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Original Article

Association between vascular growth factor gene -2578C>A, +1612G>A polymorphism and the risk of renal cell carcinoma: a meta-analysis

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Abstract: Previous studies have investigated the associations between polymorphisms of vascular growth factor (VEGF) gene and risk of renal cell carcinoma (RCC). However, the results were inconsistent. The present meta-analysis was therefore designed to clarify these controversies. The meta-analysis was performed by searching Pub Med, Web of Science and Embase databases. Odds ratio (OR) and corresponding 95% confidence interval (95% CI) as well as effect size were calculated by a fixed-effect model according to the I^2 value. A total of 6 studies including 1397 cases and 2094 controls for -2578C>A of VEGF and 1184 cases and 1862 controls for +1612G>A of VEGF were combined. The pooled results showing evidence of association between VEGF gene -2578C>A polymorphism and RCC risk (for A/A vs. C/C: OR=1.69, 95% CI=1.37-2.07, P<0.00001; for C/A vs. C/C: OR=1.31, 95% CI=1.12-1.52, P=0.0006; for C/A+A/A vs. C/C: OR=1.39, 95% CI=1.21-1.61, P<0.00001; for A/A vs. C/A+C/C: OR=1.43, 95% CI=1.19-1.73, P=0.0002; for A allele vs. C allele: OR=1.31, 95% CI=1.19-1.45, P<0.00001). However, there was no significant association between VEGF +1612G>A polymorphism and RCC except comparing additive model (for A/A vs. G/G: OR=1.33, 95% CI=1.02,1.74, P=0.03; for G/A vs. G/G: OR=1.09, 95% CI=0.93-1.27, P=0.30; for G/A+A/A vs. G/G: OR=1.12, 95% CI=0.96-1.30, P=0.14; for A/A vs. G/A+G/G: OR=1.27, 95% CI=0.99-1.64, P=0.06; for A allele vs. G allele: OR=1.12, 95% CI=1.00-1.25, P=0.05). In conclusion, our results indicated that VEGF -2578C>A polymorphism, but not VEGF +1612G>A polymorphism was associated with the risk of RCC.

Keywords: Vascular growth factor gene, polymorphism, renal cell carcinoma, meta-analysis

Introduction

Renal cell carcinoma (RCC) is a common urological tumor and it accounts for 3% of all human malignancies and for >80% of all malignant kidney tumors [1, 2]. RCC continues to be a devastating cancer and the worldwide incidence and mortality rates are rising at a rate of 2-3% per decade [3]. Although increased studies are conducted on the etiology of RCC, the real causes of this cancer are not well understood. Previous studies showed that smoking, alcohol consumption, hypertension, obesity, occupational exposures, and family history of cancer are established risk factors that play key roles in the development of RCC [4-8]. Increasing evidences have indicated that the growth of tumors is associated with increased angiogenesis [9].

Vascular endothelial growth factor (VEGF) is one of the key initiators of angiogenesis. Previous experimental studies reported that the growth and metastasis of tumor expression can affected by VEGF, and inhibition of VEGF signaling can control the angiogenesis and growth of tumor cells [10-12]. The VEGF gene has been localized on chromosome 6p21.3 and at least 30 single-nucleotide polymorphisms have been described [13]. The functional polymorphisms, that is, the gene variants that effect the expression and/or the function of the encoded proteins, are most likely to contribute to an individual's susceptibility to a disease. Several previous studies reported that polymorphisms in VEGF play an important role in the development of renal cell carcinoma [14-21]. But the results of association between VEGF gene polymorphism with RCC were controver-

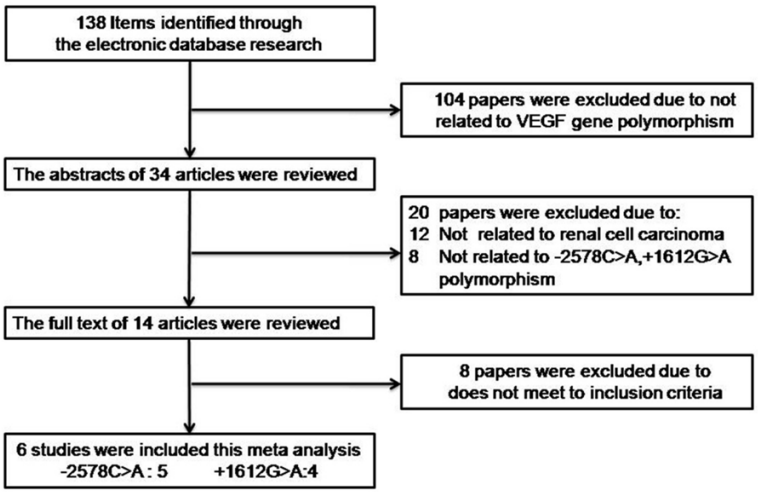

Figure 1. Flow chart of literature search and study selection. Six case-control studies were included in this meta-analysis.

sial. Therefore, we conducted a meta-analysis to draw a more reliable conclusion about association between -2578C>A, +1612G>A polymorphism and risk of RCC.

Materials and methods

Literature search

We searched eligible literatures published up to November 2015 in Pub Med, Web of Science and Embase using the following keywords: ("Vascular growth factor" OR "VEGF") AND ("polymorphism" OR "SNP" OR "mutation" OR "variant") AND ("renal cell cancer" OR "renal cell carcinoma" OR "RCC"). Eligible reports were restricted to English language articles, unpublished articles did not included.

Inclusion and exclusion criteria

To be included in this meta-analysis, studies had to meet the following criteria: (1) association of VEGF gene -2578C>A and +1612G>A polymorphism with renal cell carcinoma; (2) published case-control studies; (3) studies with full text articles; (4) all patients must have met the diagnostic criteria for renal cell cancer; (5) the study must provide total number of cases and controls, and the number for each genotype; (6) odds ratio (OR) with 95% confidence interval (CI), were provided or could be calculated. Articles that did not meet these inclusion criteria and animal studies were excluded. If authors published several studies using the same data, the most recent or largest sample size publication was included.

Data extraction

Data were systematically extracted from each included study by two authors using a standardized form. If these two authors could not reach a consensus, disagreements were discussed and resolved by a third author. The following data were extracted: first author's name, publication year of article, country of the first author, ethnicity, source of controls, sample size and genotype frequencies.

Quality score assessment

Qualities of the included studies assessed using the Newcastle-Ottawa Scale (NOS). Total NOS scores range from 0 to 9 with a score ≥7 indicating good quality.

Statistical analysis

All the above statistical analyses were performed using Review Manager 5.3.3 software and Stata 12.0. Odds ratios (OR) and their corresponding 95% confidence intervals (95% CI) were calculated. The pooled ORs were performed for five genetic models (-2578C>A, +1612G>A): additive model (A/A vs. C/C, A/A vs. G/G), codominant model (C/A vs. C/C, G/A vs. G/G), dominant model (C/A+A/A vs. C/C, G/A+A/A vs. G/G), recessive model (A/A vs. C/A+C/C, A/A vs. G/A+G/G), and allelic model (A allele vs. C allele, A allele vs. G allele). The Z test was used to estimate the statistical significance of pooled ORs. The genotype frequencies of healthy controls were tested for their conformity to Hardy-Weinberg equilibrium (HWE) using the χ^2 test. Heterogeneity was investigated and measured using Cochran's Q statistic [22] and the I^2 statistic [23], $P<0.10$ and $I^2>50\%$ indicated evidence of heterogeneity and the random effects model was used. Otherwise the fixed-effects model was used in the absence of between-study heterogeneity. We also calculated HWE in control group. Sensitivity analysis was performed, by limiting the meta-analysis to the studies conforming to

Table 1. Baseline characteristics and methodological quality of all included studies

First Author	Year	Country	Ethnicity	Source of controls	Sample Size		Genotyping method	NOS
					Case	Control		
B.L. Shen	2015	China	Asian	HB	360	360	PCR-RFLP	8
Sadia Ajaz	2011	Pakistan	Asian	HB	143	106	PCR-RFLP	7
W. Xian	2015	China	Asian	HB	266	532	PCR-RFLP	7
Akihiko Abe	2002	Japan	Asian	HB	145	145	PCR-RFLP	6
Pablo Sáenz-López	2013	Spain	Caucasian	PB	216	280	TaqMan	8
Guangjian Lu	2015	China	Asian	HB	412	814	PCR-RFLP	8

HB: Hospital based; PB: population based; NOS: Newcastle-Ottawa Scale; PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism.

Table 2. Comparison of genotype distributions and allele frequencies between case and control group. For VEGF gene -2578C>A polymorphism

First Author	Year	N	Case					N	Control					HWE
			Genotype (n)			Allele			Genotype (n)			Allele		
			GG	GA	AA	G	A		GG	GA	AA	G	A	
B.L Shen	2015	361	152	170	39	474	248	360	166	164	30	496	224	0.23
W. Xian	2015	266	113	123	30	349	183	532	248	243	41	739	325	0.08
Akihiko Abe	2002	145	113	31	1	257	33	145	109	33	3	251	39	0.788
Guangjian Lu	2015	412	172	191	49	535	289	825	365	375	85	1105	545	0.43

HWE: Hardy-Weinberg equilibrium.

Table 3. Comparison of genotype distributions and allele frequencies between case and control group. For VEGF gene +1612G>A polymorphism

First Author	Year	N	Case					N	Control					HWE
			Genotype (n)			Allele			Genotype (n)			Allele		
			CC	CA	AA	C	A		CC	CA	AA	C	A	
B.L Shen	2015	360	150	149	61	449	271	360	178	141	41	497	223	0.11
Sadia Ajaz	2011	143	30	81	32	141	145	106	44	41	21	129	83	0.053
W. Xian	2015	266	99	119	48	317	215	532	243	225	64	711	353	0.29
Pablo Sáenz-López	2013	216	54	114	48	222	210	272	77	142	53	296	248	0.388
Guangjian Lu	2015	412	171	174	67	516	308	824	397	332	95	1126	522	0.06

HWE: Hardy-Weinberg equilibrium.

HWE and the high quality studies, to evaluate the stability of the results. The Begg's funnel plot and Egger's test were used to assess the publication bias.

Results

Eligible studies

Firstly, we identified 138 items through the electronic databases with the relative keywords and 104 of them were excluded because of not related to the VGEF gene polymorphism. The abstracts of the 34 article were reviewed and 20 papers were excluded due to they were not related to the renal cell carcinoma and -2578C>A, +1612G>A polymorphism of VEGF gene. After that, 14 studies were identified as related to our topics and full texts were reviewed. Furthermore, 8 studies don't meet to our inclusion criteria and excluded. After all, 6 eligible studies (5 were related to -2578C>A polymorphism and 4 were related to +1612G>A polymorphism) were selected to this Meta analysis (**Figure 1**). Among them, three studies from China, one from Pakistan, one from Japan and one from Spain. All studies were case-control in design. Including 1397 cases and 2094 controls for -2578C>A and 1184 cases and 1862

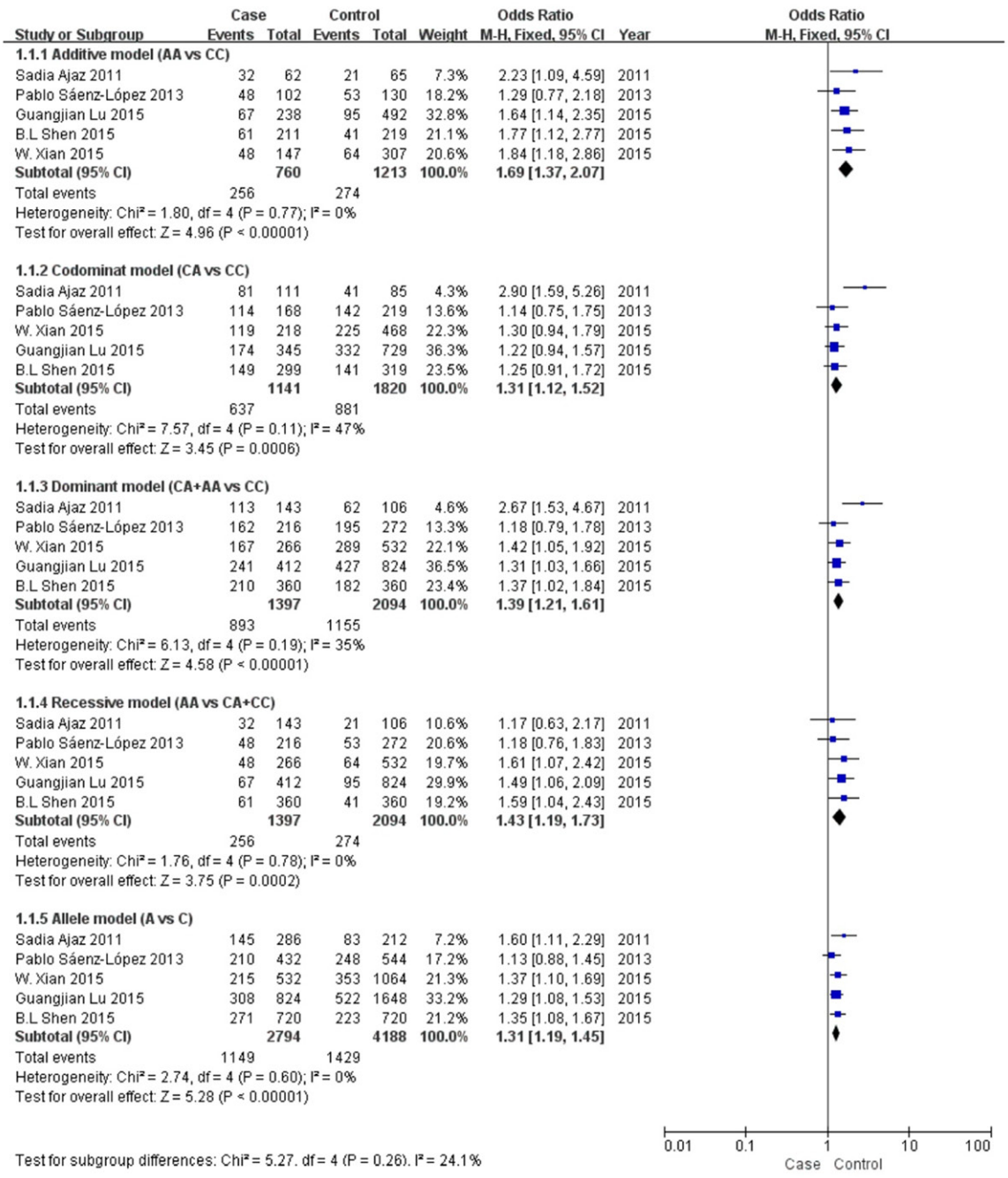

Figure 2. Forest plots for VEGF gene -2578C>A polymorphism and RCC risk in different genetic models.

controls for +1612G>A. In the control group, 1 study was public based and 5 studies were drawn from health check visits or outpatient departments, healthy volunteers and healthy blood donors, classified as a hospital basic controls. Five studies used polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method and one study used TaqMan method. Almost all of the research was high quality (**Table 1**). Genotypes distributions and HWE of included studies is provided in the **Tables 2** and **3**. The genotype distribution in the controls was consistent with HWE.

Quantitative data synthesis

Five studies were included in the meta-analysis of -2578C>A of VEGF gene. A heterogeneity test

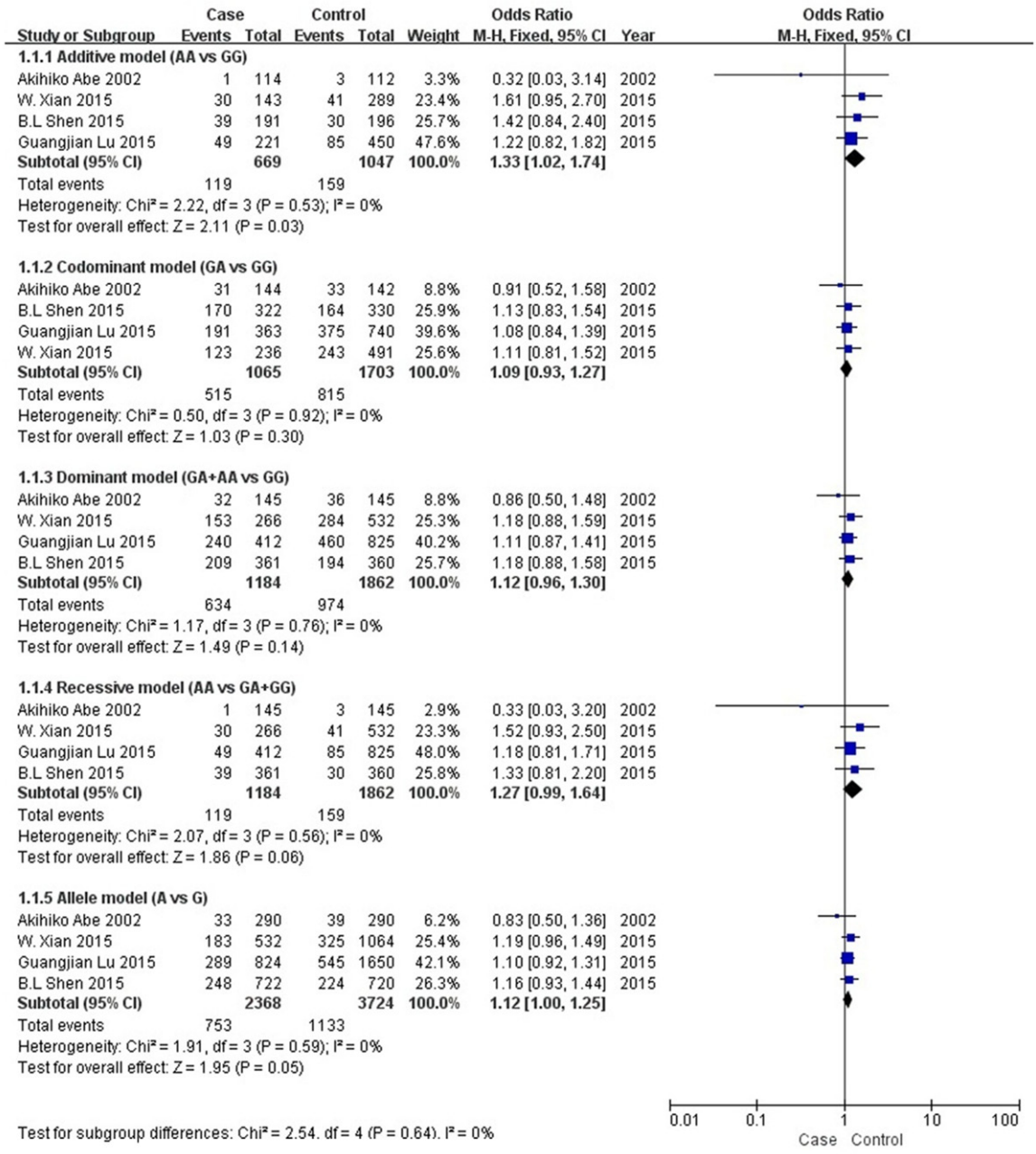

Figure 3. Forest plots for VEGF gene +1612G>A polymorphism and RCC risk in different genetic models.

showed that there were no significant heterogeneity among the studies and fixed-effected model was used. Pooling results shows that -2578C>A polymorphism of the VEGF gene was associated with risk of renal cell carcinoma (**Figure 2**) (for A/A vs. C/C: OR=1.69, 95% CI= 1.37-2.07, P<0.00001; for C/A vs. C/C: OR= 1.31, 95% CI=1.12-1.52, P=0.0006; for C/A+A/A vs. C/C: OR=1.39, 95% CI=1.21-1.61, P< 0.00001; for A/A vs. C/A+C/C: OR=1.43, 95% CI=1.19-1.73, P=0.0002; for A allele vs. C allele: OR=1.31, 95% CI=1.19-1.45, P<0.00001). **Figure 3** shows that there was no relationship between +1612G>A polymorphism of the VEGF with renal cell carcinoma except comparing additive model for A/A vs. G/G: OR=1.33, 95% CI=1.02, 1.74, P=0.03; for G/A vs. G/G: OR= 1.09, 95% CI=0.93-1.27, P=0.30; for G/A+A/A vs. G/G: OR=1.12, 95% CI=0.96-1.30, P=0.14; for A/A vs. G/A+G/G: OR=1.27, 95% CI=0.99-

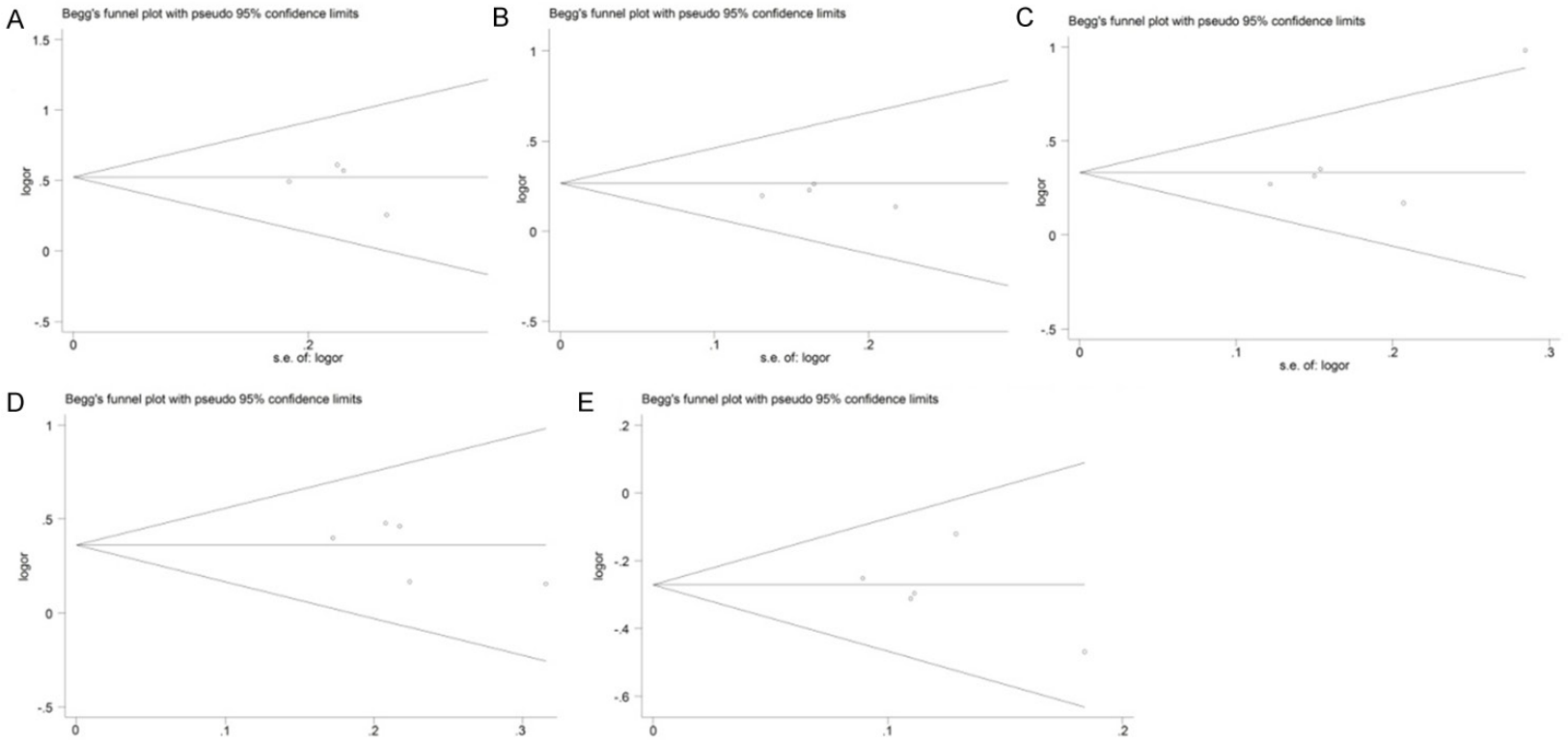

Figure 4. Begg's funnel plot with pseudo 95% confidence limits under different genetic models for VEGF gene -2578C>A polymorphism. A: Additive model (A/A vs. C/C), B: Codominant model (C/A vs. C/C), C: Dominant model (C/A+A/A vs. C/C), D: Recessive model (A/A vs. C/A+C/C), E: Allelic model (A allele vs. C allele). Horizontal axis represents the standard error of log OR. Vertical axis represents the log OR. The s.e. denotes standard error.

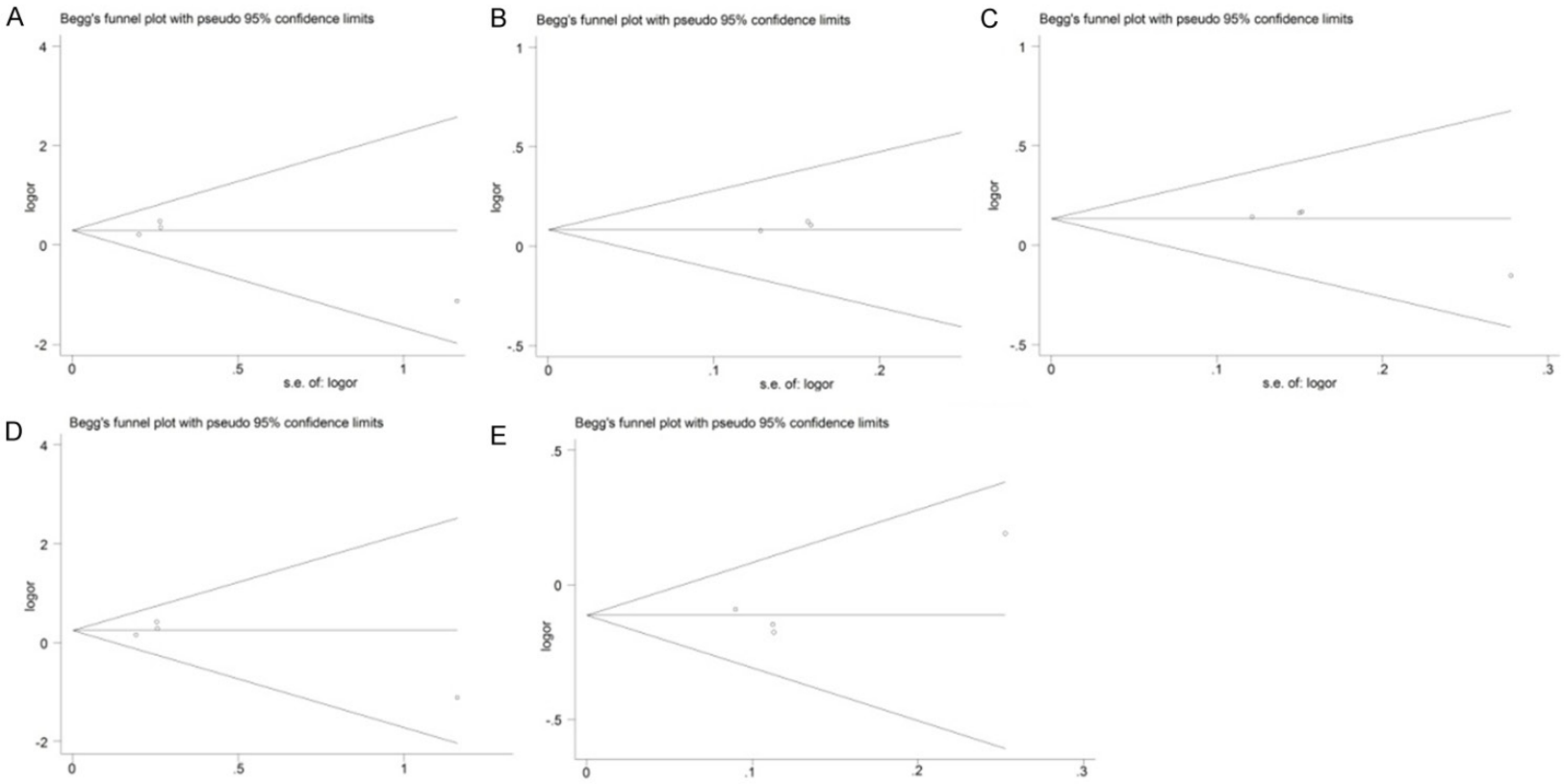

Figure 5. Begg's funnel plot with pseudo 95% confidence limits under different genetic models for VEGF gene +1612G>A polymorphism. A: Additive model (A/A vs. G/G), B: Codominant model (G/A vs. G/G), C: Dominant model (G/A+A/A vs. G/G), D: Recessive model (A/A vs. G/A+G/G), E: Allelic model (A allele vs. G allele). Horizontal axis represents the standard error of log OR. Vertical axis represents the log OR. The s.e. denotes standard error.

1.64, P=0.06; for A allele vs. G allele: OR=1.12, 95% CI=1.00-1.25, P=0.05).

Sensitivity analysis

The sensitivity analyses were performed by limiting studies to those conforming to HWE and those with high NOS score. All of the included studies were consist with HWE and one studies was relatively low NOS score (NOS=6) were excluded for sensitivity analysis. The pooled OR changed quite little, indicating that our results were statistically robust.

Publication bias

The Begg's funnel plot and Egger's test were used to assess the publication bias. As shown in **Figures 4** and **5**, no visual evidence of publication bias was observed by the funnel plots. The Egger's test was not significant for the meta-analyses of -2578C>A (P=0.520) and +1612G>A (P=0.283) of VEGF gene.

Discussion

To clarify the role of VEGF gene -2578C>A, +1612G>A polymorphism in RCC, we have carried out this meta-analysis. Analysis comparisons of all five genetic models were performed, which provided enough information to detect the association. In this study, we found significant associations between -2578C>A polymorphism and RCC. However, +1612G>A polymorphism was not associated with the risk of RCC. There was a meta-analysis about VEGF gene polymorphism and risk of RCC have reported in 2013 [24]. What's different from the previous work, we added 4 latest studies which didn't include in the previous meta-analysis and enlarged sample size to 3779 participants (1542 cases and 2237 controls).

Early studies reported association of angiogenesis with the development of many tumors, and VEGF is one of the key regulator of angiogenesis [25, 26]. As a promoter of endothelial cell proliferation in the blood vessels, functional gene variations of the VEGF gene could influence the gene expression and the plasma VEGF levels, result in acceleration of carcinogenesis [18, 19, 22]. A meta-analyses has reported that VEGF gene polymorphisms are correlated with the risk of various diseases, including cardiovascular disease [27], pre-eclampsia [28], gastric cancer [29] and amyotrophic lateral sclerosis [30].

There were five studies reported the association between-2578C>A polymorphism of VEGF gene with RCC [14-16, 18, 19] and four of them suggested there were a correlation of VEGF gene -2578C>A polymorphism and RCC, the A allele was a risk factor same as our results [14-16, 19]. This discrepant may be caused by the ethnicity of the participants, because of the four studies which found a correlation were about the Asians and another one was studied Caucasian. We also analyzed the association of VEGF gene +1612G>A polymorphism with RCC and four eligible studied included in this study [14-16, 21]. All of these studies revealed the lack of association. The results drawn by our meta-analysis is consisting with the latest meta-analysis reports [24] and indicated that results of both meta-analysis was stable. In this meta-analysis, we have not carried out ethnicity subgroup analysis because of limited number of articles about various ethnicities.

Heterogeneity is an important factor that influence the results, caused by insufficient sample size, diversity in ethnicity, genotyping method and study design. In our study, there is no heterogeneity in overall comparison. Therefore we have applied fixed effect model for pooled analysis. Although, no visual evidence of publication bias was observed by the funnel plots, but selection bias may inevitably exist in this meta-analysis since we have restricted to the English articles, missing some reports in other languages, it was possible that some eligible studies were not included because of the limitations of data bases.

There were some limitations in this meta-analysis. First, the number of relative studies about our topic is comparatively small, more studies are needed. Second, as a retrospective study, meta-analysis involved selection bias. Third, our meta-analyses have not performed adjustment, may cause serious confounding bias. Fourth, insufficient data of individual participant's has restricted the further evaluation of the potential roles of VEGF genetic polymorphisms in the development of RCC.

In conclusion, our meta-analysis suggests that VEGF gene -2578C>A polymorphism is associated with increased risk of renal cell carcinoma

and may be a marker for use in clinical evaluation. However, due to the limitations mentioned above, further larger sample size studies must be conducted to obtain a more representative statistical analysis. There is a greater need in genetics epidemiology to help conclude more conclusive results.

Disclosure of conflict of interest

None.

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