

Current Evidence on VEGF+405G/C Polymorphism and Malignancy Susceptibility: A Meta-Analysis Involving 30 Studies

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The association of VEGF+405G/C (where VEGF is vascular endothelial growth factor) polymorphism and malignancy susceptibility attracts considerable attention because VEGF is one of the most potent angiogenic factors and plays a critical role in the onset and development of malignancy. However, the published findings remain inconclusive. In order to derive a more precise assessment of the association, we performed a meta-analysis including 30 published case-control studies from PubMed, Embase, and Ovid databases. Odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the strength of the association. In the pooled analyses, no significant association was found between VEGF+405G/C polymorphism and malignancy susceptibility in different genetic models (G-allele vs. C-allele: OR = 1.00, 95% CI: 0.93–1.07; CC vs. GG: OR = 1.01, 95% CI: 0.88–1.15; GC+CC vs. GG: OR = 1.00, 95% CI: 0.91–1.10; CC vs. GC+GG: OR = 1.01, 95% CI: 0.90–1.13). When stratified by ethnicity, a weak association between this polymorphism and malignancy susceptibility was found in African under allelic frequency comparison (OR = 0.65, 95% CI: 0.43–0.98) and dominant genetic model comparison (OR = 1.95, 95% CI: 1.09–3.50). In summary, although our meta-analysis indicated a weak association of VEGF+405G/C polymorphism with malignancy susceptibility in African, no persuasive evidence of association between the polymorphism and malignancy susceptibility was detected in the pooled analyses. Therefore, more studies with larger scale of participants, especially Africans, are required to further evaluate gene-environment interaction on this polymorphism and malignancy susceptibility.

■ **Keywords:** vascular endothelial growth factor, single nucleotide polymorphism, malignancy, susceptibility

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor, was discovered by Ferrara and Henzel in 1989 (Ferrara & Henzel, 1989) and is not only an angiogenic cytokine that plays a pivotal role in normal and pathologic angiogenesis with a specific mitogenicity for endothelial cells (Leung et al., 1989), but also a potent permeability factor that stimulates an increase in vascular permeability via prostacyclin and nitric oxide (Murohara et al., 1998).

The VEGF gene is located on chromosome 6p12 and consists of a 14 kb coding region with eight exons and seven introns (Vincenti et al., 1996). Many single nucleotide polymorphisms (SNPs) have been described in the 5'-untranslated region (UTR), as well as in the promoter region (Brogan et al., 1999). One of these, +405G/C polymorphism (rs2010936, at position –634 before transcrip-

tion initiation site in the 5'-UTR) is of particular note. To our knowledge, there are several studies which have investigated the association between VEGF+405G/C polymorphism and malignancy susceptibility (Guan et al., 2009; Kim et al., 2010; Maltese et al., 2009; Zhou et al., 2011). Whereas, the data reported are conflicting and inconclusive. The lack of concordant conclusion can be partly explained by the relatively small sample size, differences in ethnic compositions, and research methodology among some studies.

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Since a meta-analysis could summarize effects of the same genetic variation across various populations, it is a powerful approach for exploring their relationship. We carried out this meta-analysis of all eligible studies to derive a more robust estimation of the association between VEGF+405G/C polymorphism and malignancy susceptibility.

Materials and Methods

Literature Search

A systematic literature search was conducted to identify all studies published between January 1989 and May 2011 that investigated the association of VEGF+405G/C polymorphism with malignancy susceptibility.

We carried out a search in PubMed, Embase, and Ovid, using the search terms: 'Vascular endothelial growth factor' or 'Vascular endothelial growth factor A' or 'Vascular permeability factor' or 'VEGF' or 'VEGF-A', 'polymorphism' or 'haplotype', 'malignancy' or 'cancer' or 'carcinoma' or 'tumor' or 'neoplasms'. All eligible studies were retrieved, and their bibliographies were checked for other relevant publications. Only published studies with full-text articles were included. If studies had partly overlapped subjects, only the most recent or complete study was selected.

Inclusion Criteria

The inclusion criteria were as follows: (1) published in English; (2) evaluation of the VEGF+405G/C polymorphism and malignancy susceptibility; (3) case-control study design; and (4) sufficient published data for estimating an odds ratio (OR) with 95% confidence interval (CI).

Data Extraction

Data extraction was performed independently by two of the authors (Kai Hu and Yong Zhang) and compared afterwards. For each study, the following data were recorded: first author's last name, year of publication, country of origin, ethnicity, malignancy type, control source, total number of cases and controls, and frequencies of GG, GC, and CC genotype in cases and controls.

Statistical Analysis

Hardy–Weinberg equilibrium (HWE) of genotype distribution among controls of each study was carried out using the Pearson chi-squared test and a p value $\geq .05$ was considered as meeting HWE. The Chi-squared-based Q -test and I^2 statistic were used for the assessment of the between-study heterogeneity, and it was considered significant for $p \leq .1$ and I^2 value is larger than 50%. When study groups are homogeneous, the fixed-effect model and the random-effect model are similar. Otherwise, the random effects model is best used in the presence of significant between study heterogeneity.

A pooled OR and 95% CI were used to evaluate the strength of association between VEGF+405G/C polymorphism

and susceptibility to malignancy under allelic frequency comparison (G-allele vs. C-allele), homozygote comparison (CC vs. GG), recessive genetic mode comparison (CC vs. GG+GC), and dominant genetic model comparison (GC+CC vs. GG). The significance of the combined OR was determined by the Z -test, in which $p < .05$ was considered significant. Stratified analysis was also performed by ethnicity, cancer type, sample size, control source, and HWE status. The potential publication bias was assessed by Egger's linear regression test and Begg's funnel plots. All statistical tests were performed with Review Manager (version 5.1.4, Cochrane Collaboration) Oxford, UK.

Results

Characteristics of Studies

Totally, 533 studies about VEGF polymorphisms with respect to malignancy were searched and screened for retrieval, of which 454 irrelevant studies and 32 papers of abstract, review comment, and case report were excluded. Then, five studies were excluded because they did not have control group; five studies were excluded since they were about the prognosis of cancer; six studies were excluded due to their insufficient genotyping information, and one study was excluded because the authors published two papers on the same patients, and only one complete study was selected (Figure 1). Finally, 30 case-control studies were selected and study characteristics are summarized in Table 1. Of these studies, 11 studies were about Asian; 14 studies were about Caucasian; one study was about African, and four studies were about mixed ethnicity. The genotype distributions among the control of most of studies met HWE except for 5 studies (Dassoulas et al., 2009; Guan et al., 2009; Kammerer et al., 2010; Oliveira et al., 2011; Zhou et al., 2011). Overall, our study included 12,345 cases and 12,517 controls for studying the relationship between VEGF+405G/C polymorphism and malignancy susceptibility.

Quantitative Synthesis

The appraisal of association between VEGF+405G/C polymorphism and malignancy susceptibility was presented in Table 2. In the pooled analysis, no significant association was found between VEGF+405G/C polymorphism and malignancy susceptibility in different genetic models (G-allele vs. C-allele: OR = 1.00, 95% CI: 0.93–1.07, $p = .95$, $P_{\text{heterogeneity}} < .01$; Figure 2; CC vs. GG: OR = 1.01, 95% CI: 0.88–1.15, $p = .92$, $P_{\text{heterogeneity}} < .01$; GC+CC vs. GG: OR = 1.00, 95% CI: 0.91–1.10, $p = .99$, $P_{\text{heterogeneity}} < .01$; CC vs. GC+GG: OR = 1.01, 95% CI: 0.90–1.13, $p = .92$, $P_{\text{heterogeneity}} < .01$). The effect of the polymorphism was further evaluated in stratification analysis. According to the ethnicity, a weak association between this polymorphism and malignancy susceptibility was found in African under allelic frequency comparison (OR = 0.65, 95% CI: 0.43–0.98, $p = .04$) and dominant

TABLE 1
Characteristics of All Studies Included in Our Meta-Analysis

Author	Year	Ethnicity	Cancer type	Source of control	Case			Control			Case/control	HWE ^a
					GG	CC	GC	GG	CC	GC		
Garcia-Closas et al.	2007	Caucasian	Bladder cancer	Hospital	388	98	395	387	93	396	881/876	0.58
Al-Moundhri et al.	2009	Asian	Gastric cancer	Population	49	22	59	62	14	54	130/130	0.66
Amano et al.	2008	Asian	Endometrial carcinoma	Population	25	28	52	58	42	79	105/179	0.14
Balassubramanian et al.	2007	Caucasian	Breast cancer	Population	226	57	207	209	64	225	490/498	0.78
Bruyère et al.	2010	Caucasian	Renal cell carcinoma	Population	15	8	25	86	20	92	48/198	0.52
Chae et al.	2006	Asian	Gastric cancer	Population	129	31	253	106	84	223	413/413	0.09
Salajegheh et al.	2010	Caucasian	Papillary thyroid cancer	Hospital	39	17	31	12	23	36	87/71	0.74
Dassoulas et al.	2009	Caucasian	Colorectal cancer	Population	128	59	125	145	76	141	312/362	0.00
Guan et al.	2009	Mixed	Gastric cancer	Population	69	30	72	180	74	99	171/353	0.00
Hofmann et al.	2008	Caucasian	Colorectal cancer	Population	193	47	192	192	43	195	432/430	0.52
Howell et al.	2002	Caucasian	Cutaneous malignant melanoma	Population	63	18	56	121	30	90	137/241	0.05
Hsiao et al.	2007	Asian	Thyroid cancer	Hospital	104	49	160	67	44	119	313/230	0.49
Jacobs et al.	2006	Mixed	Breast cancer	Population	221	52	222	232	47	221	495/500	0.59
Jin et al.	2005	Caucasian	Breast cancer	Population	488	85	363	492	82	367	936/941	0.25
Kammerer et al.	2010	Caucasian	Oral squamous cell cancer	Population	37	20	23	18	10	12	80/40	0.02
Kataoka et al.	2006	Asian	Breast cancer	Population	395	192	508	418	182	598	1095/1198	0.18
Ke et al.	2008	Asian	Breast cancer	Population	161	92	287	186	97	278	540/561	0.69
Kim et al.	2010	Asian	Early cervical cancer	Population	63	31	102	76	36	103	196/215	0.91
Langsenlehner et al.	2008	Caucasian	Prostate cancer	Population	303	83	315	314	71	313	701/698	0.59
Lee et al.	2005	Asian	Lung cancer	Population	108	76	247	108	92	232	431/432	0.12
Maltese et al.	2008	Caucasian	Colorectal cancer	Population	118	48	135	30	15	46	301/91	0.71
Oliveira et al.	2011	Mixed	Breast cancer	Population	95	38	102	82	24	129	235/235	0.01
Sfar et al.	2006	African	Prostate cancer	Population	29	15	57	44	10	46	101/100	0.69
Li et al.	2011	Asian	Glioma	Hospital	247	120	393	306	115	379	760/800	0.89
Tzanakis et al.	2006	Caucasian	Gastric cancer	Population	41	19	40	52	9	39	100/100	0.67
Galimberti et al.	2010	Caucasian	Mantle cell lymphoma	Population	29	0	3	32	6	20	32/58	0.30
Chae et al.	2008	Asian	Colorectal cancer	Population	166	106	193	106	84	223	465/413	0.09
Zhai et al.	2008a	Caucasian	Non-small cell lung cancer	Population	805	247	848	650	164	644	1900/1458	0.81
Zhai et al.	2008b	Mixed	Esophageal adenocarcinoma	Population	155	29	124	233	62	251	308/546	0.65
Zhou et al.	2011	Asian	Gastric cancer	Population	74	29	47	76	30	44	150/150	0.00

Note: ^aHWE: Hardy-Weinberg equilibrium. A *p*-value $\geq .05$ was considered as meeting HWE.

TABLE 2
Meta-Analysis for the Association of VEGF+405G/C Polymorphism with Malignancy Susceptibility

Variables	NO (case/control)	G vs. C		CC vs. GG		GC+CC vs. GG		CC vs. GC+GG	
		OR (95%CI) ^a	<i>p</i> ^b	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>
Total	30 (12345/12517)	1.00 (0.93–1.07)	$\leq .10$	1.01 (0.88–1.15)	$\leq .10$	1.00 (0.91–1.10)	$\leq .10$	1.01 (0.90–1.13)	$\leq .10$
Ethnicity									
Asian	11 (4598/4721)	1.01 (0.91–1.12)	$\leq .10$	0.95 (0.74–1.21)	$\leq .10$	1.01 (0.87–1.18)	$\leq .10$	0.93 (0.75–1.16)	$\leq .10$
Caucasian	14 (6437/6062)	1.01 (0.90–1.12)	$\leq .10$	1.03 (0.85–1.24)	$\leq .10$	0.97 (0.85–1.12)	$\leq .10$	1.06 (0.94–1.20)	$\leq .37$
African	1 (101/100)	0.65 (0.43–0.98)^c	NA ^d	2.28 (0.90–5.75)	NA	1.95 (1.09–3.50)	NA	1.57 (0.67–3.68)	NA
Mixed	4 (1209/1634)	1.00 (0.86–1.18)	$\leq .12$	1.03 (0.78–1.35)	$\leq .31$	0.98 (0.72–1.33)	$\leq .10$	1.04 (0.76–1.43)	$\leq .14$
Cancer type									
Breast cancer	6 (3791/3933)	0.99 (0.92–1.05)	$\leq .76$	1.07 (0.93–1.24)	$\leq .77$	0.98 (0.89–1.08)	$\leq .40$	1.10 (0.96–1.26)	$\leq .46$
Gastric cancer	5 (964/1146)	0.90 (0.65–1.26)	$\leq .10$	1.06 (0.50–2.25)	$\leq .10$	1.20 (0.87–1.67)	$\leq .10$	0.95 (0.48–1.86)	$\leq .10$
Colorectal cancer	4 (1510/1296)	1.08 (0.97–1.21)	$\leq .65$	0.89 (0.71–1.12)	$\leq .79$	0.83 (0.65–1.06)	$\leq .10$	1.04 (0.85–1.27)	$\leq .73$
Urogenital cancer	4 (1731/1872)	0.88 (0.75–1.03)	$\leq .13$	1.27 (0.95–1.70)	$\leq .25$	1.18 (0.93–1.48)	$\leq .10$	1.16 (0.94–1.44)	$\leq .62$
Head and neck cancer	3 (480/341)	1.39 (0.89–2.19)	$\leq .10$	0.56 (0.26–1.20)	$\leq .10$	0.60 (0.29–1.26)	$\leq .10$	0.74 (0.52–1.05)	$\leq .46$
Thoracic cancer	3 (2639/2436)	1.05 (0.87–1.27)	$\leq .10$	0.94 (0.66–1.33)	$\leq .10$	0.95 (0.74–1.21)	$\leq .10$	0.95 (0.71–1.27)	$\leq .10$
Gynecologic cancer	2 (301/394)	0.88 (0.71–1.10)	$\leq .40$	1.23 (0.79–1.92)	$\leq .38$	1.28 (0.92–1.77)	$\leq .42$	1.05 (0.71–1.53)	$\leq .54$
Other cancer ^e	3 (929/1099)	1.17 (0.69–1.97)	$\leq .10$	1.14 (0.67–1.93)	$\leq .18$	0.84 (0.43–1.65)	$\leq .10$	1.08 (0.79–1.47)	$\leq .33$
Control source									
Population-based	26 (10304/10540)	0.99 (0.92–1.06)	$\leq .10$	1.03 (0.90–1.19)	$\leq .10$	1.01 (0.92–1.11)	$\leq .10$	1.02 (0.90–1.16)	$\leq .10$
Hospital-based	4 (2041/1977)	1.13 (0.88–1.46)	$\leq .10$	0.80 (0.49–1.30)	$\leq .10$	0.84 (0.58–1.23)	$\leq .10$	0.94 (0.73–1.21)	$\leq .16$
Sample size									
Total subject ≥ 800	14 (9847/9764)	1.02 (0.95–1.09)	$\leq .10$	0.97 (0.82–1.13)	$\leq .10$	0.98 (0.89–1.07)	$\leq .10$	0.98 (0.85–1.14)	$\leq .10$
Total subject < 800	16 (2498/2753)	0.96 (0.83–1.12)	$\leq .10$	1.09 (0.85–1.41)	$\leq .10$	1.04 (0.84–1.29)	$\leq .10$	1.04 (0.87–1.25)	$\leq .15$
Meet HWE ^f									
Yes	25 (11397/11377)	1.00 (0.93–1.08)	$\leq .10$	1.00 (0.86–1.17)	$\leq .10$	0.99 (0.90–1.10)	$\leq .10$	1.01 (0.89–1.14)	$\leq .10$
No	5 (948/1140)	0.98 (0.87–1.12)	$\leq .81$	1.02 (0.80–1.30)	$\leq .83$	1.04 (0.82–1.32)	$\leq .15$	1.00 (0.77–1.29)	$\leq .30$

Note: ^aOR = odds ratio, CI = confidence interval; ^b*p*-value of Q-test for heterogeneity; ^cpositive results shown in bold; ^dNA = not applicable; ^eother cancer including cutaneous malignant melanoma, glioma, and mantle cell lymphoma; ^fPearson chi-squared test was used to verify HWE and a *p*-value $\geq .05$ was considered as meeting HWE.

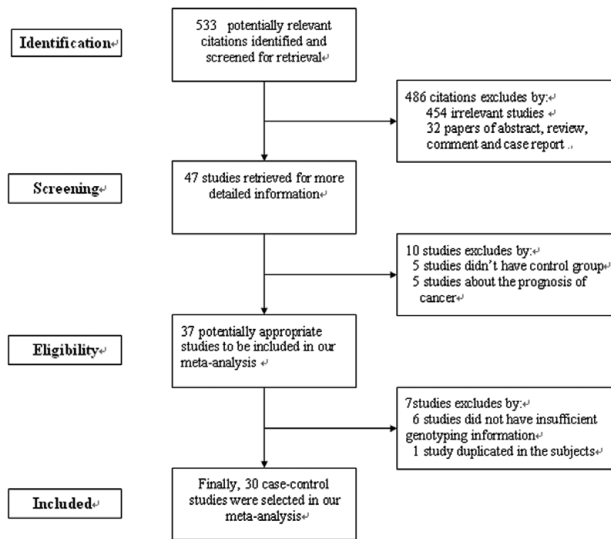


FIGURE 1
Flow diagram of the study selection process.

genetic model comparison (OR = 1.95, 95% CI: 1.09–3.50, $p = .03$). However, we failed to detect other effects for VEGF+405G/C polymorphism on malignancy susceptibility in different genetic models when stratified by cancer type, sample size, control source, or HWE status.

Heterogeneity

There was substantial heterogeneity among these studies in overall comparisons. Thus, we assessed the source of heterogeneity by ethnicity, cancer type, control source, sample size, and HWE status. Through the stratified analysis, heterogeneity decreased significantly in some sub-groups and still remained in the others.

Sensitivity Analysis and Publication Bias

Sensitivity analysis was performed to assess whether alteration of the selected studies affected the pooled results. None of the pooled results were materially altered when we repeated the meta-analysis by sequential removal of individual studies and stratified analyses by HWE status. It was showed that our results were statistically stable.

Funnel plot and Egger’s test were used to evaluate the publication and other bias in the meta-analysis. As shown in Figure 3, shape of the funnel plots did not reveal any evidence of apparent asymmetry in the allelic frequency comparison. There is also no evidence of publication bias as measured by Egger’s test in our study.

Discussion

In the early 1970s, Judah Folkman first proposed the hypothesis that solid tumor growth depends on the formation of new blood vessels (Folkman, 1971). The supporting vasculature of solid tumors not only delivers nutrient and

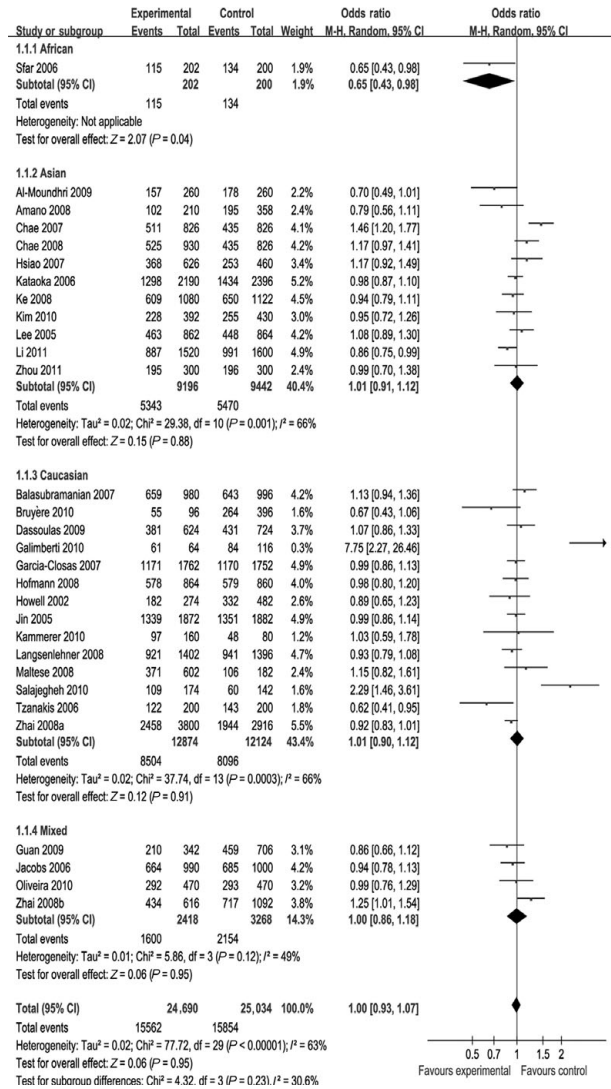
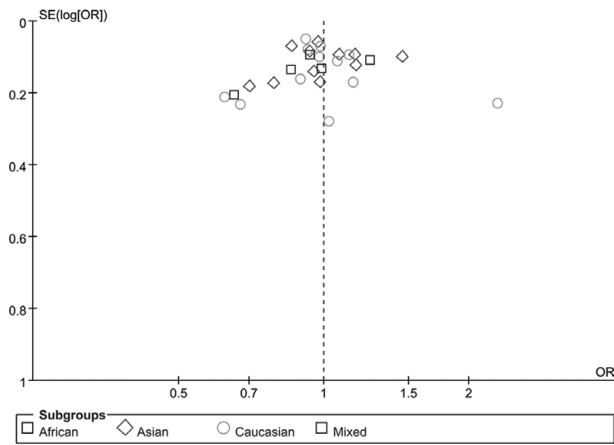


FIGURE 2
Forest plot for association between VEGF+405G/C polymorphism and malignancy susceptibility in different ethnicities (Comparison: G-allele vs. C-allele).

oxygen to the growing tumor cells but also provides a route for excreting metabolites (Dass & Su, 2000). Strong pre-clinical and clinical evidence from many studies describes that VEGF is a dominant angiogenic factor in solid tumors (Yancopoulos et al., 2000).

Some evidence from in vitro and in vivo experiments demonstrated that upregulation of VEGF is associated with the onset and development of malignancy, and several potentially functional SNP in VEGF gene have been reported to be associated with VEGF gene expression. For example, the -2578C and -1154G alleles in the promoter region of VEGF were associated with higher VEGF production in vitro, whereas the +936T allele located in the 3'-untranslated region has been shown to decrease VEGF plasma levels (Koukourakis et al., 2004; Wolf et al., 2004).

**FIGURE 3**

Funnel plot of publication bias in all studies. Every point represents an independent study for the indicated association.

Also, +405G/C polymorphism in the 5'-UTR region were found to be associated with variations in VEGF gene expression. Awata et al. reported that carriers of the +405CC genotype were associated with higher levels of plasma VEGF than other genotype carriers (Awata et al., 2002). Accordingly, Steffensen et al. also showed that VEGF serum levels were significantly higher in carriers of +405C allele compared with non-carriers (Steffensen et al., 2010). While in another study, Watson et al. demonstrated that the +405GG genotype was obviously correlated with the highest VEGF production in peripheral blood mononuclear cells when stimulated by lipopolysaccharide (Watson et al., 2000).

There are two possible interpretations for the potential mechanism that VEGF+405G/C polymorphisms influence gene expression. Brogan et al. identified that the +405G/C polymorphism resulted in the loss of a potential Estrogen Receptor binding site (Brogan et al., 1999). The polymorphism would alter the estrogen responsiveness of the promoter and this could have significant implications for regulation of VEGF expression. Meanwhile, by applying the software program TFSEARCH ver.1.3 and the TRANSFAC database which identifies transcription factor binding motifs, Watson et al. reported that the polymorphism at +405 was predicted to lie within a potential myeloid zinc finger protein (MZF1) binding site, with C-allele possibly reducing the binding specificity of this motif (Watson et al., 2000).

In order to test the hypothesis that VEGF+405G/C polymorphism influence gene expression and associate with malignancy susceptibility, we carried out this meta-analysis of all eligible studies. Finally, our results didn't indicate significant association of VEGF+405G/C polymorphism with malignancy susceptibility in the pooled analyses. When stratified by ethnicity, a weak association of this polymorphism with malignancy susceptibility was observed in African under the allelic frequency comparison and domi-

nant genetic model comparison. However, only one study of African was included in our meta-analysis and involved merely 101 cases and 100 controls in this study. These limitations suggest that positive results are not robust and could be due to chance. Our meta-analysis does not forcefully support the genetic association between VEGF+405C/G polymorphism and malignancy susceptibility in African. More studies based on a larger population in Africa could be added to further analyze the relationship.

Analysis of the possible reasons as follows: To begin with, various types of malignancy may have different mechanism of carcinogenesis and the polymorphism may have different functions in various types of malignancy. Besides, different ethnic compositions, genetic background among different populations may lead to disparity in genotype and allele frequencies of VEGF+405G/C polymorphism and then lead to inconsistent conclusion. Furthermore, small size of some study population can't discover the tiny relationship. Last but the most important, since cancer is a multistep and multigene disease, the effort of single gene is prone to be mixed with complicated gene-gene and gene-environmental interactions, it requires a research with larger samples among different populations and combinations of multiple genes and environmental factors to assess the true risk effects.

Our results should be explained in light of some inherent limitations. First, only English language studies were included in our meta-analysis may have led to publication bias, and the exclusion of unpublished data was usually associated with an overestimation of the real effect. Second, we endeavor to contact the authors of the six studies to obtain individual patient data for our meta-analysis; however, we could not obtain the replies from them. Third, in the sub-group analyses, the size of some sub-groups was relatively small and didn't have enough statistical power to explore the actual association. Fourth, obvious heterogeneity was observed in our study. Although we use random-effect model and stratified analysis by ethnicity, cancer type, sample size, control source, and HWE status to calculate the combined OR, heterogeneity is a potential problem when explaining the results of our meta-analysis. Unquestionably, these limitations may influence our final conclusions.

On the other hand, our meta-analysis that included 12,345 patients with malignancy and 12,517 healthy subjects from all available studies represents the most comprehensive meta-analysis performed in the relationship between VEGF+405G/C polymorphism and malignancy susceptibility. In addition, the selected studies in our meta-analysis were all case-control studies and contained available genotype information, which met the inclusion criterion very well.

In conclusion, although our meta-analysis indicated a weak association of VEGF+405G/C polymorphism with malignancy susceptibility in African, no persuasive evidence of association between the polymorphism and malignancy

susceptibility was detected in the pooled analyses. Therefore, more studies with larger scale of participants, especially Africans, are required to further evaluate gene-environment interaction on this polymorphism and malignancy susceptibility.

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