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Review Article

Gene polymorphisms of vascular endothelial growth factor and the susceptibility to osteosarcoma in Chinese population: a meta-analysis

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Abstract: Osteosarcoma (OS) is a kind of malignant bone tumor accounting for ~6% of all cancer in children. The precise mechanism of occurrence and development of OS remains under investigation. Vascular endothelial growth factor (VEGF), known for the role as a key mediator of angiogenesis, has been shown to have some associations with a variety of malignant tumors, including OS. VEGF gene polymorphisms may involve in the carcinogenesis by influencing the formation of blood vessels. In order to estimate the correlation between VEGF gene polymorphisms and the risk of OS, we performed the meta-analysis based on five case-control studies from PubMed and CNKI. The results of this study indicated that in Chinese populations, the VEGF 1612G/A, -1156G/A gene polymorphisms are not associated with the susceptibility to OS and the -2578C/A and -460T/C gene polymorphism are associated with the risk of OS, and for -634G/C and -936C/T, there may be exist an association in OS risk. Due to the limitations of this study, further larger-size, multi-center and higher-quality case-control studies are warranted to validate our findings.

Keywords: Vascular endothelial growth factor, polymorphism, osteosarcoma, susceptibility, meta-analysis

Introduction

Osteosarcoma (OS) is an aggressive bone cancer, it is the most common primary malignant bone tumor in children and adolescents. It is arising from mesenchymal tissues, the long bone is the most common lesion site. The annual incidence of OS is estimated about 4-5/10^5 globally, which assumes a proportion of ~6% of childhood cancer [1-3]. The process of OS is complex, multistep and multifactorial, the precise mechanism of progression of OS is not well understood [4, 5]. Many studies have showed that environmental and genetic factors were involved in the pathogenesis of OS [6-8]. In addition to that, a growing body of research suggests that microenvironment and cancer stem cells may have play a crucial role in the occurrence and development of OS [9-11].

Some previous studies have reported that the development of OS was related with many genetic factors, including CTLA-4, MDM2 and TNF-α gene polymorphisms [12-14], they indicated that single nucleotide polymorphisms were associated with the risk of OS. Recent years, some studies indicated that VEGF are associated with the pathogenesis of OS [15-17], and some persuasive evidences have indicated that VEGF expression has very important effects on prognosis in patients with OS, especially in the aspect of overall survival [18-21]. However, the association with VEGF gene polymorphisms and the risk on OS has not yet been assessed comprehensively.

Recently, some case-control studies have been conducted to investigate the potential association between VEFG gene polymorphism and OS. However, the results are not consistent or even contradictory. Therefore, for deriving more reliable conclusions and clarifying its relationship with OS risk, we conducted this meta-analysis from the published literatures to present the genetic knowledge on the VEGF polymorphisms and the susceptibility to OS.

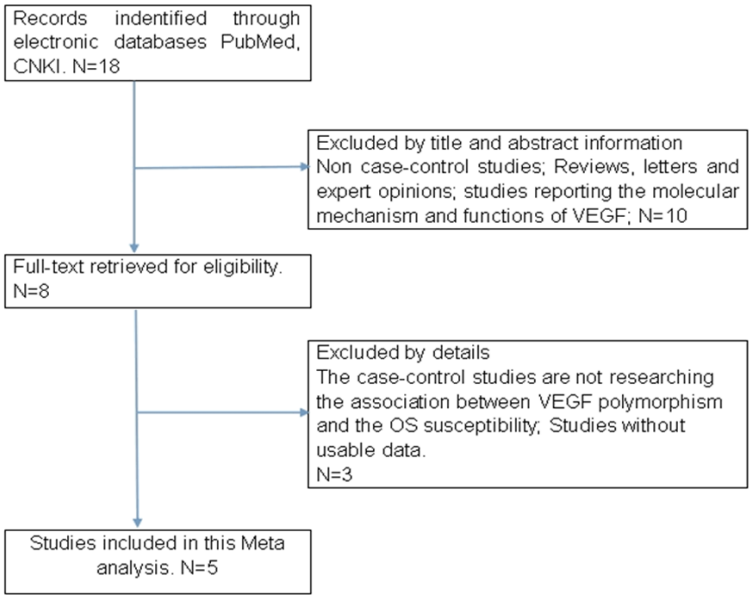

Figure 1. Flowchart presenting the steps of literature search and selection.

Materials and methods

Search strategy

A comprehensive online search was performed in PubMed and China National Knowledge Infrastructure (CNKI) databases for relevant articles. The search time is up to October 1, 2015. The following search terms were used for the publication search: ("vascular endothelial growth factor" or "vascular endothelial growth factor" or "vegf" or "VEGF") and ("polymorphism, genetic" or "polymorphism") and ("genetic" or "genetic polymorphism" or "polymorphism") and ("osteogenic sarcoma" or "osteosarcoma"). In addition, reference lists of retrieved articles were also examined.

Inclusion and exclusion criteria

Any study that met the following criteria was included: (1) case-control studies design; (2) Studies are evaluating the association between VEGF gene polymorphisms and osteosarcoma in humans; (3) The literatures have the basic information, and enough data was recorded for estimating of odds ratio (OR) and 95% confidence interval (CI); (4) osteosarcoma confirmed by the accepted diagnostic criteria.

Date extraction and quality assessment

Date extraction: Two investigators (ZL and XGF) extracted data from the eligible studies independently. From each study, the following basic information and data were extracted: gene polymorphisms, first author's last name, year of publication, country, racial, genotyping method, source of controls, the number of cases and controls, genotype distribution, P for HWE in controls. For any disagreements, a consensus was reached by a third investigator (ZY).

Assessment of study quality: The quality of all studies included is assessed by two reviewers (ZL and XGF) according to a set of criteria described by Wang et al [22]. A total of five aspects included in the predetermined criteria are as following: the source of cases and controls, case-control matching, the source of obtained specimens used for determining genotypes, total sample size, evidence of Hardy-Weinberg equilibrium (HWE). For disagreements, a consensus was reached by the third investigator (ZY). The quality of the studies was scored from 0 to 18, with higher scores indicating a better quality (high quality ≥12, low quality <12).

Statistics analysis

Statistical analyses in this Meta-analysis were performed by using STATA version 12.0 (Stata Corporation LP, College Station, TX). HWE was evaluated for controls in each study by the Chi-square test. The heterogeneity between different studies was assessed by using Q-statistic and the I^2 test [23]. If the heterogeneity was significant ($P \leq 0.1$, $I^2 > 50\%$), the random effects model was used [24], otherwise, the fixed effects model was used [25]. The crude odds ratio (OR) and 95% confidence interval (CI) was used to assess the strength of association between VEGF gene polymorphisms and OS susceptibility under five genetic models: the allele model, dominant model, recessive model, heterozygous model and homozygous model. In addition, sensitivity analysis and publication bias were assessed in the present meta-analysis. All *P* values were two-tailed, and $P<0.05$ was considered statistically significant.

Table 1. Main characteristics of the included studies of VEGF polymorphisms (+1612G/A, -2578C/A, -460T/C) in the meta-analysis

Gene polymorphism	Country	Racial	Genotyping method	Source of controls	No. cases/ controls	Cases			Controls			P_{HWE}	Score
						GG	GA	AA	GG	GA	AA		
+1612G/A (rs10434)	China	Asian	PCR-RFLP	HB	164/330	68	76	20	151	146	33	0.791	13
	China	Asian	PCR-RFLP	HB	330/342	95	157	78	97	172	73	0.841	14
	China	Asian	PCR-RFLP	HB	180/360	77	80	23	163	155	42	0.579	14
	China	Asian	PCR-RFLP	HB	176/176	77	80	19	80	78	18	0.874	13
						CC	CA	AA	CC	CA	AA		
-2578C/A (rs699947)	China	Asian	PCR-RFLP	HB	165/330	64	72	29	159	136	35	0.464	13
	China	Asian	PCR-RFLP	HB	182/182	68	79	35	88	71	23	0.153	13
	China	Asian	PCR-RFLP	HB	176/176	62	78	36	84	71	21	0.322	13
						TT	TC	CC	TT	TC	CC		
-460T/C (rs833061)	China	Asian	PCR-RFLP	HB	182/182	65	91	26	72	86	24	0.832	13
	China	Asian	PCR-RFLP	HB	176/176	48	89	39	66	85	25	0.777	13

Table 2. Meta-analysis of VEGF polymorphisms (+1612G/A, -2578C/A, -460T/C) and osteosarcoma risk in Chinese populations

Gene polymorphism	Comparison	Test of association			Model	Test of heterogeneity	
		OR	95% CI	*P*		P	I^2
+1612G/A (rs10434)	A vs. G	1.08	0.94-1.23	0.269	F	0.944	0.00%
	AG/AA vs. GG	1.08	0.09-1.30	0.424	F	0.896	0.00%
	AA vs. AG/GG	1.14	0.89-1.47	0.300	F	0.986	0.00%
	AG vs. GG	1.05	0.86-1.28	0.621	F	0.873	0.00%
	AA vs. GG	1.15	0.87-1.52	0.315	F	0.956	0.00%
-2578C/A (rs699947)							
	C vs. A	0.67	0.57-0.80	0.000	F	0.904	0.00%
	AA/CA vs. CC	1.56	1.23-1.97	0.000	F	0.899	0.00%
	AA vs. CA/CC	1.78	1.28-2.46	0.001	F	0.942	0.00%
	AA vs. CC	2.11	1.49-2.99	0.000	F	0.93	0.00%
	CA vs. CC	1.4	1.09-1.81	0.008	F	0.917	0.00%
-460T/C (rs833061)							
	T vs. C	0.79	0.64-0.97	0.027	F	0.216	34.60%
	TT/CT vs. CC	0.71	0.48-1.07	0.103	F	0.279	14.60%
	TT vs. CT/CC	0.73	0.54-1.00	0.050	F	0.333	0.00%
	TT vs. CC	0.62	0.39-0.96	0.034	F	0.206	37.40%
	CT vs. CC	0.8	0.52-1.22	0.299	F	0.391	0.00%

Results

According to the above inclusion criteria, a total of five studies were finally identified in this Meta-analysis [26-30]. Six gene polymorphisms of the VEFG gene were investigated, and the participants in all included case-control studies are Chinese. A flowchart showing the steps of literature search and selection is presented in **Figure 1**. The quality score of all included case-control studies ranged 13-14, with classified as high quality (≥12).

Meta-analysis results

All studies were published in English and conducted in China. The controls were all healthy individuals and were matched for age and gender. The genotypes of all studies were analyzed using PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism). The studies in the meta-analysis are all case-control studies with high quality.

Zhang T [26] studied five polymorphisms, -2578C/A (rs699947), -1156G/A (rs1570360),

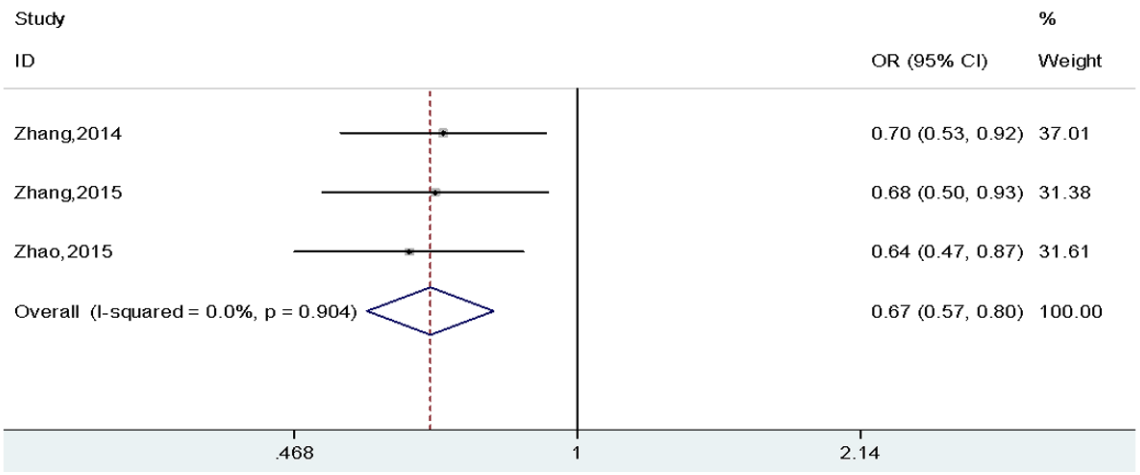

Figure 2. Forest plot of the association between the rs699947 polymorphism and the OS risk under the allele model (C vs. A).

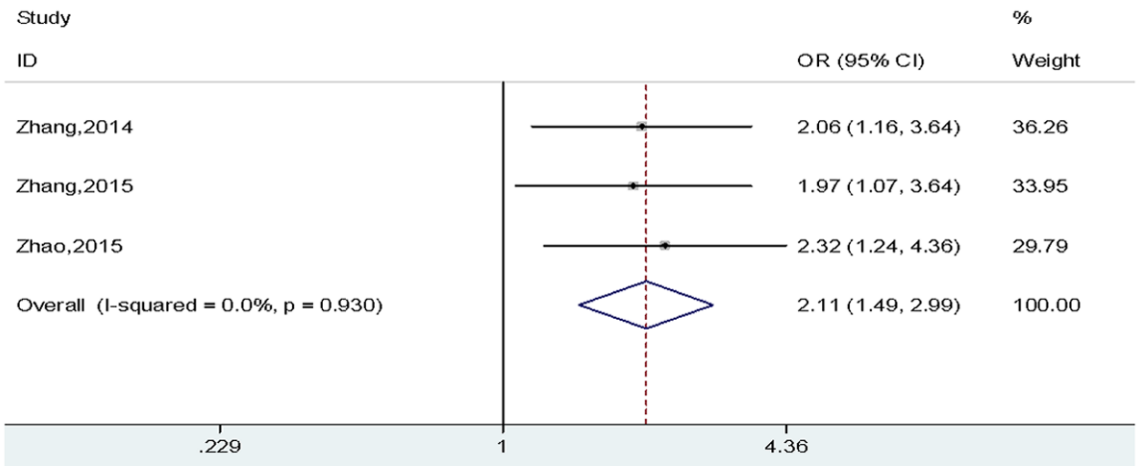

Figure 3. Forest plot of the association between the rs699947 polymorphism and the OS risk under the codominant model (AA vs. CC).

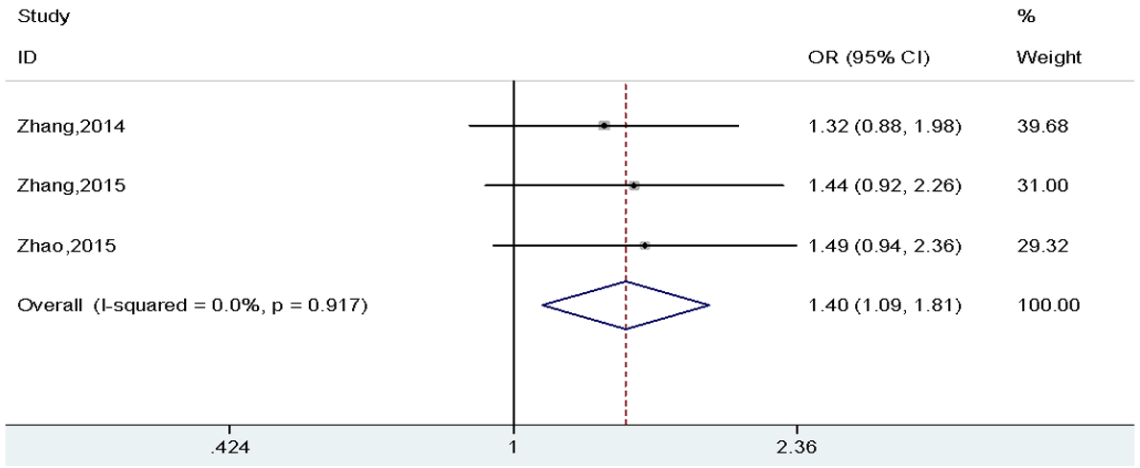

Figure 4. Forest plot of the association between the rs699947 polymorphism and the OS risk under the codominant model (AC vs. CC).

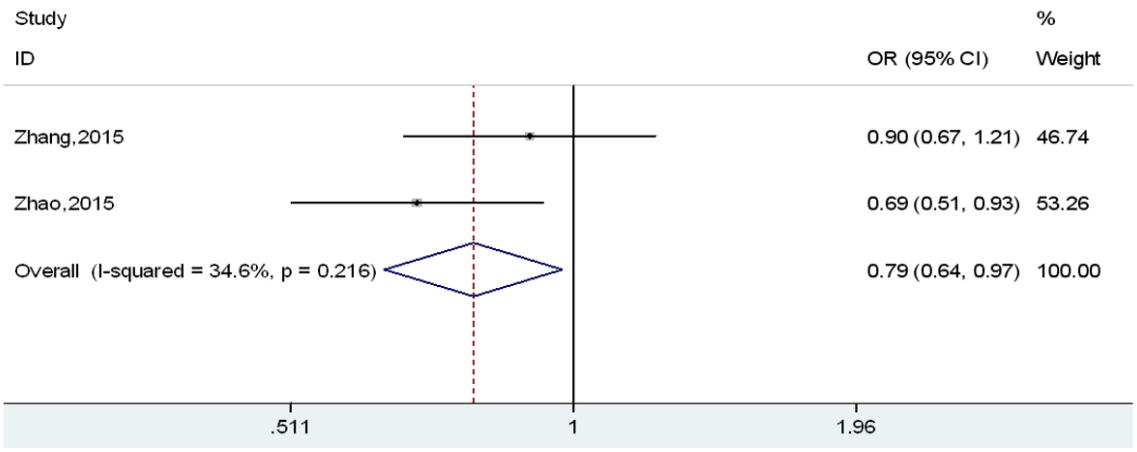

Figure 5. Forest plot of the association between the rs833061 polymorphism and the OS risk under the allele model (T vs. C).

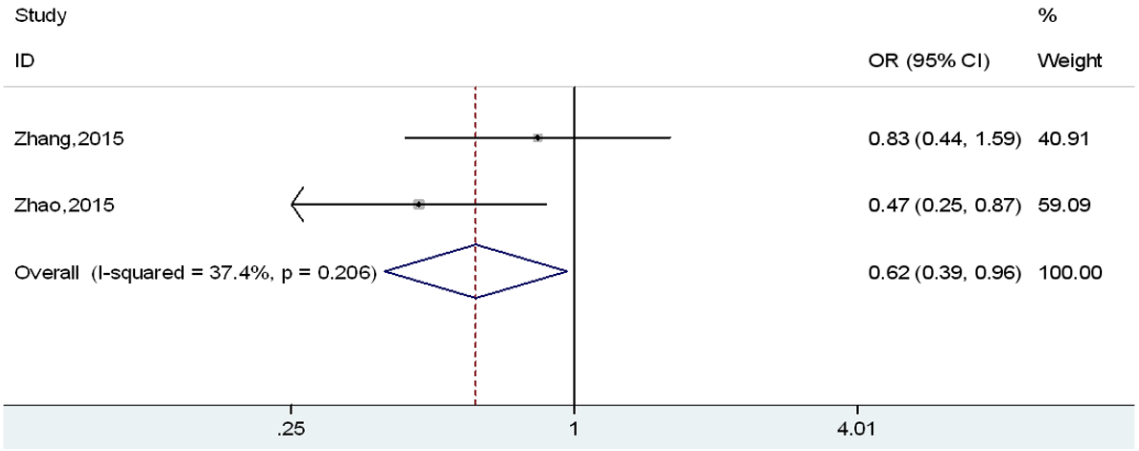

Figure 6. Forest plot of the association between the rs833061 polymorphism and the OS risk under the codominant model (TT vs. CC).

+1612G/A (rs10434), +936C/T (rs3025039) and -634G/T (rs2010963). Two studies respectively by Wang Z [27] and Zhang G [28] were all concerned with three polymorphism, +1612G/A (rs10434), +936C/T (rs3025039) and -634G/T (rs2010963). Zhang HF [29] surveyed three polymorphisms, -2578C/A (rs699947), 936C/T (rs3025039) and -460T/C (rs833061). Zhao LL [30] inquired about six polymorphisms, -2578C/A (rs699947), -1156G/A (rs1570360), +1612G/A (rs10434), +936C/T (rs3025039), -634G/T (rs2010963) and -460T/C (rs833061).

+1612G/A polymorphism and osteosarcoma

A total of four studies [26-28, 30] investigated the 1612G/A polymorphism in osteosarcoma (**Table 1**). As there is no heterogeneity, the fixed effects models were used for all five genetic models. The results indicate that the VEGF gene 1612G/A polymorphism was not associated with the risk of osteosarcoma (**Table 2**).

-2578C/A polymorphism and osteosarcoma

A total of three studies [26, 29, 30] investigated the -2578C/A polymorphism and osteosarcoma (**Table 1**). Based on the fixed effects model for no heterogeneity, the results show that the genotype at the -2578C/A polymorphism exhibited a significant association between -2578C/A polymorphism and the risk of osteosarcoma. The C allele was associated with a reduced risk of osteosarcoma (**Table 2**; **Figure 2**). When comparing with the carrier with

CC, the carriers with AA or CA all have an increased susceptibility to osteosarcoma (**Table 2**; **Figures 3**, **4**).

-460T/C polymorphism and osteosarcoma

A total of two studies [29, 30] investigated the -460T/C polymorphism and osteosarcoma (**Table 1**). **Table 2** shows the results of the meta-analysis based on the fixed effects models. The results indicated that there was a positive association between the VEGF gene -460T/C polymorphism and the risk of osteosarcoma only in T vs. C and TT vs. CC gene model (**Figures 5**, **6**), suggesting T allele may have the potential values as a protective factor.

-634G/C polymorphism and osteosarcoma

A total of four studies [26-28, 30] investigated the -634G/C polymorphism and osteosarcoma. **Table 3** shows the main characteristics of the 634G/C polymorphism in the meta-analysis and **Table 4** shows the results of the 634G/C polymorphism in overall and removed by Zhang T, respectively. The results indicated that there was a positive association between the VEGF gene-634G/C polymorphism and the risk of osteosarcoma in C vs. G, CG/CC vs. GG, CC vs. CG/GG and CC vs. GG gene model in overall, but when deletion of the study by Zhang T, a positive association only found in CC vs. CG/GG.

-936C/T polymorphism and osteosarcoma

A total of five studies [26-30] investigated the -936C/T polymorphism and osteosarcoma. Among them, the genotype distributions of rs3025039 in two studies (Zhang T; Zhang HF) were not in HWE in the control subjects, and they all detected non-statistically significantly association between 936C/T polymorphism and osteosarcoma. For deriving a more reliable conclusion, an analysis was performed with three studies which are in HWE in the controls. **Table 5** shows the main characteristics of the 936C/T polymorphism in the meta-analysis.

We detected a statistically significantly increased risk of osteosarcoma only in T vs. C (OR=1.21, 95% CI: 1.03-1.41, P=0.019), TT vs. CC (OR=1.69, 95% CI: 1.15-2.50, P=0.008) and TT vs. CT/CC (OR=1.57, 95% CI: 1.09-2.28, P=0.016), but when deletion of the study by Wang Z, the results showed that the genotype at the -936C/T polymorphism exhibited negative association between -936C/T polymorphism and osteosarcoma in T vs. C (OR=1.15, 95% CI: 0.94-1.41, P=0.185), TT vs. CC (OR=1.33, 95% CI: 0.83-2.15, P=0.239), TT vs. CT/CC (OR=1.23, 95% CI: 0.78-1.93, P=0.371).

-1156G/A polymorphism and osteosarcoma

A total of two studies [26, 30] investigated the -1156G/A polymorphism and osteosarcoma. The genotype distributions of rs1570360 in one studies (Zhang T) was in line with HWE, the other (Zhao LL) was not. They all reported that there was no association between -1156G/A polymorphism and osteosarcoma.

Sensitivity analysis

Sensitivity analysis was conducted by omitting every study by sequence from the pooled analysis to examine the influence of the removed data set to the pooled OR. The result showed no individual study significantly affected the overall ORs in specific genotype in gene polymorphism (+1612G/A, -2578C/A, -460T/C), suggesting that the results of the meta-analysis were steady.

Publication bias

Each genetic model were used by funnel plot, Egger' test and Begg' test, respectively (Not shown). The results all showed there were no publication biases of all included studies in gene polymorphism.

Discussion

This study comprehensively assessed the associations between VEFG polymorphism and OS susceptibility in Chinese populations. A total of six gene polymorphisms of the VEFG gene were investigated, and the five case-control studies included were all Chinese participants. This meta-analysis we performed addresses the potential association between six VEGF gene polymorphisms and OS risk in Chinese populations. We evaluated the genetic associations between six VEGF gene polymorphisms and OS by pooling the data from published literatures, the six VEGF gene polymorphisms were -2578C/A, -1156G/A, +1612G/A, +936C/T, -634G/T and -460T/C. There were no associations iden-

Table 3. The main characteristics of the 634G/C polymorphism in the meta-analysis

Gene polymorphism	Author, year	Country	Racial	Genotyping method	Source of controls	No. cases/ controls	Cases			Controls			P_{HWE}	Score
							CC	CG	GG	CC	CG	GG		
634G/C (rs2010963)	Zhang, 2014	China	Asian	PCR-RFLP	HB	165/330	42	80	43	120	151	59	0.34	13
	Wang, 2014	China	Asian	PCR-RFLP	HB	330/342	50	165	115	58	166	118	0.976	14
	Zhang, 2015	China	Asian	PCR-RFLP	HB	180/361	48	90	42	138	170	53	0.956	14
	Zhao, 2015	China	Asian	PCR-RFLP	HB	176/176	61	85	30	67	81	28	0.671	13

Table 4. The results of the 634G/C polymorphism in overall and in part (omitting one study by Zhang T)

Gene polymorphism	Comparison	Test of association			Model	Test of heterogeneity	
		OR	95% CI	*P*		P	I^2
634G/C (rs2010963)	C vs. G	0.79	0.65-0.96	0.020	R	0.077	56.20%
	CG/CC vs. GG	0.79	0.64-0.97	0.025	F	0.145	44.40%
	CC vs. CG/GG	0.71	0.58-0.87	0.001	F	0.347	9.20%
	CG vs. GG	0.86	0.69-1.08	0.192	F	0.437	0.00%
	CC vs. GG	0.64	0.49-0.83	0.001	F	0.118	48.90%
Removed by Zhang T							
	C vs. G	0.83	0.66-1.05	0.126	R	0.082	59.90%
	CG/CC vs. GG	0.81	0.57-1.16	0.248	F	0.135	50.20%
	CC vs. CG/GG	0.75	0.59-0.95	0.017	F	0.299	17.20%
	CG vs. GG	0.9	0.70-1.16	0.428	F	0.35	4.70%
	CC vs. GG	0.69	0.44-1.09	0.111	R	0.108	55.10%

Table 5. The main characteristics of the 936C/T polymorphism in the meta-analysis

Gene Polymorphism	Author, Year	Country	Racial	Genotyping Method	Source of Controls	No. Cases/ Controls	CC	CT	TT	CC	CT	TT		
+936C>T (rs3025039)	Wang, 2014	China	Asian	PCR-RFLP	HB	330/342	185	116	29	207	123	12	0.224	14
	Zhang, 2015	China	Asian	PCR-RFLP	HB	180/360	66	92	22	148	175	37	0.158	14
	Zhao, 2015	China	Asian	PCR-RFLP	HB	176/176	85	75	16	92	71	13	0.89	13

tified between the 1612G/A, -1156G/A polymorphisms and OS. However, a significant association was revealed by the results between -2578C/A, -460T/C and OS. For the -634G/C polymorphism and OS, the results indicated that there was an significant association between the VEGF gene -634G/C polymorphism and the risk of OS in allele, dominant, recessive and codominant model in overall, but when omitting the study by Zhang Tie, a statistical significance only found in recessive model, suggesting the results should be treated with caution. And for -936C/T polymorphism and OS, a statistically significance were found in some gene models, but when deletion of the study by Wang Zhen, the results showed no association between -936C/T polymorphism and OS, suggesting the results may be not steady, more case-control studies should be brought into the analysis in the future.

This meta-analysis we present still has some strengths. Firstly, all included studies are methodologically rigorous and high quality (score ≥12). The heterogeneity was also checked by Q and I^2 test, and the heterogeneity of the meta-analysis was not significant. The sensitivity analysis was also conducted by omitting one study by sequence to ensure the steadiness of the pooled results. Secondly, all five genetic models were performed in six gene polymorphism, which offered enough information to detect the association. At last, the literature search was comprehensive and extensive, including all studies published about VEGF gene polymorphisms and OS. Six VEFG gene polymorphisms were identified in this study, it was essential for us to get a comprehensive understanding of the relationships between VEGF gene polymorphisms and OS.

Also, there are still a few limitations of this study that the findings should be interpreted with caution. Firstly, there may exist some potential bias for only including published literatures and all included studies are hospital-based. Secondly, this meta-analysis is based on unadjusted estimates, a more precise analysis are needed. Thirdly, the number of studies and subjects was relatively small and limited, for this reason, more case-control studies with larger sample size and higher quality are required. Finally, because of lacking of relevant enough information provided in all included studies, other influence factors such as gene-environment were fail to investigated.

In conclusion, the current meta-analysis we performed suggested that in a Chinese population, the 1612G/A, -1156G/A gene polymorphisms are not associated with the risk of OS, while there was a significant association between -2578C/A polymorphism and OS, and for -460T/C, T allele may reduce the susceptibility to OS for Chinese. But the results about -634G/C and -936C/T should be treated with caution. Due to the limitations of this study, further larger-size, multi-center and higher-quality case-control studies are required to validate these findings in the future.

Disclosure of conflict of interest

None.

Authors' contribution

Zhu ZM was involved in the design of this meta-analysis and revision of this manuscript, he has also given final approval for submission. Zhou L, Xia GF and Zhang Y were involved in publication collection and assessment of study quality. Liu FT was involved in data analysis of the study, and wrote this manuscript, he has also assisted in the design of this work. Luo HL and Zhu PQ assisted in the revision of this manuscript. All authors read and approved the final manuscript.

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