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Vascular endothelial growth factor gene polymorphisms and colorectal cancer risk: a meta-analysis

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ABSTRACT. Vascular endothelial growth factor (VEGF) is an endothelial cell-specific mitogen involved in a number of pathologic processes, including angiogenesis, tumor growth and metastasis. Polymorphisms of the VEGF gene have been associated with susceptibility to colorectal cancer (CRC). However, the specific association still remains controversial. We made a meta-analysis of the association between VEGF gene polymorphisms and CRC risk. Only eight case-control studies were retrieved, with a total of 2337 CRC patients and 2032 healthy controls. Six VEGF gene polymorphisms were addressed in all studies included, +936C>T (rs3025039), -2578C>A (rs699947), -1154G>A (rs1570360), -634G>C (rs2010963), -460C>T (rs833061), and +405C>G (rs2010963). There was a significant association between -2578C>A polymorphism and susceptibility to CRC in the comparison of C allele carriers (CC + CA) versus AA (odds ratio = 0.77, 95% confidence interval = 0.62-0.96, P = 0.02). No association was found between +936C>T, -1154G>A, -634G>C, -460C>T, and +405C>G with susceptibility to CRC. We conclude that the C allele carrier (CC + CA) of VEGF -2578C>A polymorphism appears to be a protective factor for CRC.

Key words: Colorectal cancer; Polymorphism; VEGF; Meta-analysis

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INTRODUCTION

Every year, more than 945,000 people develop colorectal cancer (CRC) worldwide, and approximately 492,000 patients die (Weitz et al., 2005). CRC is the second most common cancer in developed countries, with a lifetime risk of 5% and about 1 million new cases each year (Rothwell et al., 2010). CRC has predominantly been considered a genetic disease, characterized by sequential accumulation of genetic alterations (van Engeland et al., 2011). Growing evidence indicates that epigenetic alterations add an additional layer of complexity to the pathogenesis of CRC (Venkatachalam et al., 2010). Epigenetic dysregulation in CRC is organized at multiple levels, involving DNA methylation, histone modifications, nucleosomal occupancy and remodeling, chromatin looping, and noncoding RNAs (Taby and Issa, 2010; Venkatachalam et al., 2010; Duthie 2011). Interactions between these processes and complex associations with genetic alterations have recently been unraveled (van Engeland et al., 2011).

Angiogenesis, the formation of new capillaries from existing blood vessels, is essential for the growth and metastasis of a solid tumor (Folkman and Shing, 1992). It is generally assumed that microvessel formation around a tumor is stimulated by various angiogenic factors secreted by the tumor cells (Takahashi et al., 1995). Among them, vascular endothelial growth factor (VEGF) is considered one of the strongest promoters of tumor angiogenesis (Hanahan and Folkman, 1996). VEGF is an endothelial cell-specific mitogen involved in a number of pathologic processes, including angiogenesis, tumor growth and metastasis (Ferrara, 1999; Schott and Morrow, 1993). Numerous studies have shown that growing tumors require the establishment of a blood supply, and VEGF is often up-regulated in cancer (Carmeliet and Jain, 2000; Ferrara, 2000). VEGF plays an essential role in the development and differentiation of the cardiovascular system. Markers in the VEGF gene have been associated with increased risk of developing cancer, and recent studies have also demonstrated that the expression of the VEGF had a prognostic significance in patients with cancer (Gasparini et al., 1997). Several studies have also suggested a strong correlation between VEGF expression and both poor prognosis and metastasis in CRC (Des Guetz et al., 2006). Increased VEGF expression in CRC may predict the risk of multiple liver metastases and play a role in the spread of CRC cells to the lymph nodes (Tanigawa et al., 1997; Kuramochi et al., 2006; Saad et al., 2006).

The VEGF gene is located on chromosome 6p12 and includes a 14-kb coding region with eight exons and seven introns (Vincenti et al., 1996). At least 30 single nucleotide polymorphisms (SNP) in VEGF gene have been described in the literature (Brogan et al., 1999; Renner et al., 2000; Watson et al., 2000). Polymorphisms of VEGF gene have been associated with susceptibility to several types of cancer. Some of these polymorphisms (+936C>T rs3025039, -2578C>A rs699947, -1154G>A rs1570360, -634G>C rs2010963, -460C>T rs833061, +405C>G rs2010963) have been related to protein expression of VEGF in CRC. Despite numerous studies that have evaluated the association between VEGF gene polymorphisms and susceptibility to CRC, the specific association still remains controversial. Since VEGF is significant in the angiogenesis of CRC, it is reasonable to hypothesize that VEGF gene polymorphisms are good candidates for predicting the risk of developing CRC. The aim of this meta-analysis study was to investigate the association between VEGF gene polymorphisms and its susceptibility to CRC by conducting a meta-analysis from all eligible case-control studies published to date.

MATERIAL AND METHODS

Literature search strategy

We performed an electronic search of the PubMed Embase and CBM to retrieve papers linking VEGF gene polymorphisms and susceptibility to CRC available until January 2011 without language restrictions, using the following query: ["VEGFs" or "VEGF" or "Vascular Endothelial Growth Factors"] and ["Polymorphism, Single Nucleotide" or "SNPs" or "Polymorphism, Genetic"] and ["Colorectal Cancer" or "Colorectal Tumors" or "Colorectal Neoplasms"]. The reference lists of major textbooks, reviews, and included articles were identified through manual searches to find other potentially eligible studies. Studies reported by the same authors, although published in different journals, were checked for possible overlapping participant groups. When pertinent data were not included, or data that were presented were unclear, the authors were contacted directly.

Inclusion and exclusion criteria

To be eligible for inclusion in this meta-analysis, the following criteria were established: i) case-control studies that addressed CRC cases and healthy controls; ii) studies that evaluated the association between VEGF gene polymorphisms and CRC risk; iii) studies that included sufficient genotype data for extraction. Studies were excluded when: i) not case-control studies that evaluated the association between VEGF gene polymorphisms and CRC risk; ii) case reports, letters, reviews, meta-analysis and editorial articles; iii) studies that were based on incomplete raw data and those with no usable data reported; iv) duplicate data were contained in the studies; v) family-based design was used.

Data extraction

Using a standardized form, data from published studies were extracted independently by two reviewers (Dong XH and Jin GJ) to acquire the necessary information. From each of the included articles the following information was retrieved: first author, year of publication, country, language, ethnicity, study design, diagnostic criteria, source of cases and controls, number of cases and controls, male/female ratio, mean age, sample, detection methods, polymorphisms, genotypes frequency and evidence of Hardy-Weinberg equilibrium (HWE) in controls. For conflicting evaluations, an agreement was reached following a discussion.

Quality assessment of the studies included

The quality of papers was also independently assessed by two reviewers (Dong XH and Jin GJ) based on the STROBE quality score systems (Vandenbroucke et al., 2007). Thirty items relevant to the quality appraisal were used for assessment in this meta-analysis. Quality scores ranged from 0 to 30. We defined 10, 20 and 30 scores as low, moderate and high grade respectively. Any discrepancies between the two reviewers were resolved by discussion and consultation with a third reviewer (Shang H).

Statistical analysis

Meta-analysis was performed using the RevMan 5.0.25 (provided by The Cochrane Collaboration) and STATA package version 9.2 (Stata Corporation, College Station, Texas). The strength of the associations between VEGF gene polymorphisms and susceptibility to CRC were estimated by odds ratio (OR) and 95% confidence interval (95%CI). Between-study heterogeneities were estimated using Cochran's *Q* test (Higgins and Thompson, 2002; Zintzaras and Ioannidis, 2005). We also quantified the effect of heterogeneity by I^2 test. I^2 ranges between 0 and 100% and represents the proportion of inter-study variability that can be attributed to heterogeneity rather than chance. I^2 values of 25, 50 and 75% were defined as low, moderate and high estimates, respectively. When a significant Q test ($P < 0.10$ or $I^2 > 50\%$) indicated heterogeneity across studies, the random effects model was used for meta-analysis, or else the fixed effects model was used (Viechtbauer, 2007). Before the effect estimation of associations between VEGF gene polymorphisms and susceptibility to CRC, we tested whether genotype frequencies of controls were in HWE using the χ^2 test. Subgroup analysis based on ethnicity was used to explore and to explain the diversity among the results of different studies. Sensitivity analysis was mainly performed by sequential omission of individual studies or non-HWE studies. Publication bias was investigated by Begg's funnel plot, and funnel plot asymmetry was assessed by Egger's linear regression test (Peters et al., 2006), statistical significance was considered when the P value of Egger's test was ≤0.10. All the P values were two-sided. To ensure the reliability and the accuracy of the results, two reviewers (L.P. Zhou and H. Luan) entered the data in the statistical software programs independently and obtained the same results.

RESULTS

Characteristics of the studies included

The search strategy retrieved 29 potentially relevant studies. Base onthe inclusion criteria, only 8 case-control studies (Wu et al., 2006; Park et al., 2007; Bae et al., 2008; Cacev et al., 2008; Chae et al., 2008; Hofmann et al., 2008; Dassoulas et al., 2009; Maltese et al., 2009) with full-text were included in this meta-analysis and 21 studies were excluded. The flow chart of study selection is summarized in Figure 1. These 8 case-control studies included a total of 2,337 CRC cases and 2,032 healthy controls. All included studies were case-control studies which evaluated the association between VEGF gene polymorphisms and susceptibility to CRC. The published year of the included studies ranged from 2006 to 2009. All included articles were written in English except one (Wu et al., 2006) in Chinese. The source of controls was based on a healthy population. Diverse genotyping methods mainly used polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). Six VEGF gene polymorphisms were addressed in all included studies, including +936C>T, -2578C>A, -1154G>A, -634G>C, -460C>T and +405C>G. HWE test was performed on all included studies, all of them showed in HWE ($P > 0.05$) except one by Dassoulas et al. (2009). The baseline characteristics and methodological quality of all included studies are summarized in Table 1. The genotype distribution and frequency of are summarized in Table 2.

Table 1. Baseline characteristics of included studies in meta-analysis.

First author (year) [Ref]	Country	Ethnicity	Tumor types	VEGF gene polymorphisms	Number of subjects		Sex (male/female)		Age (mean ± SD)		Detection method	Quality scores
					Cases	Controls	Cases	Controls	Cases	Controls		
Wu et al., 2006	German	German	CRC	+936C>T	157	117	100/57	74/43	-	-	PCR-RFLP	37
Park et al., 2007	Korea	Korean	CC	-2578C>A	246	203	128/118	71/132	59.3 ± 13.3	46.6 ± 16.5	PCR-RFLP	45
Bae et al., 2008	Korea	Korean	CC	+936C>T	262	229	136/126	112/117	60.2 ± 13.2	59.6 ± 11.8	PCR-RFLP	43
Cacev et al., 2008	Croatia	Croatian	CC	-1154G>A, -460C>T	160	160	92/86	86/74	60.1 ± 15.3	64.7 ± 10.9	RT-PCR	39
Chae et al., 2008	Korea	Korean	CRC	+936C>T, -634G>C	465	413	241/224	333/80	-	-	PCR-DHPLC	41
Hofmann et al., 2008	Austria	Austrian	CRC	+936C>T, -2578C>A, -634G>C	433	433	175/258	175/258	61.1 ± 12.1	61.0 ± 10.9	-	42
Dassoulas et al., 2009	Greece	Greek	CRC	+936C>T, -2578C>A, -634G>C,								
-1154G>A, -460C>T	312	362	-	-	-	-	PCR	36				
Maltese et al., 2009	Italy	Italian	CRC	-2578C>A, -460C>T, +405C>G	302	115	177/125	54/61	-	-	PCR-RFLP	40

VEGF = vascular endothelial growth factor; CRC = colorectal cancer; CC = colon cancer; PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism; RT = real time; DHPLC = denaturing high-performance liquid chromatography.

Table 2. The genotype distribution and frequency of all included studies.

First author (year)	Cases					Controls					HWE test of controls	
+936C>T polymorphism	Total	CC	CT	TT	T frequency	Total	CC	CT	TT	T frequency	χ^2	P value
Wu et al., 2006	157	123	31	3	0.118	117	88	28	1	0.128	0.583	0.445
Bae et al., 2008	262	170	83	9	0.193	229	169	57	3	0.138	0.551	0.458
Chae et al., 2008	465	293	156	16	0.202	413	252	149	12	0.209	3.304	0.069
Hofmann et al., 2008	427	331	88	8	0.122	427	308	108	11	0.152	0.172	0.679
Dassoulas et al., 2009	312	135	103	74	0.402	362	185	98	79	0.354	60.197	<0.001
-2578C>A polymorphism	Total	CC	CA	AA	A frequency	Total	CC	CA	AA	A frequency	χ^2	P value
Park et al., 2007	246	149	83	14	0.226	203	106	82	15	0.276	0.025	0.875
Hofmann et al., 2008	433	80	225	128	0.555	427	85	238	104	0.522	2.842	0.092
Dassoulas et al., 2009	312	151	116	45	0.330	362	199	121	42	0.283	11.292	0.001
Maltese et al., 2009	302	97	150	55	0.430	115	43	60	12	0.365	1.804	0.179
-1154G>A polymorphism	Total	GG	GA	AA	A frequency	Total	GG	GA	AA	A frequency	χ^2	P value
Cacev et al., 2008	152	60	73	19	0.365	156	52	81	23	0.407	0.892	0.345
Dassoulas et al., 2009	312	126	138	48	0.375	362	152	156	54	0.365	1.772	0.183
-634G>C polymorphism	Total	GG	GC	CC	C frequency	Total	GG	GC	CC	C frequency	χ^2	P value
Chae et al., 2008	465	166	193	106	0.435	413	106	223	84	0.473	2.844	0.092
Hofmann et al., 2008	432	193	192	47	0.331	430	192	195	43	0.327	0.406	0.524
Dassoulas et al., 2009	312	128	125	59	0.389	362	145	141	76	0.405	13.293	<0.001
-460C>T polymorphism	Total	CC	CT	TT	T frequency	Total	CC	CT	TT	T frequency	χ^2	P value
Cacev et al., 2008	155	40	84	31	0.471	160	45	83	32	0.459	0.315	0.574
Dassoulas et al., 2009	312	47	104	161	0.683	362	42	121	199	0.717	11.292	0.001
Maltese et al., 2009	299	76	153	70	0.490	111	10	54	47	0.667	0.993	0.319
+405C>G polymorphism	Total	CC	CG	GG	G frequency	Total	CC	CG	GG	G frequency	χ^2	P value
Maltese et al., 2009	301	48	135	118	0.616	91	15	46	30	0.582	0.140	0.708

HWE = Hardy-Weinberg equilibrium.

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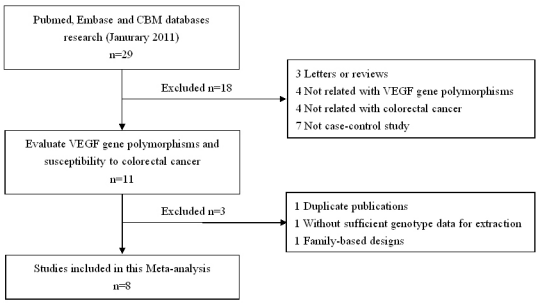

Figure 1. Flow-chart showing study selection procedure.

Association between +936C>T polymorphism and susceptibility to CRC

There were five included studies that reported the association between VEGF +936C>T polymorphism and susceptibility to CRC (Figure 2). Meta-analysis results identified no significant association between VEGF +936C>T polymorphism and susceptibility to CRC in the comparisons of C allele versus T allele (OR = 0.95, 95%CI = 0.76-1.20, P = 0.68), C allele carrier (CC + CT) versus TT (OR = 0.87, 95%CI = 0.65-1.17, P = 0.36), and T allele carrier (CT + TT) versus CC (OR = 1.04, 95%CI = 0.79-1.38, P = 0.76).

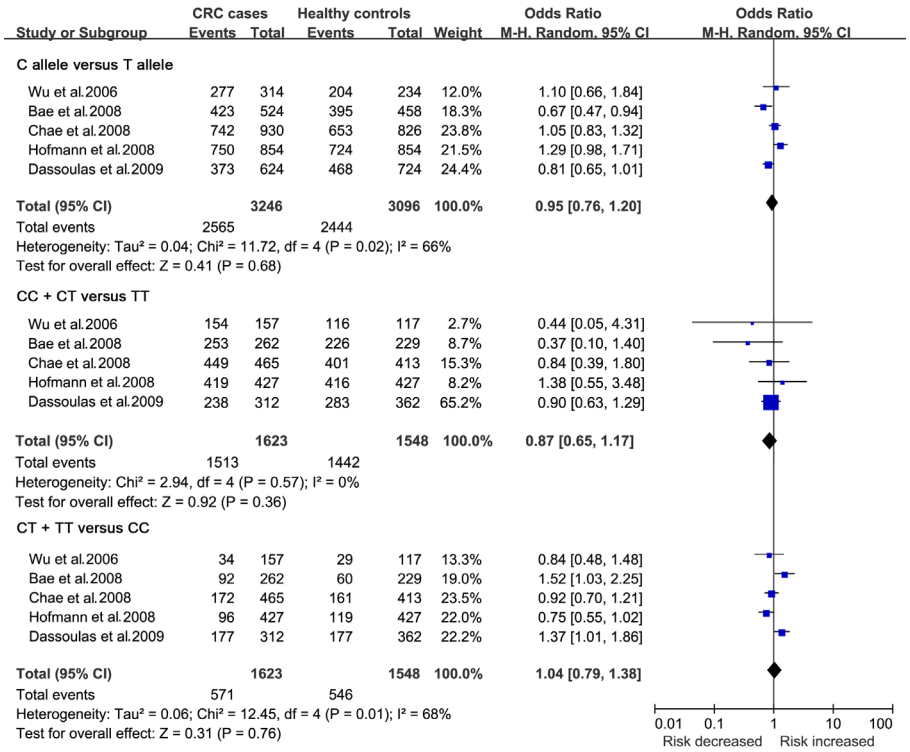

Figure 2. Forest plot showed the association between +936C>T polymorphism and CRC risk.

Association between -2578C>A polymorphism and susceptibility to CRC

Four included studies reported the association between -2578C>A polymorphism and susceptibility to CRC (Figure 3). A significant association was found between -2578C>A polymorphism and susceptibility to CRC in the comparison of C allele carrier (CC + CA) versus AA (OR = 0.77, 95%CI = 0.62-0.96, P = 0.02). However, no association was found in the comparisons of C allele versus A allele (OR = 0.91, 95%CI = 0.74-1.11, P = 0.34) and A allele carrier (CA + AA) versus CC (OR = 1.07, 95%CI = 0.82-1.40, P = 0.63). In addition, we have also used the Fisher's exact test for re-analysis of the association between -2578C>A polymorphism and susceptibility to CRC. The results showed that C allele versus A allele (P = 0.062); CC + CA versus AA (P = 0.051); CA + AA versus CC (P = 0.273).

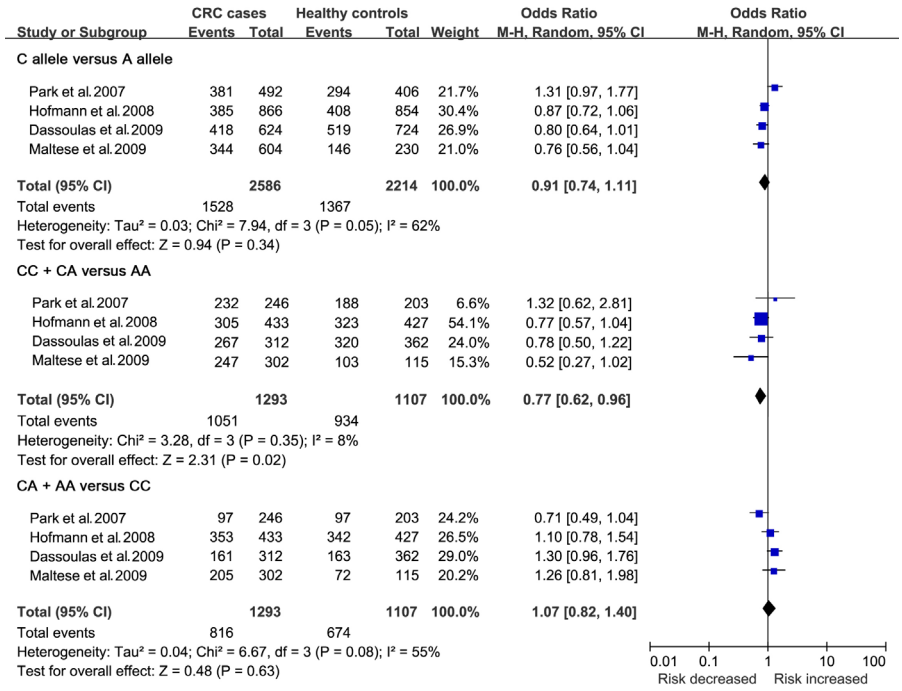

Figure 3. Forest plot showed the association between -2578C>A polymorphism and CRC risk Fisher's-exact test suggested that C allele versus A allele (P = 0.062); CC + CA versus AA (P = 0.051); CA + AA versus CC (P = 0.273).

Association between -1154G>A polymorphism and susceptibility to CRC

There were only two included studies that reported the association of -1154G>A polymorphism and susceptibility to CRC (Figure 4). Meta-analysis results showed no association between -1154G>A polymorphism and susceptibility to CRC in the comparisons of G allele versus A allele (OR = 1.03, 95%CI = 0.85-1.23, P = 0.78), G allele carrier (GG + GA) versus AA (OR = 1.03, 95%CI = 0.72-1.47, P = 0.86), and A allele carrier (GA + AA) versus GG (OR = 0.97, 95%CI = 0.75-1.25, P = 0.79).

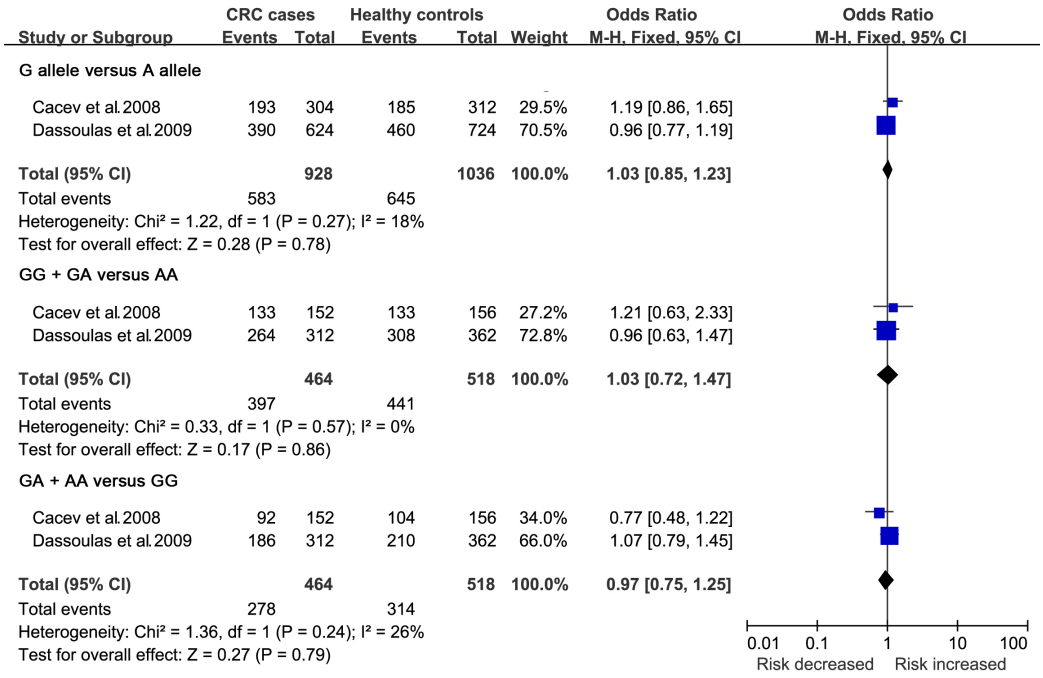

Figure 4. Forest plot showing the association between -1154G>A polymorphism and CRC risk.

Association between -634G>C polymorphism and susceptibility to CRC

Only three included studies reported the association between -634G>C polymorphism and susceptibility to CRC (Figure 5). No association was also found between -634G>C polymorphism and susceptibility to CRC in the comparisons of G allele versus C allele (OR = 1.07, 95%CI = 0.95-1.20, P = 0.24), G allele carrier (GG + GC) versus CC (OR = 0.96, 95%CI = 0.77-1.18, P = 0.68), C allele carrier (GC + CC) versus GG (OR = 0.85, 95%CI = 0.72-1.00, P = 0.05).

Association between -460C>T polymorphism and susceptibility to CRC

There were also only three included studies that investigated the association between -460C>T polymorphism and susceptibility to CRC (Figure 6). Unfortunately, we also found no association between -460C>T polymorphism and susceptibility to CRC in the comparisons of C allele versus T allele (OR = 1.32, 95%CI = 0.87-2.01, P = 0.19), C allele carrier (CC + CT) versus TT (OR = 1.40, 95%CI = 0.84-2.33, P = 0.19), and C allele carrier (CT + TT) versus CC (OR = 0.65, 95%CI = 0.33-1.29, P = 0.22).

Association between +405C>G polymorphism and susceptibility to CRC

There was only one study by Maltese et al. (2009) who investigated the association of between +405C>G polymorphism and susceptibility to CRC (Figure 7). Similarly, we found

no association between +405C>G polymorphism and susceptibility to CRC in the comparisons of C allele versus G allele (OR = 0.87, 95%CI = 0.62-1.22, P = 0.41), C allele carrier (CC + CG) versus GG (OR = 0.76, 95%CI = 0.47-1.25, P = 0.28), and G allele carrier (CG + GG) versus CC (OR = 1.04, 95%CI = 0.55-1.96, P = 0.90).

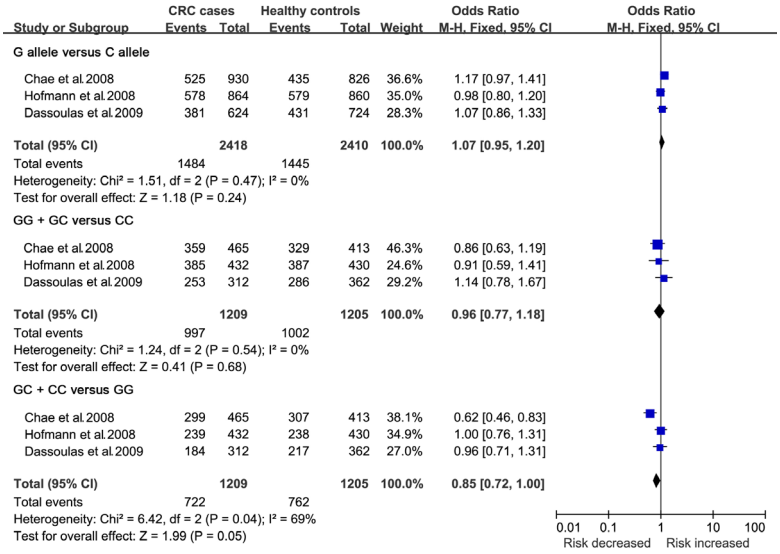

Figure 5. Forest plot showing the association between -634G>C polymorphism and CRC risk.

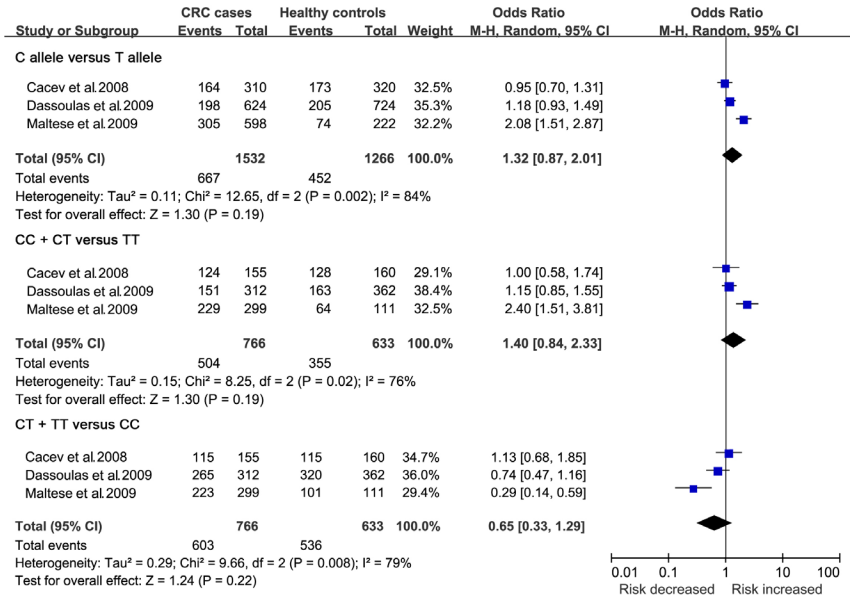

Figure 6. Forest plot showing the association between -460C>T polymorphism and CRC risk.

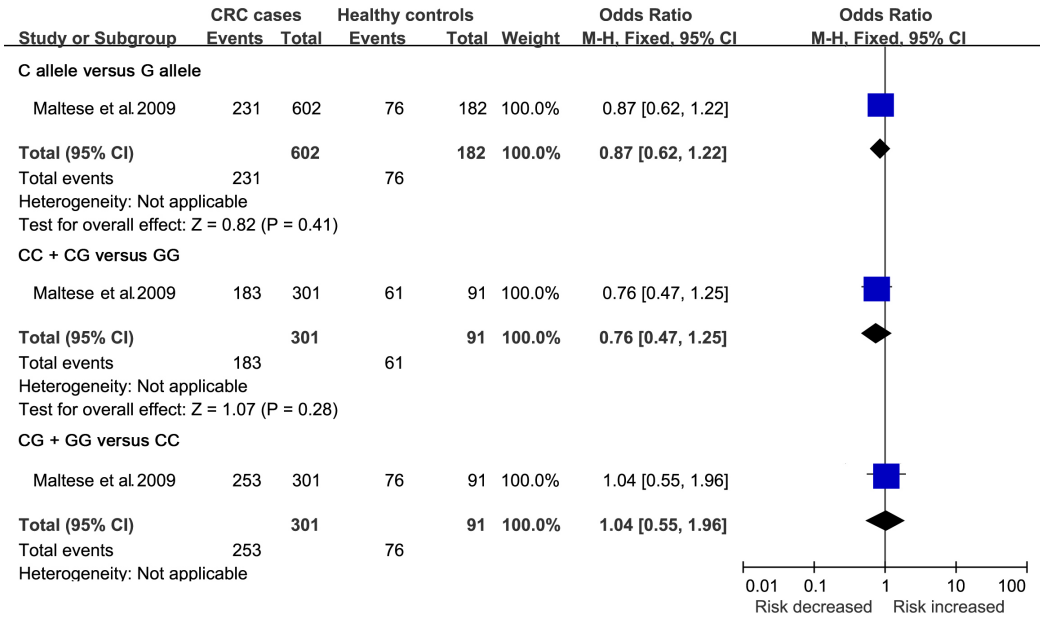

Figure 7. Forest plot showing the association between +405C>G polymorphism and CRC risk.

Subgroup analysis and sensitivity analysis

A summary of subgroup analysis of the associations between VEGF gene polymorphisms and susceptibility to CRC is provided in Table 3. In the subgroup analysis based on ethnicity, included studies were divided into Caucasian and Asian populations. Subgroup analysis results showed that the C allele and C allele carrier (CC + CA) of -2578C>A polymorphism might be protective factors for CRC in Caucasian populations (OR = 0.83, 95%CI = 0.73-0.95, P = 0.006; OR = 0.73, 95%CI = 0.58-0.92, P = 0.008; respectively). In addition, the G allele carrier (GG + GC) of -634G>C polymorphism might also be a protective factor for CRC in Asian populations (OR = 0.62, 95%CI = 0.46-0.83, P = 0.001).

Sensitivity analysis was performed by sequential omission of individual studies. The significance of pooled OR in all individuals analysis and subgroup analysis was not influenced excessively by omitting any single study. Furthermore, we also performed a sensitivity analysis by omission of one non-HWE study (Dassoulas et al., 2009). There was also no obvious influence on all individuals' analysis and subgroup analysis.

Publication bias

Publication bias in the literature was accessed by Begg's funnel plot and Egger's linear regression test. Egger's linear regression test was used to measure the asymmetry of the funnel plot. Due to the limitation in the number of included studies, the publication bias was detected on +936C>T and -2578C>A polymorphisms (Table 4, Figure 8). Results showed that there was no publication bias (all P > 0.05).

Table 3. Subgroup analysis based on ethnicity.

Comparisons	OR	95%CI	P value	Heterogeneity		Effects model
				I^2	P value	
+936C>T						
C allele versus T allele	0.95	[0.76-1.20]	0.68	66%	0.02	Random
Caucasian	1.03	[0.74-1.44]	0.85	71%	0.03	
Asian	0.85	[0.55-1.32]	0.47	78%	0.03	
CC + CT versus TT	0.87	[0.65-1.17]	0.36	0%	0.57	Fixed
Caucasian	0.93	[0.67-1.30]	0.69	0%	0.56	
Asian	0.67	[0.35-1.28]	0.23	9%	0.30	
CT + TT versus CC	1.04	[0.79-1.38]	0.76	68%	0.01	Random
Caucasian	0.97	[0.63-1.48]	0.88	74%	0.02	
Asian	1.16	[0.71-1.90]	0.56	77%	0.04	
-2578C>A						
C allele versus A allele	0.91	[0.74-1.11]	0.34	62%	0.05	Random
Caucasian	0.83	[0.73-0.95]	0.006	0%	0.71	
Asian	1.31	[0.97-1.77]	0.08	-	-	
CC + CA versus AA	0.77	[0.62-0.96]	0.02	8%	0.35	Fixed
Caucasian	0.73	[0.58-0.92]	0.008	0%	0.56	
Asian	1.32	[0.62-2.81]	0.47	-	-	
CA + AA versus CC	1.07	[0.82-1.40]	0.63	55%	0.08	Random
Caucasian	1.22	[0.99-1.49]	0.06	0%	0.75	
Asian	0.71	[0.49-1.04]	0.08	-	-	
-1154G>A*						
G allele versus A allele	1.03	[0.85-1.23]	0.78	18%	0.27	Fixed
GG + GA versus AA	1.03	[0.72-1.47]	0.86	0%	0.57	
GA + AA versus GG	0.97	[0.75-1.25]	0.79	26%	0.24	
-634G>C						
G allele versus C allele	1.07	[0.95-1.20]	0.24	0%	0.47	Fixed
Caucasian	1.02	[0.88-1.18]	0.80	0%	0.58	
Asian	1.17	[0.97-1.41]	0.11	-	-	
GG + GC versus CC	0.96	[0.77-1.18]	0.68	0%	0.54	Fixed
Caucasian	1.03	[0.78-1.38]	0.82	0%	0.45	
Asian	0.86	[0.63-1.19]	0.38	-	-	
GC + CC versus GG	0.85	[0.72-1.00]	0.05	69%	0.04	Random
Caucasian	0.98	[0.80-1.20]	0.86	0%	0.85	
Asian	0.62	[0.46-0.83]	0.001	-	-	
-460C>T*						
C allele versus T allele	1.32	[0.87-2.01]	0.19	84%	0.002	Random
CC + CT versus TT	1.40	[0.84-2.33]	0.19	76%	0.02	
CT + TT versus CC	0.65	[0.33-1.29]	0.22	79%	0.008	
+405C>G*						
C allele versus G allele	0.87	[0.62-1.22]	0.41	-	-	Fixed
CC + CG versus GG	0.76	[0.47-1.25]	0.28	-	-	
CG + GG versus CC	1.04	[0.55-1.96]	0.90	-	-	

OR = odds ratio; 95%CI = 95% confidence interval; * = only included Caucasian populations.

Table 4. Evaluation of publication bias by Egger's linear regression test.

Polymorphisms	Coefficient	SE	*t*	P > |t|	95%CI
+936C>T					
C allele versus T allele	0.20	3.35	0.06	0.96	[-10.45, 10.85]
CC + CT versus TT	-0.63	0.74	-0.84	0.46	[-3.00, 1.74]
CT + TT versus CC	0.58	4.05	0.14	0.90	[-12.32, 13.48]
-2578C>A					
C allele versus A allele	2.17	4.59	0.47	0.68	[-17.60, 21.94]
CC + CA versus AA	0.44	1.81	0.24	0.83	[-7.33, 8.21]
CA + AA versus CC	-2.43	6.35	-0.38	0.74	[-29.76, 24.91]

SE = standard error; 95%CI = 95% confidence interval.

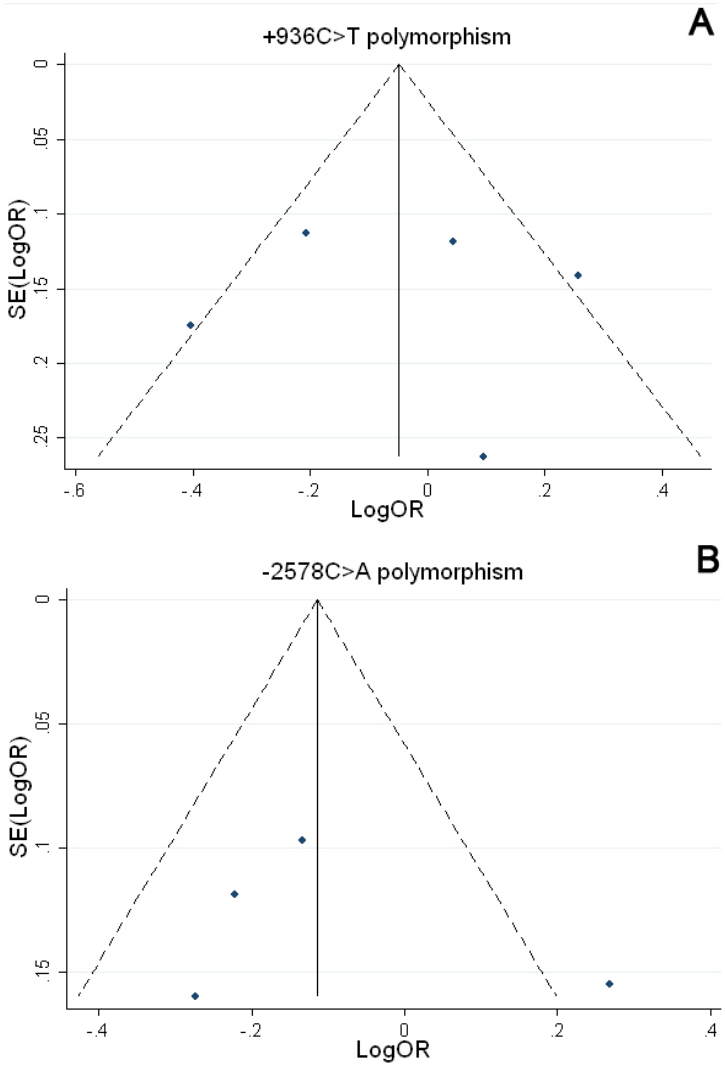

Figure 8. Funnel plot of publication bias for the association between VEGF gene polymorphisms and susceptibility to CRC (A: +936C>T polymorphism; B: -2578C>A polymorphism).

DISCUSSION

There is growing evidence that genetic variation plays an important role in the determination of individual susceptibility to complex disease traits (Knight, 2005). Functional polymorphisms, which affect the regulation of gene expression, can contribute to differences between individuals in susceptibility to various cancers (Ponder, 2001). The effect may be seen with one polymorphism alone or in combination with other polymorphisms (Clapper, 2000). Several studies have shown that polymorphisms in the promoter as well as in the 5'-

and 3'-untranslated regions of the VEGF gene are associated with the production of the VEGF protein in colorectal carcinogenesis (Watson et al., 2000; Bae et al., 2008). VEGF expression was associated with both poor prognosis and metastasis in CRC. In a large meta-analysis, including 27 studies (Des Gustz et al., 2006), demonstrated that VEGF over-expression is significantly correlated with poor overall survival and with an increased risk of relapse. Recently, a number of molecular epidemiological studies have been conducted to examine the association between VEGF gene polymorphisms and CRC susceptibility (Cao et al., 2010; Liu et al., 2010). However, the possible influence of VEGF gene polymorphisms on VEGF production as well as tumor development and progression in CRC still remains controversial. Therefore, the aim of this study was to investigate the influence of VEGF gene polymorphisms on susceptibility to CRC by means of meta-analysis.

Our meta-analysis quantitatively assessed the association between VEGF gene polymorphisms and susceptibility to CRC. Finally, only 8 case-control studies were included and comprised of a total of 2,337 CRC cases and 2,032 healthy controls. In this meta-analysis, six VEGF gene polymorphisms were addressed and evaluated in colorectal carcinogenesis, including +936C>T, -2578C>A, -1154G>A, -634G>C, -460C>T and +405C>G. Meta-analysis results showed that the C allele carrier (CC + CA) of -2578C>A polymorphism might be a protective factor for CRC. However, we found no association between +936C>T, -1154G>A, -634G>C, -460C>T and +405C>G with susceptibility to CRC. In addition, we performed a subgroup analysis based on ethnicity. Interestingly, subgroup analysis results showed that the C allele and C allele carrier (CC + CA) of -2578C>A polymorphism might be might be protective factors for CRC in Caucasian populations, while the G allele carrier (GG + GC) of -634G>C polymorphism might be a protective factor for CRC in Asian populations, suggesting a possible role of ethnic differences in genetic backgrounds and the environment they lived in. Although a significant association was found between -634G>C polymorphism and susceptibility to CRC, only one eligible study number was included, however this result still requiresfurther investigation. Unfortunately, there was also no association between +936C>T, -1154G>A, -460C>T and +405C>G with susceptibility to CRC in further subgroup analysis. Between-study heterogeneity was found in meta-analysis of VEGF +936C>T, -2578C>A, -634G>C and -460C>T polymorphisms. Therefore, the random effects model was used to minimize potential bias. No evidence showed publication bias in this meta-analysis for the association between VEGF gene polymorphisms and susceptibility to CRC.

In addition, VEGF gene polymorphisms may also be associated with many clinicopathologic features of CRC. Chae et al. demonstrated that the TT genotype of 936C>T polymorphism was significantly associated with advanced stage, distant metastasis, high serum level of CA19-9 and higher grade in CRC patients (Chae et al., 2008). Park et al. (2007) found that the AA genotype and A allele carrier (CA + AA) of -2578C>A polymorphism might be protective factors for Korean women with proximal CRC. Moreover, Bae et al. (2008), confirmed that the CT genotype and T allele carrier (CT +TT) of 936C>T polymorphism were associated with increased risk for CRC in females with a distal lesion or age less than 55 years-old. Wu et al. (2006) conducted a subgroup analysis on anastomotic leakage, and their results showed that the C allele and CC genotype were associated with less frequency of anastomotic leakage in patients with CRC. Furthermore, the -2578AA, -634CC and +936TT genotypes were found to be related with a significantly lower overall survival (Dassoulas et al., 2009).

Such evidence on the functionality of VEGF gene polymorphisms might lead to a bet-

ter understanding of CRC biology and behavior. Also it was also a strong rationale for the development of novel anti-angiogenesis drugs interfering with the VEGF protein production in colorectal carcinogenesis. At the same time, findings about SNPs influencing VEGF-targeted therapies as predictive markers would be of great help for doctors to choose therapies in an individual manner (Hofmann et al., 2008).

Some limitations of this meta-analysis should be acknowledged. Firstly, because of incomplete raw data or publication limitations, some relevant studies could not be included in our analysis. Secondly, the small sample size available was not ideal for detecting small genetic effects. Thirdly, we were not able to address all the sources of heterogeneity that existed among studies for most polymorphisms, although we could have made subgroup stratifications analysis for the limited number of published studies. In addition, the lack of genotype frequency information provided by some published studies did not allow the estimation of the best genetic model of inheritance to follow. Finally, although all cases and controls of each study were well defined with similar inclusion criteria, there may be potential factors that were not taken into account that may have influenced our results.

In conclusion, our meta-analysis of 8 case-control studies demonstrated that the C allele carrier (CC + CA) of VEGF -2578C>A polymorphism might be a protective factor for CRC, especially in Caucasian populations. As few studies are available in this field and current evidence remains limited, this conclusion should be further confirmed by large case-control studies with an adequate methodological quality and proper controlling for possible confounders.

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