ORIGINAL ARTICLE

Vascular endothelial growth factor gene polymorphisms and gastric cancer risk: a meta-analysis

Meng Zhuang¹, Zhi Peng², Jian Wang¹, Xiangqian Su¹

¹Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Gastrointestinal Surgery IV, Peking University Cancer Hospital and Institute, Beijing 100142, China; ²Department of Gastrointestinal Oncology, Peking University Cancer Hospital & Institute, Beijing, 100142, China

Summary

Purpose: Vascular endothelial growth factor (VEGF) plays important roles in the process of tumor growth and metastasis. Although the association between VEGF polymorphisms and gastric cancer risk has been extensively studied, available results remain controversial. To derive a convincing estimation of the relationship, a meta-analysis containing 6 VEGF (+936C/T, -634G/C, -460T/C, +1612G/A, -2578C/A and -1154G/A) gene polymorphisms was performed.

Methods: We conducted a systematic search of PubMed and Chinese National Knowledge Infrastructure (CNKI) to select relevant articles. Nine available case-control studies with 2,281 gastric cancer cases and 2,820 healthy controls met the inclusion criteria. The odds ratio (OR) and 95% confidence interval (95%CI) were used to evaluate the strength of the association.

Results: This meta-analysis indicated that the VEGF-634 *G* allele carrier represented a risk factor for gastric cancer

(GG+GC vs CC: OR=1.23, 95%CI=1.02-1.49, p=0.03). The VEGF +1612G/A polymorphism was associated with risk of gastric cancer (G allele vs A allele: OR=0.62, 95%CI=0.49-0.79, p<0.0001; GG+GA vs AA: OR=0.16, 95%CI=0.05-0.51, p=0.002 and GA+AA vs GG : OR=1.57, 95%CI=1.21-2.04, p=0.008). For polymorphisms of VEGF +936C/T, -460C/T, -2578C/A, -1154G/A, no association was found with gastric cancer risk.

Conclusion: Our meta-analysis suggests that VEGF-634 G allele carrier may increase gastric cancer risk, whereas the VEGF +1612 G/A G allele and G allele carrier may decrease the risk. No association between+936C/T, -460C/T, -2578C/A, -1154G/A polymorphisms and susceptibility to gastric cancer was found.

Key words: gastric cancer, gene polymorphisms, meta-analysis, risk, vascular endothelial growth factor

Introduction

Although the incidence and mortality of gastric cancer has gradually decreased over the past several decades, gastric cancer, the second most common cause of cancer mortality [1,2], remains a major health problem worldwide due to its poor prognosis. Gastric cancer is a result of a multifactorial process including many biological changes [3].

Published articles revealed that VEGF gene plays an important role in angiogenesis which is

essential for the development, growth and progression of malignant tumors [4-7]. Although more than 30 single nucleotide polymorphisms (SNPs) have been described in VEGF gene [8], the association between VEGF polymorphisms and the risk of gastric cancer remains controversial. Some of these polymorphisms (+936C/T rs3025039,-634G/C rs2010963, - 460T/C rs833061, and -2578C/A rs699947) have been found to be associated with the susceptibility, development,

Correspondence to: Xiangqian Su, MD. Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Gastrointestinal Surgery IV, Peking University Cancer Hospital & Institute,0 52 Fucheng Road, Haidian District, Beijing 100142, China. Tel: + 86 10 88196579, Fax: +86 10 88122437, E-mail: suxiangqian@bjmu.edu.cn Received: 29/10/2016; Accepted: 11/11/2016 prognosis and metastasis of gastric cancer [9,10]. Bae et al. found that +936 T was a susceptibility factor for gastric cancer, at least in Korean population [11], while other case-control studies in Korea showed +936T allele was associated with a decreased susceptibility to gastric cancer [12]. Moreover, Tzanakis et al. revealed a marginally significant association of the -634CC genotype with increased risk for gastric cancer development [13]. Furthermore, Zhou et al. suggested that the VEGF +1612G/A gene polymorphism was associated with gastric cancer in Chinese Han patients [14]. Tahara et al. showed that the +1612G/A gene polymorphism was associated with susceptibility to gastric cancer in Japanese population [15]. However, many studies indicated that VEGF polymorphisms have no associations with susceptibility for gastric cancer. Al-Moundhri et al. [16], Ke et al. [17] and Lin et al. [18] suggested that + 936C/T gene polymorphisms play no significant roles in gastric cancer risk. Some other studies indicated that the -634G/C gene polymorphism was not associated with risk of gastric cancer and its progression [14,17].

In this study, we aimed to carry out a meta-analysis in order to investigate the association between VEGF gene polymorphisms and gastric cancer risk.

Methods

Literature search

We performed a systematic search of PubMed and Chinese National Knowledge Infrastructure (CNKI) by using the following key words: "vascular endothelial growth factor" or "VEGF", "polymorphism", "single nucleotide polymorphism" and "gastric" or "stomach cancer". Only published studies with full-text articles were included without language restriction; all non-English articles were translated if necessary. Of all the studies with overlapping data published by the same investigators, only the most detailed article was included. When necessary, we contacted the authors of related articles to require additional information.

Inclusion criteria

The criteria of all included studies were as follows: (1) case-control studies that contained gastric cancer cases and healthy controls; (2) VEGF gene polymorphisms and its related gastric cancer risk; (3) sufficient genotype frequency data were provided with the original literature. According to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement, we made a flowchart showing the selection procedure (Figure 1).



Figure 1. Flowchart of the selection procedure of the studies and reasons for exclusion.

Data extraction

Necessary data was extracted from eligible publications including surname of first author, year of publication, numbers of genotyped cases and controls, genotypes frequencies, country, ethnicity, polymorphisms, and evidence of Hardy-Weinberg equilibrium (HWE) in controls.

Statistics

The consistency of genotype frequencies in controls, compared with HWE, was tested by x^2 test. OR with 95% CI were calculated to appraise the strength of the association between VEGF polymorphism and gastric cancer risk. Q-statistic with I² was used to assess the degree of heterogeneity between the trials. When heterogeneity existed (p<0.10 or I²>50%), we used the random-effects model to calculate OR; otherwise, the fixed-effect method was applied [19]. All statistical analyses were performed with the RevMan statistical software (version.5.0.25, provided by The Cochrane Collaboration). The Egger's test was used to assess the potential publication bias by using Stata software (version 9.0). All p values were two-sided and p<0.05 was considered as statistically significant publication bias [20].

Results

Study characteristics

Nine eligible case-control studies were selected to evaluate the association of VEGF polymorphisms with the risk of gastric cancer, which included 2,281 gastric cancer cases and 2,820 controls [11,12,14-

References	Country	VEGF gene polymorphisms	No. of cases	No. of controls
Al-Moundhri et al, 2009	Oman	+405G/C, -460T/C, +936C/T	130	130
Bae et al, 2008	Korea	+ 936C /T	154	229
Chae et al, 2006	Korea	+405G/C-460T/C, +936C/T	413	413
Guan et al, 2009	USA	-634G/C +936C/T -1498T/C	171	353
Ke et al, 2008	China	-2578C/A,-1498T/C,-634G/C,+936C/T	540	561
Nikiteas et al, 2007	Greece	-2578C/A,-1154G/A,-634G/C,+936C/T	100	100
Tahara et al, 2009	Japan	+936C/T,+1612G/A	385	459
Xia et al, 2010	China	+936C/T	238	425
Zhou et al, 2011	China	-634 G/C,+936 C/T,+1612 G/A	150	150

Table 1. Characteristics of studies included in the meta-analysis

Table 2.	. The	genotype	distribution	and freq	luency of	included	studies
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Reference	Cases		Control	s	HWE test of controls					
+936C/T polymorphism	Total	CC	СТ	TT	Total	CC	СТ	TT	x ²	p value
Al-Moundhri et al.2009	130	109	19	2	130	110	20	0	0.903	0.342
Bae et al. 2008	154	89	58	7	229	169	57	3	0.551	0.458
Chae et al. 2006	413	283	122	8	413	252	149	12	3.304	0.069
Guan et al. 2009	171	127	41	3	353	276	70	7	1.034	0.309
Ke et al. 2008	540	373	152	15	561	386	164	11	1.818	0.177
Nikiteas et al. 2007	100	41	33	26	100	51	27	22	16.84	< 0.001
Tahara et al. 2009	385	256	118	11	459	300	140	19	0.271	0.603
Xia et al. 2010	228	155	63	10	413	276	131	6	4.807	0.028
Zhou et al. 2011	150	97	45	8	150	94	49	7	0.036	0.85
-634G/C polymorphism	Total	GG	GC	CC	Total	GG	GC	CC	x ²	p value
Al-Moundhri et al.2009	130	49	59	22	130	62	54	14	0.189	0.664
Chae et al. 2006	413	129	253	31	413	106	223	84	2.844	0.092
Guan et al. 2009	171	69	72	30	353	180	99	74	51.92	< 0.001
Ke et al. 2008	540	161	287	92	561	186	278	97	0.156	0.693
Nikiteas et al. 2007	100	41	40	19	100	52	39	9	0.185	0.667
Zhou et al. 2011	150	74	47	29	150	76	44	30	18.63	< 0.001
-460C/T polymorphism	Total	CC	СТ	TT	Total	CC	СТ	TT	X ²	p value
Al-Moundhri et al. 2009	130	22	66	42	130	25	61	44	0.219	0.64
Chae et al. 2006	413	9	186	218	413	27	161	225	0.063	0.802
Ke et al. 2008	540	38	207	295	561	39	215	307	0.026	0.871
+1612G/Apolymorphism	Total	GG	GA	AA	Total	GG	GA	AA	X ²	p value
Tahara et al. 2009	385	262	121	2	459	359	100	0	6.86	0.009
Zhou et al. 2011	150	104	29	17	150	112	35	3	0.019	0.891
-2578C/A polymorphism	Total	CC	CA	AA	Total	CC	CA	AA	X ²	p value
Ke et al. 2008	540	293	216	31	561	301	234	26	5.397	0.02
Nikiteas et al. 2007	100	32	39	29	100	21	48	31	0.092	0.762
-1154G/A polymorphism	Total	GG	GA	AA	Total	GG	GA	AA	X ²	p value
Nikiteas et al. 2007	100	45	36	19	100	42	43	15	0.524	0.469

17,21-23]. Study characteristics are summarized in Table 1. The publication year of the 9 studies ranged from 2006 to 2011. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay was adopted in most articles. The distribution of genotypes in the controls were consistent with HWE (p>0.05) in all except 4 of the studies [14,15,21,22]. The characteristics of genotype distribution are summarized in Table 2.

VEGF +936C/T polymorphism was not associated with gastric cancer risk

The meta-analysis about the associations between VEGF +936C/T polymorphism and gastric cancer risk is presented in Figure 2. The results revealed that there was no significant association between +936C>T polymorphism and susceptibility to gastric cancer in the comparisons of C allele vs T allele (OR=0.93, 95%CI=0.77-1.13, p=0.48), CC+CT vs TT (OR=0.80, 95%CI=0.59-1.10, p=0.18), or CT+TT vs CC (OR=0.99, 95%CI=0.88-1.13, p=0.92).

VEGF -634 G allele carrier increased the risk of gastric cancer

The meta-analysis involved 4 studies that revealed a significant association between the -634G/C polymorphism and susceptibility to gastric cancer (Figure 3) in comparison with G allele carrier GG+GC vs CC (OR=1.23, 95%CI=1.02-1.49, p= 0.03).

(A) +936C/T C allele versus T allele

	GC cas	ses	Healthy c	ontrol		Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% Cl	M-H, Random, 95% CI
Al-Moundhri et al. 2009	237	260	240	260	5.4%	0.86 [0.46, 1.61]	
Bae et al,2008	236	308	395	458	10.2%	0.52 [0.36, 0.76]	
Chae et al. 2006	688	826	653	826	14.0%	1.32 [1.03, 1.69]	-
Guan et al 2009	295	342	622	706	9.9%	0.85 [0.58, 1.24]	-
Ke et al. 2008	898	1080	936	1122	14.8%	0.98 [0.78, 1.23]	+
Nikiteas et al 2007	115	200	129	200	9.4%	0.74 [0.50, 1.11]	-
Tahara et al.2009	630	770	740	918	14.1%	1.08 [0.85, 1.38]	+
Xia et al 2010	373	456	683	826	12.4%	0.94 [0.70, 1.27]	
Zhou et al 2011	239	300	237	300	9.6%	1.04 [0.70, 1.55]	+
Total (95% CI)		4542		5616	100.0%	0.93 [0.78, 1.10]	+
Total events	3711		4635				
Heterogeneity: Tau ² = 0.0	04; Chi ² = 1	19.63, c	f = 8 (P = 0	.01); l ² =	59%		
Test for overall effect: Z =	= 0.86 (P =	0.39)	99999-0000 8 0 - 60960			Fa	vours experimental Favours control

(B) +936C/T CC + CT versus TT

	GC cas	ses	healthy co	ntrol		Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% C	M-H, Fixed, 95% CI
Al-Moundhri et al. 2009	128	130	130	130	2.8%	0.20 [0.01, 4.14]	• • •
Bae et al,2008	147	154	226	229	9.2%	0.28 [0.07, 1.10]	
Chae et al. 2006	405	413	401	413	8.7%	1.51 [0.61, 3.75]	3
Guan et al 2009	168	171	346	353	4.4%	1.13 [0.29, 4.44]	
Ke et al. 2008	525	540	550	561	16.7%	0.70 [0.32, 1.54]	
Nikiteas et al 2007	74	100	78	100	22.6%	0.80 [0.42, 1.54]	
Tahara et al.2009	374	385	440	459	12.8%	1.47 [0.69, 3.12]	
Xia et al 2010	218	228	407	413	14.2%	0.32 [0.12, 0.90]	
Zhou et al 2011	142	150	143	150	8.5%	0.87 [0.31, 2.46]	
Total (95% CI)		2271		2808	100.0%	0.82 [0.60, 1.11]	•
Total events	2181		2721				
Heterogeneity: Chi ² = 10.	87, df = 8	(P = 0.2)	21); 12 = 26%				
Test for overall effect: Z =	= 1.27 (P =	0.20)	2005 A.M. 2002 200			F	avours experimental Favours control

(C) +936C/T CT + TT versus CC

	GC cas	ses	Healthy co	ntrol		Odds Ratio	Odds Rat	io
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% C	M-H, Fixed, 9	5% CI
Al-Moundhri et al. 2009	21	130	20	130	3.2%	1.06 [0.54, 2.06]		
Bae et al,2008	65	154	60	229	5.3%	2.06 [1.33, 3.18]		
Chae et al. 2006	130	413	161	413	20.8%	0.72 [0.54, 0.96]	-	
Guan et al 2009	44	171	77	353	7.0%	1.24 [0.81, 1.90]	+	
Ke et al. 2008	167	540	175	561	22.4%	0.99 [0.76, 1.27]	+	
Nikiteas et al 2007	59	100	49	100	3.8%	1.50 [0.86, 2.62]		
Tahara et al.2009	129	385	159	459	18.2%	0.95 [0.71, 1.27]	+	
Xia et al 2010	73	228	137	413	12.5%	0.95 [0.67, 1.34]	+	
Zhou et al 2011	53	150	56	150	6.8%	0.92 [0.57, 1.47]		
Total (95% CI)		2271		2808	100.0%	1.01 [0.90, 1.14]		
Total events	741		894					
Heterogeneity: Chi ² = 19.	00, df = 8	(P = 0.0)	01); l ² = 58%	1.				10 100
Test for overall effect: Z =	= 0.18 (P =	0.86)	20.0000 2022223			F	avours experimental Fav	ours control

Figure 2. Forest plot showing the association between +936C>T polymorphism and gastric cancer risk. **(A)** +936C/T C allele versus T allele; **(B)** +936C/T CC + CT versus TT; **(C)** +936C/T CT + TT versus CC

However, VEGF -634 G allele and C allele carrier had no influence on gastric cancer risk for G allele vs C allele (OR=0.83, 95%CI=0.60-1.15, p=0.26) and GC+CC vs GG (OR=1.14, 95%CI=0.98-1.32, p=0.09).

No association was found between -460C/T polymorphism and susceptibility to gastric cancer

The evaluation of the association between VEGF -460C/T polymorphism and gastric cancer

	GC cas	ses	Healthy co	ontrol		Odds Ratio		0	dds Ra	tio	
Study or Subgroup	Events	Total	Events	Total	Weight	M-H. Random, 95% C	I	M-H, R	andom	. 95% CI	
Al-Moundhri et al. 2009	157	260	178	260	15.7%	0.70 [0.49, 1.01]			-		
Chae et al. 2006	511	826	435	826	18.2%	1.46 [1.20, 1.77]			-		
Guan et al 2009	210	342	459	606	16.9%	0.51 [0.38, 0.68]			-		
Ke et al. 2008	609	1080	650	1122	18.5%	0.94 [0.79, 1.11]			+		
Nikiteas et al 2007	122	200	143	200	14.7%	0.62 [0.41, 0.95]					
Zhou et al 2011	195	300	196	300	16.1%	0.99 [0.70, 1.38]			+		
Total (95% CI)		3008		3314	100.0%	0.83 [0.60, 1.15]			•		
Total events	1804		2061								
Heterogeneity: Tau ² = 0.1	4; Chi ² = 4	43.18, 0	if = 5 (P < 0	.00001);	l ² = 88%		0.01	0.1	-	10	100
Test for overall effect: Z =	1.12 (P =	0.26)				F	0.01 avours e	U. I experimer	ntal Fa	VOURS COD	trol

(A) -634G/C G allele versus C allele

(B) -634G/C GG + GC versus CC

	GC cas	ses	Healthy c	ontrol		Odds Ratio		Odd	s Ratio		
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% (M-H, Fix	ed, 95%	6 CI	
Al-Moundhri et al. 2009	108	130	116	130	10.1%	0.59 [0.29, 1.22]		+		
Chae et al. 2006	382	413	329	413	12.7%	3.15 [2.03, 4.87]		-	5.	
Guan et al 2009	141	171	279	353	16.4%	1.25 [0.78, 1.99]				
Ke et al. 2008	448	540	464	561	39.9%	1.02 [0.74, 1.39]		•		
Nikiteas et al 2007	81	100	91	100	8.9%	0.42 [0.18, 0.98	1				
Zhou et al 2011	121	150	120	150	11.9%	1.04 [0.59, 1.84]	-	†		
Total (95% CI)		1504		1707	100.0%	1.23 [1.02, 1.49]	1		٠		
Total events	1281		1399								
Heterogeneity: Chi ² = 29.	52, df = 5	(P < 0.0	0001); I ² = 8	3%			0.01	0.1	1	10	100
Test for overall effect: Z =	= 2.17 (P =	0.03)				I	Favours	experimental	Favou	irs cont	rol

(C) -634G/C GC + CC versus GG

	GC ca	ses	Healthy co	ntrol		Odds Ratio		0	dds Ratio	,	
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% C	1	М-Н,	Fixed, 95	% CI	
Al-Moundhri et al. 2009	81	130	68	130	7.7%	1.51 [0.92, 2.47]			-		
Chae et al. 2006	284	413	307	413	28.8%	0.76 [0.56, 1.03]			-		
Guan et al 2009	102	171	173	353	13.7%	1.54 [1.06, 2.23]			-		
Ke et al. 2008	379	540	375	561	32.9%	1.17 [0.91, 1.51]			-		
Nikiteas et al 2007	59	100	48	100	5.9%	1.56 [0.89, 2.73]					
Zhou et al 2011	76	150	74	150	11.0%	1.05 [0.67, 1.66]			-		
Total (95% CI)		1504		1707	100.0%	1.14 [0.98, 1.32]			•		
Total events	981		1045								
Heterogeneity: Chi ² = 11.	95, df = 5	(P = 0.0)	04); l ² = 58%					01	1		100
Test for overall effect: Z =	= 1.72 (P =	0.09)				F	avours	U.I experimen	tal Favo	ours conf	trol

Figure 3. Forest plot showing the association between -634G/C polymorphism and gastric cancer risk. **(A)** -634G/C G allele versus C allele; **(B)** -634G/C GG + GC versus CC; **(C)** -634G/C GC + CC versus GG

risk is presented in Figure 4. We found that VEGF -460C/T polymorphism had no impact on the susceptibility to gastric cancer, when referring to the comparisons of C allele vs T allele (OR=0.98, 95%CI = 0.86-1.12, p=0.73), CC+CT vs TT (OR=1.04, 95%CI=0.87-1.23, p=0.68), and CT+TT vs CC (OR=1.33, 95%CI=0.96-1.85, p= 0.09).

VEGF +1612 G allele and G allele carrier decreased the risk of gastric cancer

According to the analysis of 2 included studies (Figure 5), significant association was found between +1612 G/A polymorphism and susceptibility to gastric cancer in the comparisons of G allele vs A allele (OR=0.62, 95%CI=0.49-0.79, p<0.0001), GG+GA vs AA (OR=0.16, 95%CI=0.05-0.51, p=0.002), and GA+AA vs GG (OR=1.57, 95%CI=1.21-2.04, p=0.008). The results suggested that the G allele and G allele carrier (GG+GA) of +1612 G/A might be protective factors for gastric cancer.

Susceptibility to gastric cancer was not due to VEGF -2578C/A polymorphism

There was no significant association between -2578C/A polymorphism and susceptibility to gas-

(A) -460C/T C allele versus T allele

	GC cas	ses	Healthy co	ontrol		Odds Ratio		Odds	Ratio		
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% C	1	M-H. Rand	lom, 95%	CI	_
Al-Moundhri et al. 2009	110	260	111	260	14.7%	0.98 [0.70, 1.39]			+		
Chae et al. 2006	204	826	215	826	36.1%	0.93 [0.75, 1.16]		1			
Ke et al. 2008	284	1080	293	1122	49.2%	1.01 [0.83, 1.22]			•		
Total (95% CI)		2166		2208	100.0%	0.98 [0.86, 1.12]		3	•		
Total events	598		619								
Heterogeneity: Tau ² = 0.0	0; Chi ² = ().29, df	= 2 (P = 0.8	37); l² = (0%		H-001	01	1 1	0 100	!
Test for overall effect: Z =	0.34 (P =	0.73)				F	avours e	0.1	Favours	control	ŝ.

(B) -460C/T CC +CT versus TT

	GC cas	es	Healthy c	ontrol		Odds Ratio		0	dds Rati	0	
Study or Subgroup	Events	Total	Events	Total	Weight	M-H. Fixed, 95% C	1	M-H.	Fixed, 98	5% CI	
Al-Moundhri et al. 2009	88	130	86	130	10.6%	1.07 [0.64, 1.80]			-		
Chae et al. 2006	195	413	188	413	37.7%	1.07 [0.81, 1.41]			+		
Ke et al. 2008	245	540	254	561	51.7%	1.00 [0.79, 1.27]			-		
Total (95% CI)		1083		1104	100.0%	1.04 [0.87, 1.23]			•		
Total events	528		528								
Heterogeneity: Chi ² = 0.14	4, df = 2 (F	P = 0.93	3); l² = 0%				0.01	0.1		10	100
Test for overall effect: Z =	0.41 (P =	0.68)				F	avours	experimen	ital Fav	ours cont	trol

(C) -460C/T CT + TT versus CC

	GC case	es	Healthy co	ntrol		Odds Ratio		0	dds Ratio	0	
Study or Subgroup	Events '	Total	Events	Total	Weight	M-H. Fixed, 95% (1	M-H.	Fixed, 95	% CI	
Al-Moundhri et al. 2009	108	130	105	130	28.6%	1.17 [0.62, 2.20]			-		
Chae et al. 2006	404	413	386	413	13.5%	3.14 [1.46, 6.76]	6			-	
Ke et al. 2008	502	540	522	561	57.9%	0.99 [0.62, 1.57]			-		
Total (95% CI)	8	1083		1104	100.0%	1.33 [0.96, 1.85]			٠		
Total events	1014		1013								
Heterogeneity: Chi ² = 6.5	7, df = 2 (P	= 0.04	4); l ² = 70%				0.01	01		10	100
Test for overall effect: Z =	= 1.70 (P = 0	0.09)				F	avours	experimen	tal Favo	ours cont	rol

Figure 4. Forest plot showing the association between -460C/T polymorphism and gastric cancer risk. **(A)** -460C/T C allele versus T allele; **(B)** -460C/T CC +CT versus TT; **(C)** -460C/T CT + TT versus CC.

tric cancer (Figure 6) , when it comes to C allele vs A allele (OR = 1.07, 95%CI = 0.84-1.37, p=0.59), CC +CA vs AA (OR=0.92, 95%CI=0.62-1.37,p=0.68), and CA+AA vs CC (OR=0.91, 95%CI=0.73-1.14, p=0.42).

VEGF -1154G/A polymorphism could not influence the susceptibility to gastric cancer

The meta-analysis included only one relevant study about the associations between VEGF -1154G/A polymorphism and gastric cancer risk, as presented in Figure 7. VEGF -1154G/A polymorphism was not correlated with susceptibility to gastric cancer in the comparisons of G allele vs A allele (OR=0.98, 95%CI=0.65-1.47, p=0.92), GG+-GA vs AA (OR=1.42, 95%CI=0.72-2.79, p=0.31), and GA+AA vs GG (OR=0.89, 95%CI =0.51-1.55, p=0.67).

Table S1. Supporting information. Evaluation of publication bias by Egger's linear regression test

Polymorphisms	t	p value
+936C/T		
C allele vs T allele	1.55	0.164
CC + CT vs TT	1.64	0.145
CT + TT vs CC	1.51	0.175
-634G/C		
G allele vs C allele	-1.24	0.282
GG + GC vs CC	-0.4	0.707
GC + CC vs GG	1.19	0.299

Publication bias

Egger's test to assess the publication bias for +936C/T and -634G/C polymorphisms did not show statistically evidence of publication bias, all p values were >0.05 (Table S1).

(A) +1612 G/A G allele versus A allele

	GC case	s	Healthy control			Odds Ratio	Odds Ratio				
Study or Subgroup	Events T	Total	Events	Total	Weight	M-H, Random, 95% Cl	M-H. Rand	lom, 95% Cl			
Tahara et al.2009	645	770	818	918	69.9%	0.63 [0.48, 0.84]					
Zhou et al 2011	237	300	259	300	30.1%	0.60 [0.39, 0.92]					
Total (95% CI)	1	1070		1218	100.0%	0.62 [0.49, 0.79]	•				
Total events	882		1077								
Heterogeneity: Tau ² =	0.00; Chi ² =	0.05	df = 1 (P =	0.83); l ²	= 0%	H	0.01 0.1		100		
Test for overall effect: Z = 3.97 (P < 0.0001)					Favo	ours experimental	Favours con	trol			

(B) +1612 G/A GG+GA versus AA



(C) +1612 G/A GA+AA versus GG

	GC cas	es	Healthy control			Odds Ratio		Odds	s Ratio		
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95%	CI	M-H, Fix	ed, 95% Cl		
Tahara et al.2009	123	385	100	459	70.2%	1.69 [1.24, 2.29	9]				
Zhou et al 2011	46	150	38	150	29.8%	1.30 [0.79, 2.16	6]		-		
Total (95% CI)		535		609	100.0%	1.57 [1.21, 2.04]		•		
Total events	169		138								
Heterogeneity: Chi ² =	0.72, df = 1	1 (P = (0.40); l ² = 0%	5						100	
Test for overall effect:	Z = 3.37 (F	o = 0.0	008)				Favours e	experimental	Favours	control	

Figure 5. Forest plot showing the association between +1612G/A polymorphism and gastric cancer risk. (A) +1612 G/A G allele versus A allele; (B) +1612 G/A GG + GA versus AA; (C) +1612 G/A GA + AA versus GG.

Discussion

VEGF is associated with tumor angiogenesis, and it is essential to tumor growth, invasion and metastasis [4-7]. Genetic polymorphisms are identified to associate with the translation and expression of VEGF, and have a contribution on cancer susceptibility [24]. Among several functional VEGF polymorphisms, only a small portion may have effects on the risk of malignant tumor, such as VEGF +936C/T, -634G/C, -460G/C, +1612 G/A ,-2578C/A and -1154 G/A [25-28]. A case-control study indicated that VEGF +936 C/T is likely an important genetic marker of susceptibility to breast cancer [29]. Lin et al. provided strong evidence that VEGF -2578C/A polymorphism is capable of increasing lung cancer susceptibility [18]. A meta-analysis suggested that the VEGF -460T/C, -634G/C, and -2578C/A gene polymorphisms were associated with colorectal cancer risk [30]. In gas-

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tric cancer, several case-control studies have assessed whether any relationships existed between the VEGF polymorphisms and the risk of disease. Also, some previous meta-analyses examined the association between the VEGF +936C/T or 634G/C polymorphisms and gastric cancer risk. For instance, a meta-analysis conducted by Zhou et al. showed that there was no significant association between VEGF +936C/T gene polymorphism and gastric cancer risk [31]. Similarly, another meta-analysis involving 31 case-controls studies also suggested that the VEGF +936C/T or -634G/C polymorphisms were not correlated with cancer risk in all examined patients [32]. In contrast, Liu et al. recently conducted a meta-analysis to estimate potential associations between the VEG-F+936C/T, -634G/C polymorphisms and susceptibility to gastric cancer [33]. Their results showed that the VEGF -634G/C polymorphism did contribute to gastric cancer susceptibility.

(A) -2578C/A C allele versus A allele

	GC cases		Healthy control			Odds Ratio		0	dds Rati	0	
Study or Subgroup	Events T	otal	Events	Total	Weight	M-H, Random, 95%	CI	M-H, R	andom.	95% CI	
Ke et al. 2008	802 1	080	836	1122	70.5%	0.99 [0.82, 1.2	0]				
Nikiteas et al 2007	103	200	90	200	29.5%	1.30 [0.88, 1.9	2]		-		
Total (95% CI)	1:	280		1322	100.0%	1.07 [0.84, 1.3]	7]		•		
Total events	905		926								
Heterogeneity: Tau ² =	0.01; Chi ² =	1.51,	df = 1 (P =	0.22); 12	= 34%		H			10	100
Test for overall effect: Z = 0.54 (P = 0.59)							Favour	s experimen	tal Fav	ours con	trol

(B) -2578C/A CC+CA versus AA



(C) -2578C/A CA+AA versus CC





Due to the contradictory results concluded from published meta-analyses, in this study we performed a meta-analysis to summarize all of the available data, to assess comprehensively the relationship between VEGF polymorphisms and the risk of gastric cancer. Apart from the extensively studied SNPs (+936C/T, -634G/C) , we gathered all available data to assess the association between gastric cancer and some seldom investigated SNPs (+1612 G/A -2578C/A, -460G/C and -1154 G/A). In concordance with previous meta-analyses [31-33], our results showed that +936C/T polymorphism had no significant effect on gastric cancer risk and we found -634G/C polymorphism was associated with gastric cancer susceptibility, as reported by Cao et al. [32].

This meta-analysis, with 2,281 cases and 2,280 controls derived from 9 case–control studies, was performed to examine the association between gastric cancer risk and the polymorphisms

of VEGF +936C/T, -634G/C, -460T/C, +1612G/A, -2578C/A and -1154G/A. Our results showed that VEGF -634G/C and +1612G/A polymorphisms were associated with gastric cancer susceptibility. It was also found that the -634G allele carrier (GG+GC) was linked with increased risk of gastric cancer. For the VEGF +1612G/A polymorphism, our data showed that the +1612G allele and the G allele carrier (GG +GA) were associated with a significant decrease in gastric cancer risk. However, due to the limited number of studies available for the meta-analysis, the association should be confirmed by further studies based on larger populations. In the present study, there was no significant difference in the SNPs allele or genotype distribution of VEGF +936C/T, -460 C/T,-2578C/A and -1154 G/A between gastric cancer patients and controls.

Some limitations in our study should not be ignored. Firstly, the susceptibility to gastric

(A) -1154G/A G allele versus A allele

	GC cases		Healthy control		Odds Ratio			Odds Ratio			
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% C		M-H. R	andom. 9	5% CI	
Nikiteas et al 2007	126	200	127	200	100.0%	0.98 [0.65, 1.47]					
Total (95% CI)		200		200	100.0%	0.98 [0.65, 1.47]			+		
Total events	126		127								
Heterogeneity: Not app Test for overall effect:	olicable Z = 0.10 (F	P = 0.9	2)			F	0.01 avours	0.1 experiment	1 tal Favo	10 urs conf	100 trol

(B) -1154G/A GG+GA versus AA





Figure 7. Forest plot showing the association between -1154G/A polymorphism and gastric cancer risk. (A) -1154G/A G allele versus A allele; (B) -1154G/A GG + GA versus AA; (C) -1154G/A GA + AA versus GG.

cancer may be modulated by some other genetic markers and other related factors such as age, gender, major histological types and Helicobacter pylori infection. Subgroup analyses have not been conducted due to lack of relevant data. Therefore, these possible risk factors should be taken into account in the future studies. Secondly, our results should be interpreted with caution, because most of the included studies were conducted in Asians, and more races need to be further studied. Thirdly, although no noticeable publication bias was found in our meta-analysis , publication bias was inevitable for negative findings which were rarely published.

Despite these limitations, this meta-analysis provides evidence that the VEGF-634 G allele carrier is associated with increased GC risk and the VEGF +1612 G allele and G allele carrier decrease the risk. Further prospective researches with larger sample size and more details are expected to confirm these findings and make more comprehensive conclusions.

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Conflict of interests

The authors declare no confict of interests.

References

- 1. Jemal A, Bray F, Center MM et al. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
- Brenner H, Rothenbacher D, Arndt V. Epidemiology of stomach cancer. Methods Mol Biol 2009;472:467-477.
- Nagini S. Carcinoma of the stomach: A review of epidemiology, pathogenesis, molecular genetics and chemoprevention. World J Gastrointest Oncol 2012;4:156-169.
- 4. Ferrara N. VEGF and the quest for tumour angiogenesis factors. Nat Rev Cancer 2002;2:795-803.
- Makrilia N, Lappa T, Xyla V, Nikolaidis I, Syrigos K. The role of angiogenesis in solid tumours: an overview. Eur J Intern Med 2009;20:663-671.
- Toi M, Matsumoto T, Bando H. Vascular endothelial growth factor: its prognostic, predictive, and therapeutic implications. Lancet Oncol 2001;2:667-673.
- Fondevila C, Metges JP, Fuster J et al. p53 and VEGF expression are independent predictors of tumour recurrence and survival following curative resection of gastric cancer. Br J Cancer 2004;90:206-215.
- 8. Stevens A, Soden J, Brenchley PE, Ralph S, Ray DW. Haplotype analysis of the polymorphic human vascular endothelial growth factor gene promoter. Cancer Res 2003;63:812-816.
- Kim JG, Sohn SK, Chae YS et al. Vascular endothelial growth factor gene polymorphisms associated with prognosis for patients with gastric cancer. Ann Oncol 2007;18:1030-1036.
- Gao L, Nieters A, Brenner H. Meta-analysis: tumour invasion-related genetic polymorphisms and gastric cancer susceptibility. Aliment Pharmacol Ther 2008;28:565-573.
- 11. Bae SJ, Ahn DH, Hong SP et al. Gender-specific association between polymorphism of vascular endothelial growth factor (VEGF 936C>T) gene and patients with stomach cancer. Yonsei Med J 2008;49:783-791.
- 12. Chae YS, Kim JG, Sohn SK et al. Investigation of vascular endothelial growth factor gene polymorphisms and its association with clinicopathologic characteristics in gastric cancer. Oncology 2006;71:266-272.
- Tzanakis N, Gazouli M, Rallis G et al. Vascular endothelial growth factor polymorphisms in gastric cancer development, prognosis, and survival. J Surg Oncol 2006;94:624-630.
- 14. Zhou Y, Li N, Zhuang W, Wu X. Vascular endothelial growth factor (VEGF) gene polymorphisms and gastric cancer risk in a Chinese Han population. Mol Carcinog 2011;50:184-188.
- 15. Tahara T, Shibata T, Nakamura M et al. Effect of polymorphisms in the 3' untranslated region (3'-UTR) of vascular endothelial growth factor gene on gastric cancer and peptic ulcer diseases in Japan. Mol Carcinog 2009;48:1030-1037
- Al-Moundhri MS, Al-Nabhani M, Burney IA et al. Gastric cancer risk predisposition and prognostic significance of vascular endothelial growth factor (VEGF) gene polymorphisms--a case-control study in an Omani population. Mol Carcinog 2009;48:1170-1176.

- 17. Ke Q, Liang J, Wang LN et al. Potentially functional polymorphisms of the vascular endothelial growth factor gene and risk of gastric cancer. Mol Carcinog 2008;47:647-651.
- Lin L, Cao K, Chen W, Pan X, Zhao H. Four common vascular endothelial growth factor polymorphisms (-2578C>A, -460C>T, +936C>T, and +405G>C) in susceptibility to lung cancer: a meta-analysis. PLoS One 2013;8:e75123.Available:http://www.plosone.org/article/info:doi/10.1371/journal.pone.0075123. Accessed March 9, 2014
- Viechtbauer W. Confidence intervals for the amount of heterogeneity in meta-analysis. Stat Med 2007;26:37-52.
- Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629-634.
- Nikiteas NI, Tzanakis N, Theodoropoulos G et al. Vascular endothelial growth factor and endoglin (CD-105) in gastric cancer. Gastric Cancer 2007;10:12-17.
- Guan X, Zhao H, Niu J et al. The VEGF-634G>C promoter polymorphism is associated with risk of gastric cancer. BMC Gastroenterol 2009;9:77. Available: http://www.biomedcentral.com/1471-230X/9/77. Accessed October 9, 2013.
- Xia HZ, Wu Q, Liu G et al. Association of VEGF rs3025039 and rs3025021 with VEGF and COX-2 expression in gastric cancer tissues. J Clin Exp Pathol 2010;26:139-143.
- 24. Watson CJ, Webb NJ, Bottomley MJ, Brenchley PE. Identification of polymorphisms within the vascular endothelial growth factor (VEGF) gene: correlation with variation in VEGF protein production. Cytokine 2000;12:1232-1235.
- Jin Q, Hemminki K, Enquist K et al. Vascular endothelial growth factor polymorphisms in relation to breast cancer development and prognosis. Clin Cancer Res 2005;11:3647-3653.
- 26. Zhai R, Liu G, Zhou W et al. Vascular endothelial growth factor genotypes, haplotypes, gender, and the risk of non-small cell lung cancer. Clin Cancer Res 2008;14:612-617.
- 27. Jain L, Vargo CA, Danesi R et al. The role of vascular endothelial growth factor SNPs as predictive and prognostic markers for major solid tumors. Mol Cancer Ther 2009;8:2496-2508.
- Koukourakis MI, Papazoglou D, Giatromanolaki A et al. VEGF gene sequence variation defines VEGF gene expression status and angiogenic activity in nonsmall cell lung cancer. Lung Cancer 2004;46: 293-298.
- 29. Rodrigues P, Furriol J, Tormo E et al. The single-nucleotide polymorphisms +936C/T VEGF and -710C/T VEGFR1 are associated with breast cancer protection in a Spanish population. Breast Cancer Res Treat 2012;133:769-778.
- 30. Zhao Z, Ba C, Wang W et al. Vascular endothelial growth factor (VEGF) gene polymorphisms and col-

orectal cancer: a meta-analysis of epidemiologic studies. Genet Test Mol Biomarkers 2012;16:1390-1394.

- 31. Zhou Y, Hu W, Zhuang W et al. Vascular endothelial growth factor (VEGF) +936 C/T gene polymorphisms and gastric cancer risk: a meta-analysis involving 4,138 subjects. Int J Biol Markers 2010;25:213-218.
- 32. Cao C, Fang JJ, Ying T et al. Vascular endothelial growth factor +936C/T and +405G/C polymorphisms and cancer risk: a meta-analysis. Arch Med Res 2010;41:548-557.
- 33. Liu H, Wang S, Huang C. VEGFA+936C/T and -634G/C polymorphisms and gastric cancer risk: a meta-analysis. Asian Pac J Cancer Prev 2011;12:1979-1983.