	Overall	11	1.21 (0.67–2.19)	0.62	0.537	R	90.60	0	89.0
	Asian	3	0.69 (0.23–2.06)	0.66	0.511	R	12.91	0.002	84.5
	Caucasian	8	1.50 (0.74–3.05)	1.12	0.263	R	66.92	0	89.5
CG vs. GG	Overall	11	1.16 (0.86–1.56)	0.94	0.348	R	51.14	0	80.4
	Asian	3	0.79 (0.61–1.01)	1.89	0.059	F	0.74	0.691	0
	Caucasian	8	1.36 (0.94–1.96)	1.64	0.101	R	36.79	0	81.0
CC/CG vs. GG	Overall	11	1.10 (0.93–1.30)	1.12	0.263	R	20.59	0.024	51.4
	Asian	3	0.89 (0.72–1.10)	1.06	0.288	F	0.19	0.909	0
	Caucasian	8	1.20 (0.97–1.49)	1.71	0.088	R	15.63	0.029	55.2
CC vs. CG/GG	Overall	11	1.03 (0.73–1.47)	0.18	0.857	R	42.84	0	76.7
	Asian	3	0.88 (0.37–2.11)	0.29	0.771	R	9.40	0.009	78.7
	Caucasian	8	1.07 (0.71–1.62)	0.32	0.746	R	33.43	0	79.1
C vs. G	Overall	11	0.99 (0.70–1.40)	0.05	0.962	R	166.97	0	94.0
	Asian	3	1.14 (0.77–1.70)	0.66	0.507	R	10.26	0.006	80.5
	Caucasian	8	0.95 (0.61–1.47)	0.25	0.801	R	140.04	0	95.0

Note: Random effect model was chosen when P-value < 0.10 and/or I² > 50% for heterogeneity test; otherwise fixed effect model was used. N: number of comparisons; OR: odds ratio; CI: confidence interval; CC vs. GG: homozygote comparison; CG vs. GG: heterozygote comparison; CC/CG vs. GG: dominant model; CC vs. CG/GG: recessive model; C vs. G: allele comparison; F: fixed effect model; R: random effect model.

Figure 1 Forest plots of the association between VEGF +405 G>C genetic polymorphism and endometriosis (homozygote comparison [CC vs. GG])

GG]: OR = 1.10, 95% CI 0.93–1.30, Z = 1.12, P = 0.263; for recessive comparison [CC vs. CG/GG]: OR = 1.03, 95% CI 0.73–1.47, Z = 0.18, P = 0.857; for allele comparison [C vs. G]: OR = 0.99, 95% CI 0.70–1.40, Z = 0.05, P = 0.962; Table 2). Besides, in the subgroup analysis by ethnicities, we did not detected any significant association between VEGF +405G>C genetic polymorphism and the risk of endometriosis in Asians and/or Caucasians under all genetic models (all P-values > 0.05, Table 2). Figure 2 shows that the forest plots of the association VEGF +405G>C genetic

polymorphism and endometriosis risk in homozygote comparison by ethnicities (for Asians: OR = 0.69, 95% CI 0.23–2.06, Z = 0.66, P = 0.511; for Caucasians: OR = 1.50, 95% CI 0.74–3.05, Z = 1.12, P = 0.263, Table 2 and Figure 2).

Publication bias

The publication bias of the included literature was assessed by the Begg's funnel plot and Egger's test. There was no evidence of publication bias for VEGF +405G>C genetic polymorphism in all genetic models (all P-values > 0.05).

Figure 2 Forest plots of the association *VEGF* +405 G>C genetic polymorphism and endometriosis (homozygote comparison by ethnicities [CC vs. GG])

Figure 3 Begg's funnel plot for publication bias test (homozygote comparison [CC vs. GG])

Figure 3 shows the Begg's funnel plot for publication bias test in homozygote comparison.

Discussion

Growing evidence suggests that the *VEGF* is a promising candidate gene for mediating the genetic influence on the risk of endometriosis.^{3-7,9-20} The *VEGF* genetic polymorphisms have been indicated to play an important effect on the regulation of *VEGF* gene expression that results in altered levels of *VEGF*, and may contribute to the pathogenesis of

endometriosis.^{7,16,29} Several studies have reported on the potential association of *VEGF* +405G>C genetic polymorphism with endometriosis risk.^{3-7,9-17} Unfortunately, results from those published observations were controversial or ambiguous. Kim et al. demonstrated that the *VEGF* +405G>C genetic polymorphism may be associated with the risk of advanced stage endometriosis in the Korean population.³ Bhanoori et al. suggested that the -460T/+405C haplotype in the *VEGF* gene was significantly less common in women with endometriosis than in controls.⁵

Gentilini et al. supported that the VEGF +405G>C genetic polymorphism plays a role in the development of endometriosis.¹⁰ Attar et al. indicated that the CC genotype of VEGF +405G>C genetic polymorphism may be associated with the risk of endometriosis, but the G allele of VEGF +405G>C genetic polymorphism appears to be protective against endometriosis.¹² Altinkaya et al. observed that GC genotype and G allele of VEGF +405G>C genetic polymorphism may be associated with the risk of developing early and advanced stage endometriosis in the Turkish population.¹³ Emamifar et al. suggested that the VEGF +405G>C genetic polymorphism may be associated with higher risk of endometriosis in northern Iran.¹⁴ Toktam and colleagues reported that there was no significant correlation between VEGF +405G>C genetic polymorphism and endometriosis in the Iranian population.⁶ Saliminejad et al. observed that no association were found between the VEGF +405G>C genetic polymorphism and the risk of endometriosis.⁴ The present meta-analysis, including 2829 endometriosis cases and 2947 controls, explored the more reliable association between the VEGF +405G>C genetic polymorphism and the risk of endometriosis. In the overall analysis, no significant association was detected between the VEGF +405G>C genetic polymorphism and the risk of endometriosis in all genetic models. Besides, in the subgroup analysis by ethnicities, we also found that there was no significant association between VEGF +405G>C genetic polymorphism and endometriosis risk in Asians and/or Caucasians under all genetic models. Our results indicate that the VEGF +405G>C genetic polymorphism might not cause the susceptibility to endometriosis.

In the present meta-analysis, some advantages should be acknowledged. First, all the possible eligible studies have been enrolled in this meta-analysis by a strict searching strategy. Second, this meta-analysis significantly increased statistical power by pooling data from different articles as much as possible. Third, based on our inclusion and exclusion criteria, all enrolled studies had acceptable quality in this meta-analysis. Fourth, no publication bias was observed in this study, which indicated that the whole pooled findings may be unbiased. However, several limitations of this meta-analysis should be considered. First, only 11 eligible studies were eventually included in this meta-analysis, and the sample size was relatively limited. Second, the ethnicities only contained Asians and Caucasians, not mentioned about other ethnicities. Third, only published literatures were collected in this meta-analysis, and ongoing and unpublished data on this topic were not retrieved which may have biased our results.

In conclusion, the present meta-analysis provides more evidence that the VEGF +405G>C genetic polymorphism is not significantly associated with the risk of endometriosis. Considering the limited sample size and ethnicities populations in our meta-analysis, more larger-scaled and well-designed studies are required to elucidate a more precise estimation on the effects of VEGF +405G>C genetic polymorphism on the risk of endometriosis.

Authors' contributions: Each author participated in the concept and design. FF and LG drafted and revised the manuscript. XW and LZ were involved in analysis and interpretation of data. All authors approved the final version of the manuscripts.

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