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Original Article

Association between *VEGF* -634G/C polymorphism and osteonecrosis of the femoral head susceptibility: a meta analysis

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Abstract: Background: *VEGF* plays an important role in bone formation and repair. However, the effects of *VEGF* -634G/C polymorphisms on the pathogenesis of osteonecrosis of the femoral head (ONFH) were not conclusive. Our research was aimed to further analyze the association of *VEGF* -634G/C polymorphism with ONFH risk. Methods: The relevant articles were searched in PubMed, Elsevier, EMBASE, Web of Science and Chinese National Knowledge Infrastructure (CNKI) database. And a total of 692 cases and 875 controls were included. Pooled odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to assess the correlation of *VEGF* -634G/C polymorphism and ONFH susceptibility. Chi-square based Q-statistic test was used to evaluate heterogeneity among the studies. The random-effects model or fixed-effects model was used depending on heterogeneity. Results: The sensitivity analysis and publication bias test indicated that our results were stable and credible. And the results suggested that *VEGF* -634G/C polymorphism was significantly related with increased risk for ONFH in Asian population (CC versus GG: OR=1.34, 95% CI=1.02-1.76). Conclusion: The results indicated that *VEGF* -634G/C polymorphism might serve as genetic-susceptibility factor for ONFH.

Keywords: *VEGF,* polymorphism, osteonecrosis of the femoral head

Introduction

Osteonecrosis of the femoral head (ONFH), also called avascular necrosis (AVN), is a common refractory disease in orthopedic field [1, 2], which seriously affects the life quality of people, especially the young [3]. The pathogenesis of ONFH is that osteocytes and marrow death caused by blood supply interruption to bone result in structural change of femoral head, collapse of the femoral head and joint dysfunction [4, 5]. And ONFH is usually divided into two types: traumatic and non-traumatic ONFH. The former is mainly induced by hip traumas such as femoral neck fracture and hip dislocation, while the latter is usually attributed to abuse of corticosteroids and alcoholism. And it has been confirmed that 80% of the femoral heads will collapse without proper and timely treatment when the osteonecrosis process begins [6]. Hence it is urgent to find out high-risk population of ONFH and then provide timely treatment for the patients.

Vascular endothelial growth factor (*VEGF*), located on chromosome 6p31.3, decodes a signal protein, which contributes to the creation of new blood vessels during embryonic development [7]. Moreover, as one member of growth factors, *VEGF* plays an important role in bone formation and cartilage and bone repair [8]. Additionally, it has been demonstrated that overexpression of *VEGF* is related with the occurrence of vascular diseases. Amo Y et al. have reported that serum level of *VEGF*-D is significantly increased in patients with angiosarcoma [9]. And the study of Andraweera PH et al. suggested that *VEGF* showed predictive value for early-onset pre-eclampsian [10]. Since the key role of *VEGF* for diseases, the association of *VEGF* polymorphisms and diseases susceptibility has attracted a lot of attention [11-18].

The relationship of *VEGF* -634G/C polymorphism with ONFH susceptibility has been reported, however, the results were inconclusive. Therefore, the present meta-analysis was

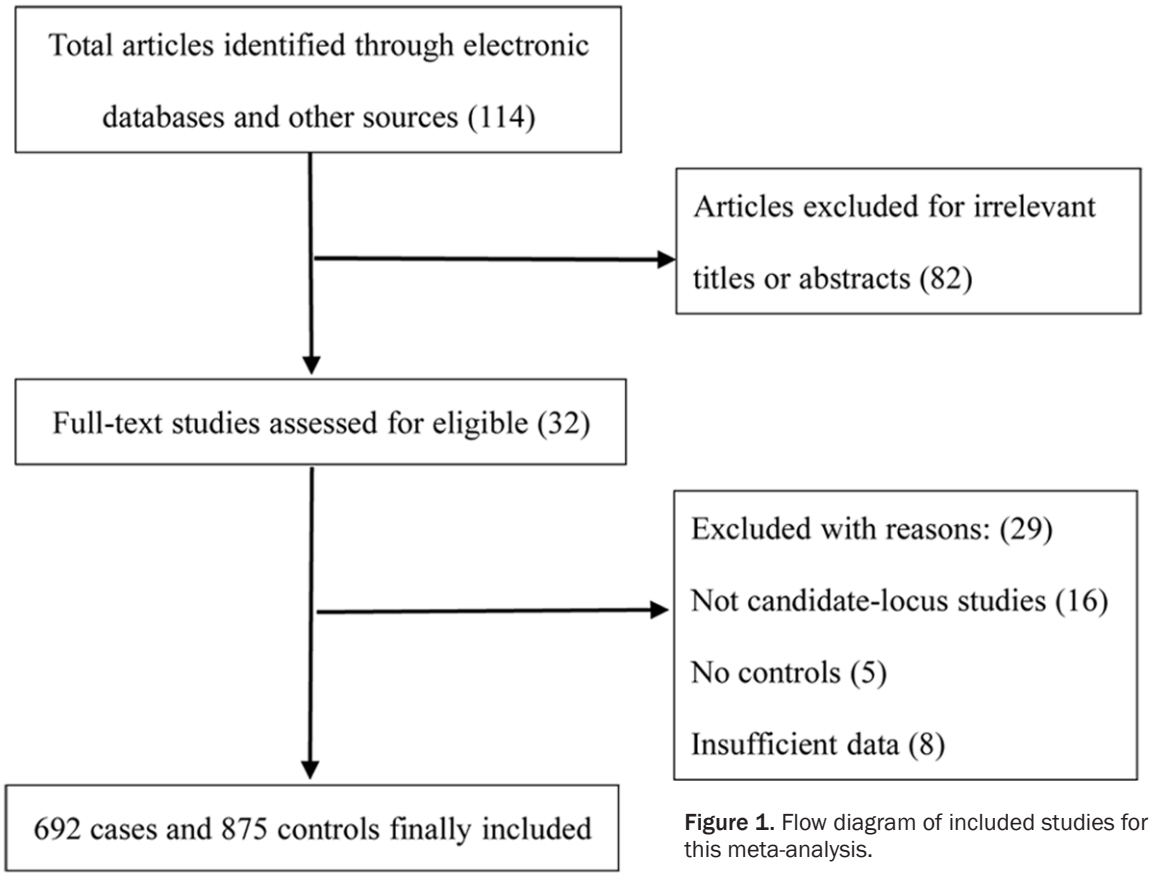

Figure 1. Flow diagram of included studies for this meta-analysis.

aimed to explore the precise correlation of *VEGF* -634G/C polymorphism and ONFH risk in Asians, which will helps for the identification of high-risk population for ONFH.

Materials and methods

Search strategy

The related articles were searched in PubMed, Elsevier, EMBASE, Web of Science and Chinese National Knowledge Infrastructure (CNKI) databases with the following terms: "*VEGF*" or "vascular endothelial growth factor", "-634G/C" or "rs2010963", "osteonecrosis of the femeral head" or "ONFH" or "avascular necrosis of femoral head", "polymorphism" or "variant". The selected studies did not include unpublished reports. The most recent publication including more information was adopted if more than one records were identified.

Inclusion criteria

Eligible studies were selected based on the following criteria: 1) case-control studies; 2) exploring the association between *VEGF* -634G/C polymorphism and ONFH risk; 3) providing detailed genotype data to calculate odds ratio (OR) and 95% confidence interval (CI); 4) being conducted in Asian populations. The precluded studies were: 1) review articles, case-only articles and family-based articles; 2) animal studies; 3) not consistent with Hardy-Weinberg equilibrium (HWE).

Data extraction

Two investigators extracted the raw data independently. The data included last name of first author, year of publication, country, ethnicity, source of control, genotyping method, number of cases and controls, genotype frequencies in case and control groups. The disagreement terms were solved by a third investigator.

Statistical analysis

Chi-square test was used to detect whether genotypes distribution of in controls were consistent with HWE. OR with 95% CI were calculated to assess the association of *VEGF* -634G/

Table 1. Principle characteristics of the studies included in the meta-analysis

First author	Year	Country	Ethnicity	Control source	Genotyping method	HWE
Liu	2012	China	Asian	Population based	PCR-RFLP	0.55
Kim	2008	Korea	Asian	Population based	Taqman	0.71
Lee	2011	Korea	Asian	Population based	PCR-RFLP	0.24

PCR: polymerase chain reaction; PCR-RFLP: PCR-restriction fragment length polymorphism; TaqMan: TaqManSNP; HWE: Hardy-Weinberg equilibrium.

Table 2. Association of *VEGF* -634G/C polymorphism and ONFH risk

	CC versus GG		CC + CG versus GG		CC versus CG + GG		C versus G		CG versus GG	
	OR (95% CI)	P_h	OR (95% CI)	P_h	OR (95% CI)	P_h	OR (95% CI)	P_h	OR (95% CI)	P_h
Total	1.34 (1.02-1.76)	0.963	1.10 (0.94-1.29)	0.948	1.29 (1.00-1.66)	0.685	1.14 (1.00-1.30)	0.984	1.11 (0.93-1.32)	0.857

P_h: *P*-value of heterogeneity test.

C polymorphism and ONFH risk. Chi-square based Q-statistic test was used to evaluate heterogeneity across the studies (P_h<0.05 was considered significant) [19]. If the test of heterogeneity was significant, the random-effects model was used; otherwise the fixed-effects model [20, 21]. Pooled ORs were presented under the following genetic models: CC versus GG, CG versus GG, CC + CG versus GG, CC versus CG + GG and C versus G. The significance of pooled ORs was estimated via Z-test, and P<0.05 was considered statistically significant. Begg's test and Egger's test were adopted to detect publication bias, and *P*<0.05 was considered significant [22]. Sensitivity analysis was performed through omitting each study in turn to assess the consistency of pooled results. All statistical analyses were done with Stata software (version 12.0).

Results

Study characteristics

As shown in **Figure 1**, a total of 114 articles were retrieved by literature search using different combinations of key terms. After reviewing the titles and abstracts, 82 articles were excluded and subsequently 29 studies were excluded with reasons of not candidate-locus studies (16), no controls (5) and insufficient data (8). Finally, 692 cases and 875 controls, including 440 Chinese and 1127 Koreans, were included. The characteristics of the included studies are summarized in **Table 1**. Genotyping methods in the studies included polymerase chain reaction-restriction fragment length polymorphis (PCR-RFLP) and TaqMan-SNP (TaqMan). HWE test was conducted for evaluating genotypes distribution of the controls in all studies. As a result, no studies deviate from HWE (*P*>0.05).

Meta-analysis results

The overall results are presented in **Table 2**. *VEGF* -634G/C polymorphism was significantly associated with increased risk for ONFH in CC versus GG genetic model (OR=1.34, 95% CI=1.02-1.76) (**Figure 2**). However, there was no evidence of significant association between the -634G/C polymorphism and risk of ONFH in other four genetic models (CC + CG versus GG: OR=1.10, 95% CI=0.94-1.29; CC versus CG + GG: OR=1.29, 95% CI=1.00-1.66; C versus G: OR=1.14, 95% CI=1.00-1.30 and CG versus GG: OR=1.11, 95% CI=0.93-1.32).

Heterogeneity and sensitivity tests

As shown in **Table 2**, no significant heterogeneity was detected in all genetic models. Thus, the fixed-effect model was used. Sensitivity analysis was performed through sequentially excluding each individual study in order to assess the stability of the combined results in the meta-analysis. The results demonstrated that no individual studies significantly affected the pooled ORs (data not shown).

Publication bias test

Publication bias was assessed by Begg's funnel plot and Egger's linear regression test. Obvious asymmetry was not shown based on shapes of the funnel plots (**Figure 3**). Moreover, Egger's test showed no statistical evidence for publication bias (*P*=0.646).

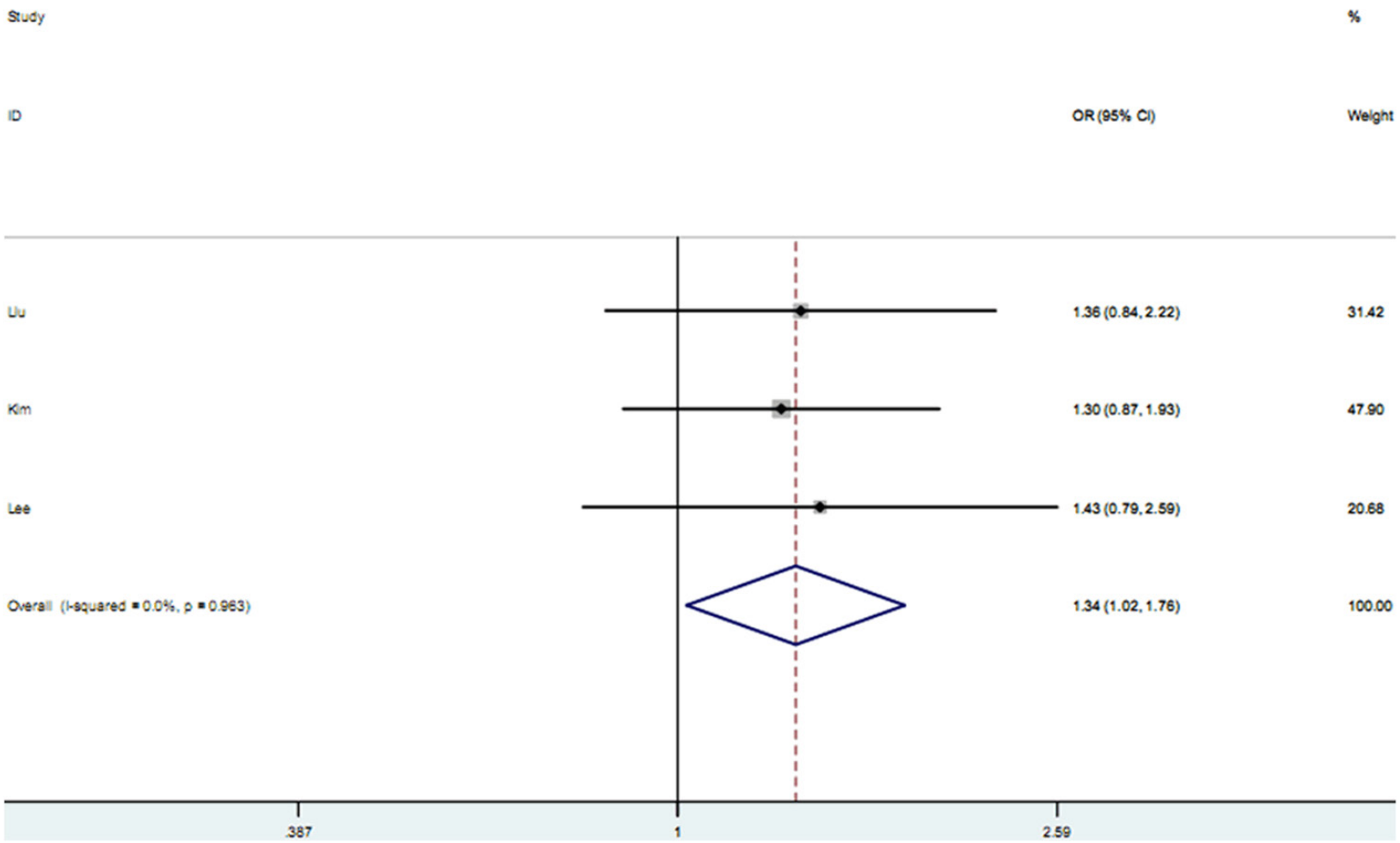

Figure 2. Forest plot of risk of ONFH associated with VEGF -634G/C polymorphism (CC versus GG) by the fixed-effects model.

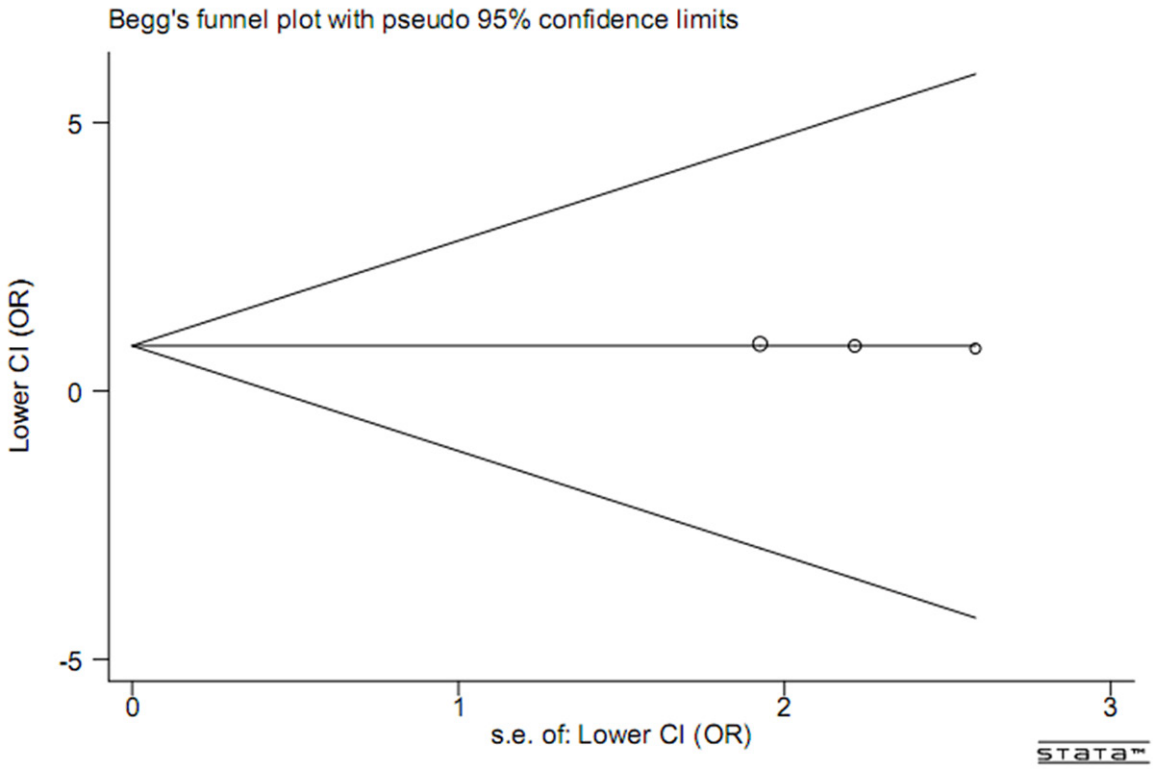

Figure 3. Begg's funnel plot for the publications bias.

Discussion

It has been demonstrated that genes play important roles in the pathophysiological process of ONFH [23-25]. ONFH commonly results from interruption of blood supply to the bone. And the angiogenic factor includes vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), epidermal growth factor (EGF), insulin-like growth factor (IGF-1) and platelet-derived growth factor (PDGF). Among these angiogenic factors, *VEGF* is one of the most potent proangiogenic growth factor, which plays crucial role in the process of blood coagulation [26]. The level of *VEGF* is up-regulated in chondrocytes in ischemic femoral heads [27].

Further studies showed that certain polymorphisms in the promoter and untranslated regions of *VEGF* could alter the expression level of *VEGF*. And -634G/C polymorphism was one of the polymorphism. Vailati FB et al. has reported that the C allele of -634G/C polymorphism is correlated with increased level of *VEGF* [28]. Additionally, several studies have reported that there exists relationship of *VEGF* -634G/C polymorphism with breast cancer, and preeclampsia (PE) [29, 30]. And the association of -634G/C polymorphism with ONFH risk was also investigated.

As for ONFH, Liu B et al. suggested that the CC genotype of *VEGF* -634G/C polymorphism was a risk factor [31]. Kim et al. also concluded that *VEGF* -634G/C polymorphism was related with ONFH susceptibility [32]. Whereas Lee et al. found that there was no association between *VEGF* -634G/C polymorphism and steroid-induced ONFH [7]. Based on published studies, our meta-analysis was aimed to evaluate the relationship of *VEGF* -634G/C polymorphism and ONFH risk in Asians. And a total of 692 cases and 875 controls were included in the analysis. The results showed that *VEGF* -634G/C polymorphism was related with increased risk for ONFH under CC versus GG genetic model.

Genotypes distribution in controls was consistent with HWE, which, to a large extent, enabled the credibility of our results. The sensitivity analysis and publication bias test both showed that our results were credible and stable.

However, there were several limitations in our analysis. Firstly, our meta-analysis only involves Asian population. Secondly, our analysis did not take gene-gene, gene-environment interaction into consideration. Lastly, the sample size was relatively small. Hence, more comprehensive studies needed to provide precise estimation of the relation.

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Disclosure of conflict of interest

None.

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