

## Review Article

# Associations between VEGF polymorphisms and the risk and prognosis of osteosarcoma: a systematic review and meta-analysis

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**Abstract:** Background: Vascular endothelial growth factor (VEGF) genetic polymorphisms play important roles in the risk and prognosis of many solid tumors. However, in osteosarcoma, the results are conflicting. Thus, a systematic review and meta-analysis was performed to investigate the effects of VEGF polymorphisms on osteosarcoma risk and prognosis. Methods: A comprehensive search was carried out in electronic databases. Odds ratio (OR) with its 95% confidence interval (CI) were calculated for the extracted data. Results: Three case-control studies on risk, including 671 cases and 848 controls, and two retrospective cohort studies on prognosis, including 456 cases, were enrolled. Meta-analysis showed a significant association between the -634 G/C polymorphism and osteosarcoma risk (CC vs. GG (OR: 0.72, 95% CI=0.53-0.97); CC vs. GC+GG (OR: 0.76 95% CI=0.60-0.97)). In addition, a significant association between osteosarcoma risk and the +936 C/T polymorphism was indicated (TT vs. CC (OR: 1.75, 95% CI=1.15-2.59); CT+TT vs. CC (OR: 1.18, 95% CI=0.96-1.46)). Furthermore, polymorphism -2578 C/A, but not +1612 G/A or -1156 G/A, was also identified as a risk factor in the original research. For osteosarcoma prognosis, meta-analysis of the +936 C/T polymorphism showed no significant associations (CT vs. CC (OR: 0.58, 95% CI=0.08-4.19), TT vs. CC (OR: 0.50, 95% CI=0.04-6.03), CT+TT vs. CC (OR: 0.53, 95% CI=0.05-5.20) and TT vs. CT+CC (OR: 0.77, 95% CI=0.32-1.87)). Polymorphisms +1612 G/A and -634 G/C also had no effects on osteosarcoma prognosis. Conclusions: Our results indicated that VEGF polymorphisms might influence osteosarcoma risk, but had no associations with its prognosis.

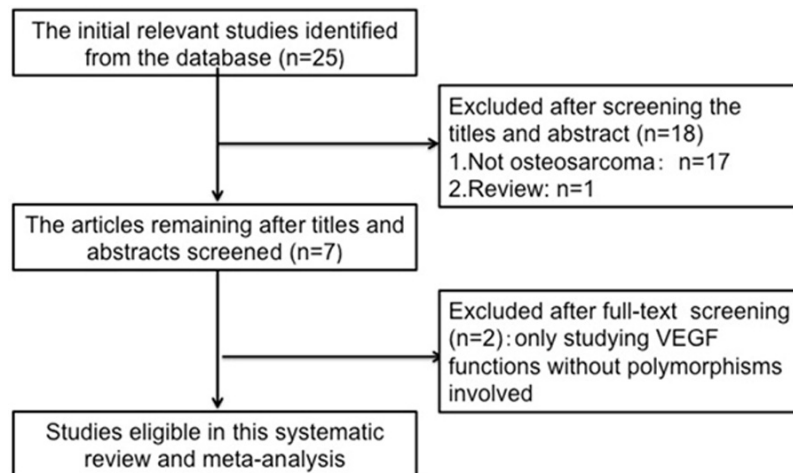
**Keywords:** Osteosarcoma, risk, prognosis, vascular endothelial growth factor polymorphism, systematic review, meta-analysis

## Introduction

Osteosarcoma, often originating in the long bones of the body, is the most common primary malignant bone tumor in children and young adults, accounting for 20% of all primary sarcomas in bone [1]. Although the etiology of osteosarcoma has been investigated by many studies, the actual mechanism remains unclear [2]. Recent studies revealed potential associations of common genetic variants with osteosarcoma risk in diverse pathways, suggesting that the pathogenesis of osteosarcoma might be regulated by genetic factors [3, 4]. To treat osteosarcoma, neoadjuvant therapy introduced by Rosen is indispensable before and after resection of the primary tumor, and the 5-year survival rate has been greatly improved to 60-70%

[5]. However, it is also reported that 40% of osteosarcoma patients show a poor response to chemotherapy, with a high risk of local recurrence and distant metastasis, even after standard treatment. Cumulative evidence also indicates that genetic factors might influence chemotherapy toxicity and the clinical outcome of osteosarcoma [6, 7].

The human vascular endothelial growth factor (VEGF) gene, consisting of 8 exons and located on chromosome 6p21.3, encodes a family of proteins generated through alternative splicing [8]. As a potent regulator of angiogenesis, VEGF has a very important role in the development and prognosis of solid tumors [9]. Thirty single nucleotide polymorphisms (SNPs) have been identified in the VEGF gene, among which sev-



**Figure 1.** Flow diagram for the identification of eligible studies for this systematic review and meta-analysis.

eral common SNPs have been investigated for their role in *VEGF* expression [10]. *VEGF* gene polymorphisms have been reported to be associated with the pathogenesis and development of a variety of cancers, such as lung, colorectal, breast, oral, ovarian, gastric, and bladder cancers, and could be used as markers for susceptibility and prognosis [11]. Recently, a number of studies have indicated that *VEGF* polymorphisms are associated with the pathogenesis and prognosis of osteosarcoma [6, 12-15]. However, some results are inconsistent, which might reflect the limitations of different numbers of subjects in each published studies. Thus, we performed a systematic review and meta-analysis of the published studies to estimate the associations more accurately.

## Materials and methods

### Literature and search strategy

We performed a meta-analysis as described previously [16]. Relevant articles were selected by searching the PubMed and Embase database (updated on August 2015). The query terms were: risk factor or susceptibility or prognosis or clinical outcome, osteosarcoma or bone tumor, *VEGF* or vascular endothelial growth factor, polymorphism or gene mutation. Two reviewers (Zitao Zhang and Jin Xiong) evaluated the titles and abstracts of the identified papers independently. Only full-text articles published in English were included in this meta-analysis. The inclusion criteria were

as follows: (1) Case-control studies including retrospective data or retrospective cohort studies; (2) evaluating the relationships between *VEGF* polymorphisms and osteosarcoma risk or prognosis; (3) containing available genotype frequencies; and (4) sufficient data was published to estimate an odds ratio (OR) with 95% confidence intervals (CIs). In case of overlapping studies, the study with the largest sample size was selected. The exclusion criteria were: 1) Not being a case-control study; 2) case reports, letters, reviews, meta-analyses and editorial articles; 3) studies reporting incomplete or insufficient data; 4) studies containing duplicate data.

### Data extraction

The data were independently examined and extracted by two investigators, based on the selection criteria. The following items were extracted: the first author's last name, year of publication, country of origin, ethnicity and the populations of cases and controls. For studies including subjects of different ethnic groups, data were extracted separately for each ethnic group, if possible. Any disagreement was resolved by discussion, and a consensus was reached for all data.

### Statistical analysis

Pooled ORs with 95% CIs were used to assess the association between *VEGF* SNPs and the risk and prognosis of osteosarcoma. SNPs were considered as binary variables. We estimated the risks or prognosis using heterozygote (-634 GC vs. GG; +936 CT vs. CC), homozygote (-634 CC vs. GG; +936 TT vs. CC), dominant (-634 GC+CC vs. GG; +936 CT+TT vs. CC) and recessive models (-634 CC vs. GC+GG; +936 TT vs. CT+CC). Heterogeneity between the studies was tested using Q statistics [17]. If the *P*-value was <0.10, the between-study heterogeneity was considered significant. When between-study heterogeneity was absent, the fixed-effects model (the Mantel-Haenszel

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**Table 1.** Characteristics of the studies included in this systematic review and meta-analysis

	First Author	Year	Country/ Ethnicity	Study Design	Methods	Cases	Controls	Gene
Risk	Wang	2014	China/Asian	CCS	PCR-RFLP	330	342	-634 G/C; +936 C/T; +1612 G/A
	Tie	2014	China/Asian	CCS	PCR-RFLP	165	330	-2578C/A; -1156G/A; +1612G/A; +936C/T; -634G/C
	Zhao	2015	China/Asian	CCS	PCR-RFLP	176	176	-2578C/A; -1156G/A; +1612G/A; +936C/T; -634G/C; -460T/C
Prognosis	Li	2015	China/Asian	RCS	PCR-RFLP		194	+1612G/A; -634C/G; +936T/C
	Zhao	2014	China/Asian	RCS	PCR-RFLP		262	+1612G/A; -634C/G +936G/C

Abbreviations: HWE, Hardy-Weinberg equilibrium; CCS, case-control study; RCS, retrospective cohort study; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

method) was used to calculate the pooled ORs [18]; otherwise, a random-effects model (the DerSimonian and Laird method) was selected [19]. Publication bias was investigated using Begg's funnel plot and  $P < 0.05$  was considered to indicate a statistically significant publication bias [20]. All analyses were performed using the Stata software, version 11.0 (Stata Corporation, College Station, TX, USA).

## Results

### Eligible studies

Twenty-five studies were selected after the initial search. In the further screening of the titles and abstracts, 18 studies were excluded: one was a review article and the tumors investigated in the others were not osteosarcoma. Among the seven remaining articles, two were excluded for addressing the VEGF functions without SNPs in osteosarcoma. Therefore, we enrolled five articles in this systematic review and meta-analysis (**Figure 1**).

### Study characteristics

Among the five enrolled articles, three were case-control studies on the association of risk of osteosarcoma risk with VEGF SNPs, and included 671 cases and 848 controls. Two studies were retrospective cohort studies concerning the effects of VEGF SNPs on prognosis of osteosarcoma and included 456 cases [6, 12-15]. All the selected studies were carried out in China. The distributions of genotypes in the controls of all the studies were in agreement with Hardy-Weinberg equilibrium, except one study when investigating the association between SNP +936 C/T and osteosarcoma risk [13]. The details of the authors, publication year, national sources, ethnicity, method and genotype of each study are shown in **Table 1**.

### Meta-analysis of VEGF SNPs-634 G/C and +936 C/T on osteosarcoma risk

Using heterozygote, homozygote, dominant and recessive models, the quantitative results for VEGF SNP -634 G/C were: GC vs. GG (OR: 0.92, 95% CI=0.72-1.19), CC vs. GG (OR: 0.72, 95% CI=0.53-0.97), GC+CC vs. GG (OR: 0.86, 95% CI=0.60-1.09) and CC vs. GC+GG (OR: 0.76 95% CI=0.60-0.97) (**Figure 2**). These results indicated that VEGF SNP-634 G/C was significantly associated with the risk of osteosarcoma and the CC or GC+CC variant genotype decreased susceptibility to osteosarcoma.

For VEGF SNP+936 C/T, the quantitative result were: CT vs. CC (OR: 1.09, 95% CI=0.87-1.36), TT vs. CC (OR: 1.75, 95% CI=1.15-2.59), CT+TT vs. CC (OR: 1.18, 95% CI=0.96-1.46) and TT vs. CT+CC (OR: 1.67, 95% CI=1.12-2.49) (**Figure 3**). Significant main effects were observed in the homozygote and recessive models; therefore, the TT variant genotype was considered a risk factor for osteosarcoma.

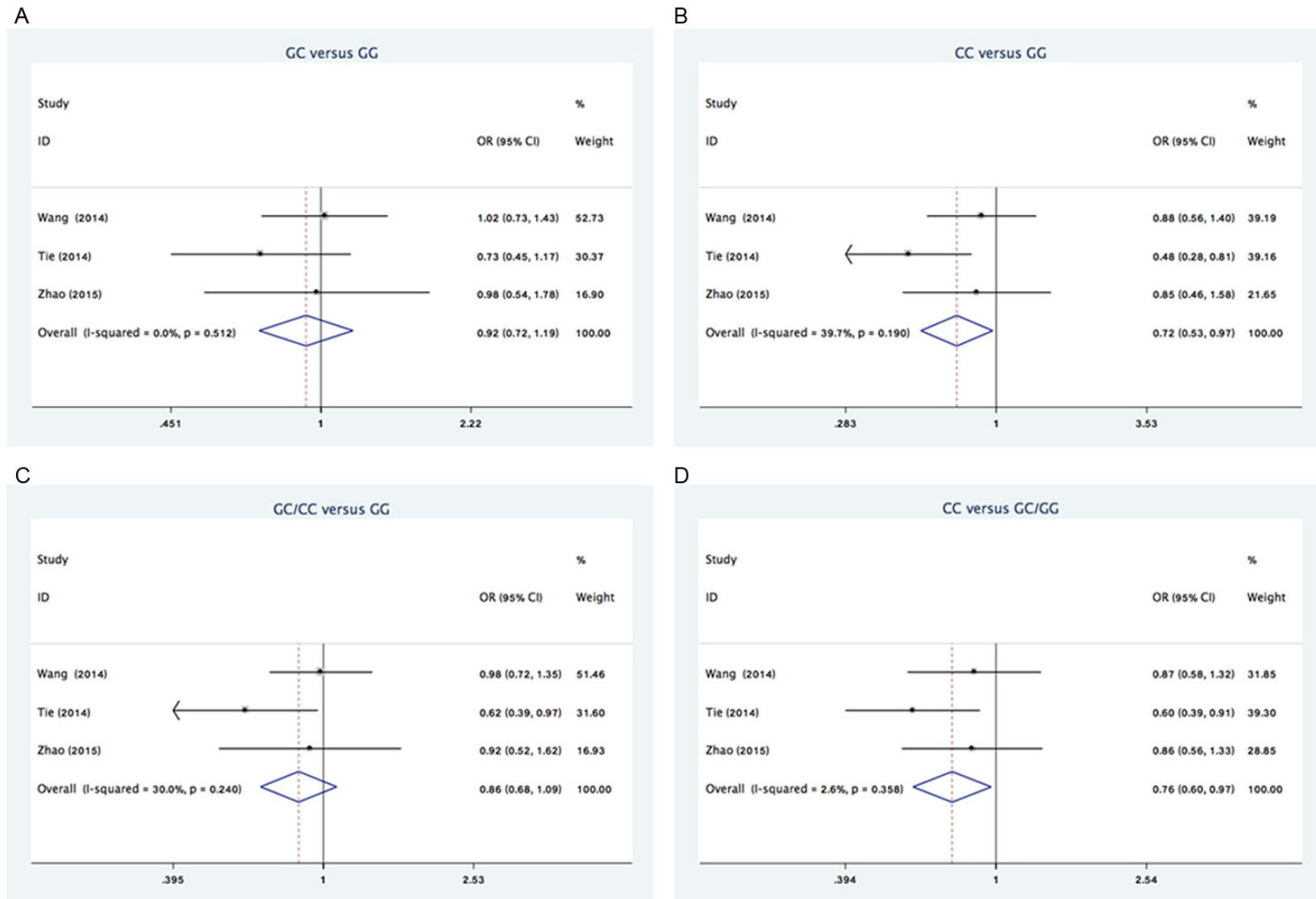
### Meta-analysis of VEGF SNP +936 C/T on osteosarcoma prognosis

Two studies investigated the association of SNP +936 C/T with osteosarcoma prognosis, with inconsistent results. The pooled ORs are shown in **Figure 4**. We found no significant associations using four different genotypes: CT vs. CC (OR: 0.58, 95% CI=0.08-4.19), TT vs. CC (OR: 0.50, 95% CI=0.04-6.03), CT+TT vs. CC (OR: 0.53, 95% CI=0.05-5.20) and TT vs. CT+CC (OR: 0.77, 95% CI=0.32-1.87).

### Other VEGF SNPs with osteosarcoma risk and prognosis

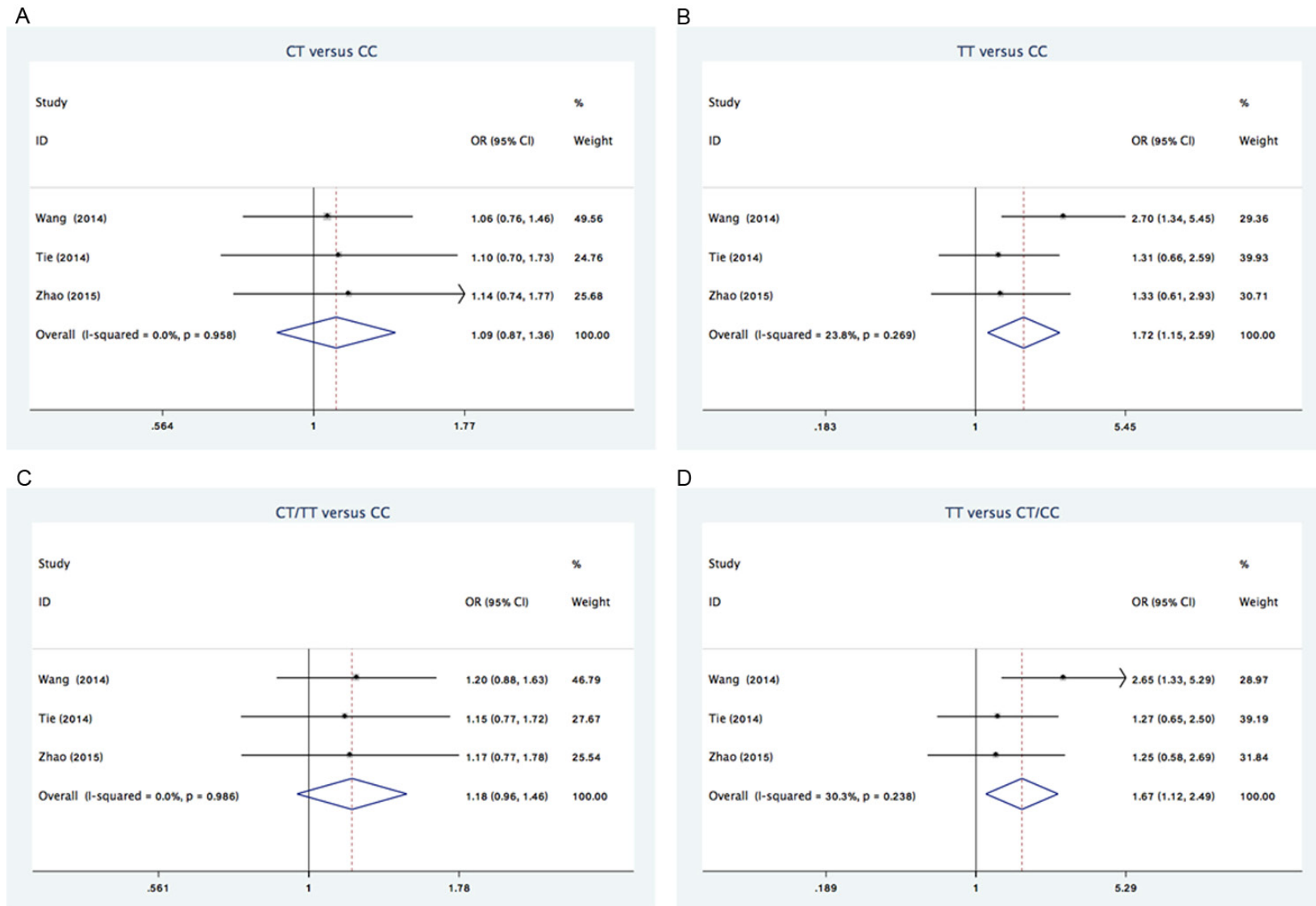
In addition to -634 G/C and +936 C/T, there were other four VEGF SNPs reported in the selected articles. The effects of those SNPs

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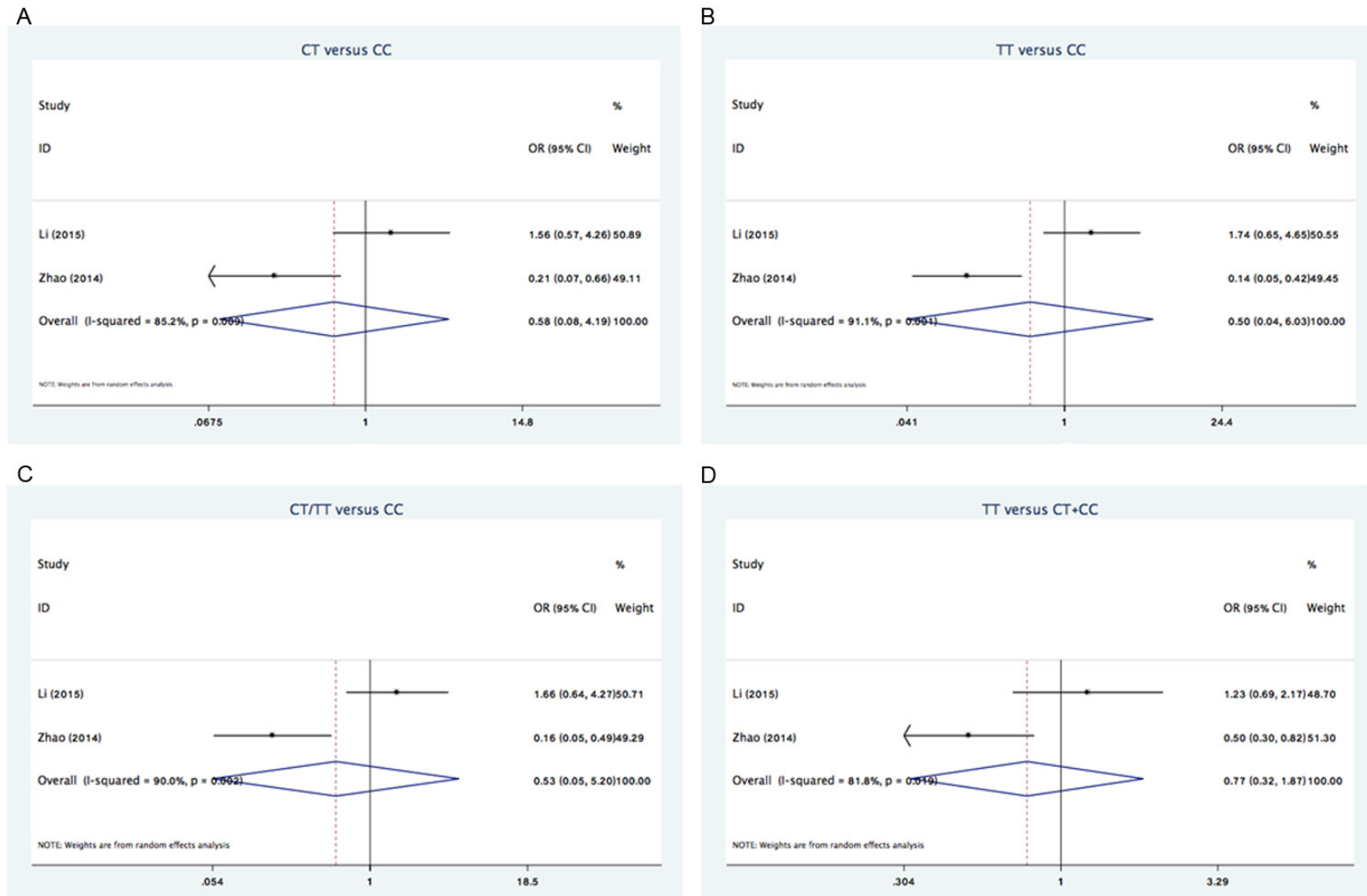
**Figure 2.** Associations between the VEGF -634 G/C polymorphism and risk of osteosarcoma. (A) Heterozygote mode: GC versus GG, (B) homozygote model: CC versus GG, (C) dominant model: GC+CC versus GG and (D) recessive model: CC versus GC+GG.

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**Figure 3.** Associations between the VEGF +936 C/T polymorphism and risk of osteosarcoma. (A) Heterozygote mode: CT versus CC, (B) homozygote model: TT versus CC, (C) dominant model: CT+TT versus CC and (D) recessive model: TT versus CC+CT.

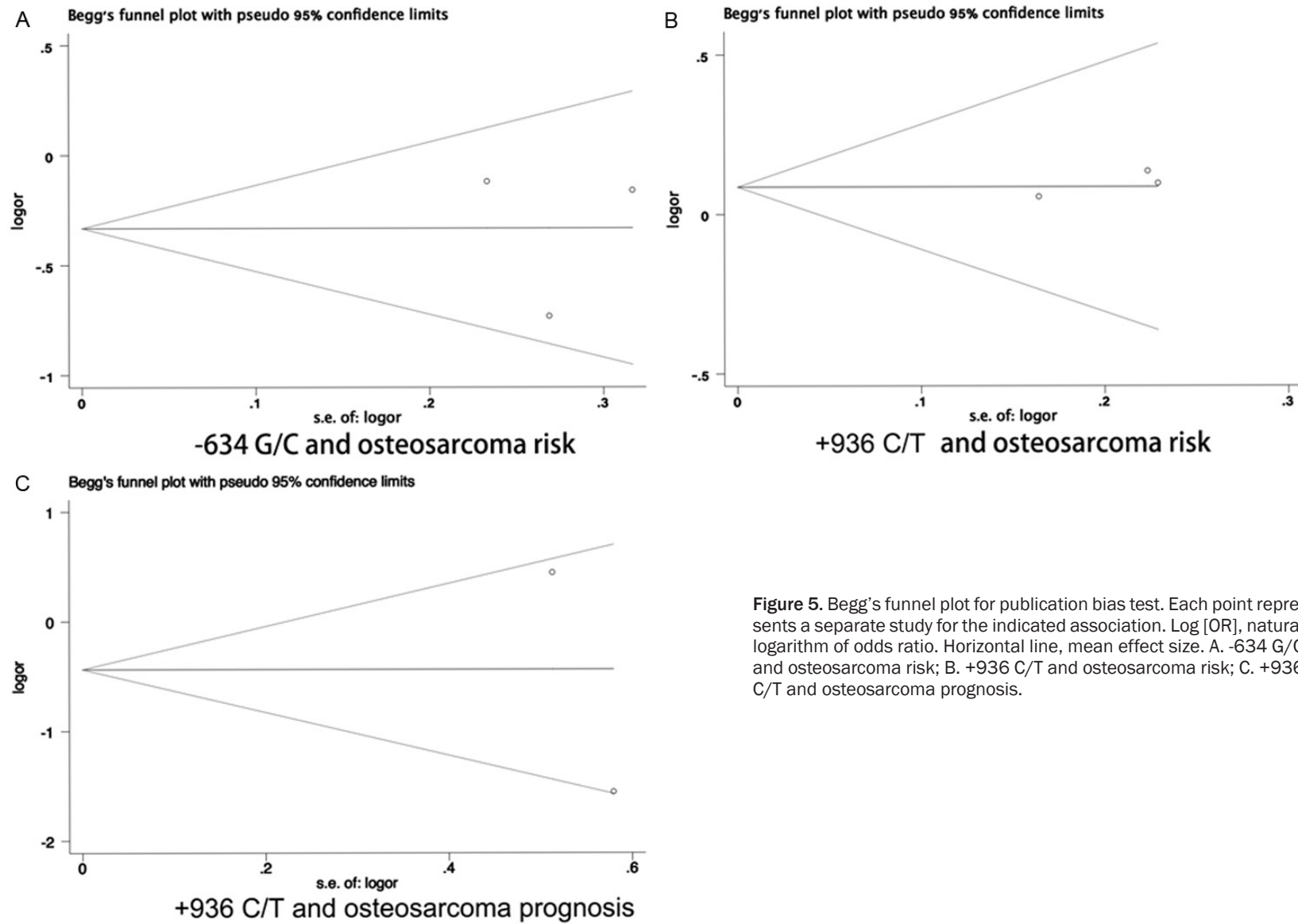
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**Figure 4.** Associations between the VEGF +936 C/T polymorphism and prognosis of osteosarcoma. (A) Heterozygote mode: CT versus CC, (B) homozygote model: TT versus CC, (C) dominant model: CT+TT versus CC and (D) recessive model: TT versus CC+CT.



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**Figure 5.** Begg's funnel plot for publication bias test. Each point represents a separate study for the indicated association. Log [OR], natural logarithm of odds ratio. Horizontal line, mean effect size. A. -634 G/C and osteosarcoma risk; B. +936 C/T and osteosarcoma risk; C. +936 C/T and osteosarcoma prognosis.

were consistent or the SNP was investigated by only one study; therefore, a meta-analysis could not be carried out. For example, all studies concluded that +1612 G/A has no association with the risk and prognosis [6, 12-15]. SNP -2578 C/A was considered to have an association with the risk and prognosis of osteosarcoma by two and one studies, respectively [13-15]. Furthermore, two studies also concluded that SNP -1156G/A had no connections with osteosarcoma pathogenesis. Finally, only one study proved that SNP-460 T/C is a risk factor for osteosarcoma.

## Publication bias

Begg's funnel plot and Egger's test were performed to assess the publication bias of the studies. No evidence of publication bias was detected in the present meta-analysis (**Figure 5**).

## Discussion

VEGF is a growth factor that regulates angiogenesis and is considered to play a pivotal role in the pathogenesis and prognosis of many solid tumors [9]. Accumulating studies have shown that *VEGF* expression is associated with higher risk of solid tumors, and higher *VEGF* expression and microvessel density in tumor tissues correlate with advanced stage disease and worse prognosis [21, 22]. Several functional SNPs in the *VEGF* gene have been reported to influence expression levels of the VEGF protein in circulating plasma and tumor tissue, and thus affect cancer susceptibility and outcome, such as in non-small cell lung, prostate and bladder cancers [23]. However, the role of some common VEGF SNPs in the pathogenesis and prognosis of osteosarcoma is still in dispute. Wang et al. found that VEGF SNP -634 G/C was a risk factor for osteosarcoma, while the other two studies showed that this SNP had no associations with susceptibility [12, 13, 15]. For osteosarcoma prognosis, VEGF SNP +936 C/T was considered to be harmful by Zhao et al., but to have no effect by Li et al. [6, 14].

To the best of our knowledge, our study is the first systematic review and meta-analysis to evaluate the associations between VEGF SNPs and the risk and prognosis of osteosarcoma. Five studies were enrolled in our analysis, of which three investigated the associations of

risk and two explored the effects on prognosis. Six VEGF SNPs were included, for which some studies came to the same conclusions while some are conflicting. Similar to its effects on glioma and breast cancer, VEGF SNP +1612 G/A was proven to have no correlations with the risk or prognosis of osteosarcoma [24, 25]. Two studies confirmed that SNP -2578 C/A, but not -1156 G/A, could be a risk factor for osteosarcoma. In addition, -460 T/C was considered to be associated with the susceptibility to osteosarcoma by only one study [15].

In contrast to the above SNPs, the effects of SNPs -634 G/C and +936 C/T on osteosarcoma were inconsistent in the original articles. Thus, the meta-analysis was performed and the results showed that -634 G/C and +936 C/T were associated the risk of osteosarcoma, but had no effects on its prognosis. The mechanism for -634 G/C and +936 C/T might be associated with their locations in the 5'- and 3'-untranslated regions (UTRs) of *VEGF*, which could affect the protein translation efficiency, the circulating plasma concentrations, and tumor tissue expression of VEGF [10, 26]. However, it was difficult to explain why they did not affect the clinical outcome of osteosarcoma patients. The limited sample sizes might be the major cause, and further study, including larger populations, is needed.

Although our results are statistically more powerful than any single study, some limitations still exist. Firstly, the number of eligible articles was limited. Three were found for risk and two were for prognosis. The distribution of genotypes in the controls of one study was in disagreement with Hardy-Weinberg equilibrium when SNP+936C/T was investigated for osteosarcoma risk. The enrolled studies were already insufficient; therefore, this group of data was also added to the meta-analysis. It is possible that the results of unpublished studies or further investigations might differ from our conclusion. Secondly, all the subjects in these five studies were Asians from China, and no Caucasians or Africans were included. Thirdly, the present results were based on unadjusted ORs, and a more precise estimation might be achieved by other confounding factors.

In conclusion, our systematic review and meta-analysis determined that SNPs -634 G/C, +936 C/T and -2578 C/A, but not -1156 G/A and



+1612 G/A, are associated with the risk of osteosarcoma; however, none of these VEGF SNPs affected the prognosis of osteosarcoma. Further studies are needed to confirm these conclusions and to determine the relationship between VEGF SNPs and the risk and prognosis of osteosarcoma.

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## Disclosure of conflicts of interest

None.

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## References

- [1] Mirabello L, Troisi RJ and Savage SA. Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program. *Cancer* 2009; 115: 1531-1543.
- [2] Ottaviani G and Jaffe N. The etiology of osteosarcoma. *Cancer Treat Res* 2009; 152: 15-32.
- [3] Bian Z, He Q, Wang X, Li M and Zhu L. Association of genetic polymorphisms with osteosarcoma risk: a meta-analysis. *Int J Clin Exp Med* 2015; 8: 8317-8328.
- [4] Savage SA, Mirabello L, Wang Z, Gastier-Foster JM, Gorlick R, Khanna C, Flanagan AM, Tirabosco R, Andrulis IL, Wunder JS, Gokgoz N, Patino-Garcia A, Sierrasesumaga L, Lecanda F, Kurucu N, Ilhan IE, Sari N, Serra M, Hattinger C, Picci P, Spector LG, Barkauskas DA, Marina N, de Toledo SR, Petrilli AS, Amary MF, Halai D, Thomas DM, Douglass C, Meltzer PS, Jacobs K, Chung CC, Berndt SI, Purdue MP, Caporaso NE, Tucker M, Rothman N, Landi MT, Silverman DT, Kraft P, Hunter DJ, Malats N, Kogevinas M, Wacholder S, Troisi R, Helman L, Fraumeni JF Jr, Yeager M, Hoover RN and Chanock SJ. Genome-wide association study identifies two susceptibility loci for osteosarcoma. *Nat Genet* 2013; 45: 799-803.
- [5] Eilber FR and Rosen G. Adjuvant chemotherapy for osteosarcoma. *Semin Oncol* 1989; 16: 312-322.
- [6] Li XD, Yu DD, Lu JR, Wu C and Jin WX. Effect of polymorphisms of vascular endothelial growth factor on prognosis in osteosarcoma patients. *Genet Mol Res* 2015; 14: 4354-4360.
- [7] Cong Y, Li CJ, Zhao JN, Liu XZ and Shi X. Associations of polymorphisms in the bone morphogenetic protein-2 gene with risk and prognosis of osteosarcoma in a Chinese population. *Tumour Biol* 2015; 36: 2059-2064.
- [8] Vincenti V, Cassano C, Rocchi M and Persico G. Assignment of the vascular endothelial growth factor gene to human chromosome 6p21.3. *Circulation* 1996; 93: 1493-1495.
- [9] Carmeliet P. VEGF as a key mediator of angiogenesis in cancer. *Oncology* 2005; 69 Suppl 3: 4-10.
- [10] Watson CJ, Webb NJ, Bottomley MJ and 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.
- [11] Eng L and Liu G. VEGF pathway polymorphisms as prognostic and pharmacogenetic factors in cancer: a 2013 update. *Pharmacogenomics* 2013; 14: 1659-1667.
- [12] Wang Z, Wen P, Luo X, Fang X, Wang Q, Ma F and Lv J. Association of the vascular endothelial growth factor (VEGF) gene single-nucleotide polymorphisms with osteosarcoma susceptibility in a Chinese population. *Tumour Biol* 2014; 35: 3605-3610.
- [13] Tie Z, Bai R, Zhai Z, Zhang H, Zhao Z, Zhou D and Liu W. Single nucleotide polymorphisms in VEGF gene are associated with an increased risk of osteosarcoma. *Int J Clin Exp Pathol* 2014; 7: 8143-8149.
- [14] Dong-Ju Z, Ai-Ju X, Yun-Jiao T and Ming-Qiu Z. Polymorphisms of vascular endothelial growth factor on prognosis in osteosarcoma patients. *Pak J Med Sci* 2014; 30: 1072-1076.
- [15] Li-Lian Z, Lin W, Lei S and Yao-Nan Z. Investigation on the role of VEGF gene polymorphisms in the risk of osteosarcoma. *Pak J Med Sci* 2015; 31: 364-368.
- [16] Zhang Z, Fan J, Ding Q, Wu M and Yin G. Risk factors for new osteoporotic vertebral compression fractures after vertebroplasty: a systematic review and meta-analysis. *J Spinal Disord Tech* 2013; 26: E150-157.
- [17] Lau J, Ioannidis JP and Schmid CH. Quantitative synthesis in systematic reviews. *Ann Intern Med* 1997; 127: 820-826.
- [18] Mantel N and Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst* 1959; 22: 719-748.
- [19] DerSimonian R and Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; 7: 177-188.

- [20] Song F and Gilbody S. Bias in meta-analysis detected by a simple, graphical test. Increase in studies of publication bias coincided with increasing use of meta-analysis. *BMJ* 1998; 316: 471.
- [21] Ferrara N. VEGF and the quest for tumour angiogenesis factors. *Nat Rev Cancer* 2002; 2: 795-803.
- [22] Bando N, Hayashi T, Takahara M, Kishibe K, Ogino T, Katayama A, Imada M, Nonaka S and Harabuchi Y. VEGF and bFGF expression and microvessel density of maxillary sinus squamous cell carcinoma in relation to p53 status, spontaneous apoptosis and prognosis. *Cancer Lett* 2004; 208: 215-225.
- [23] Jain L, Vargo CA, Danesi R, Sissung TM, Price DK, Venzon D, Venitz J and Figg WD. The role of vascular endothelial growth factor SNPs as predictive and prognostic markers for major solid tumors. *Mol Cancer Ther* 2009; 8: 2496-2508.
- [24] Jiang H, Lian M, Xie J, Li J and Wang M. Three single nucleotide polymorphisms of the vascular endothelial growth factor (VEGF) gene and glioma risk in a Chinese population. *J Int Med Res* 2013; 41: 1484-1494.
- [25] Luo T, Chen L, He P, Hu QC, Zhong XR, Sun Y, Yang YF, Tian TL and Zheng H. Vascular endothelial growth factor (VEGF) gene polymorphisms and breast cancer risk in a Chinese population. *Asian Pac J Cancer Prev* 2013; 14: 2433-2437.
- [26] Renner W, Kotschan S, Hoffmann C, Obermayr-Pietsch B and Pilger E. A common 936 C/T mutation in the gene for vascular endothelial growth factor is associated with vascular endothelial growth factor plasma levels. *J Vasc Res* 2000; 37: 443-448.