

## RESEARCH ARTICLE

# Associations of Single Nucleotide Polymorphisms in miR-146a, miR-196a, miR-149 and miR-499 with Colorectal Cancer Susceptibility

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### Abstract

**Background:** MicroRNAs (miRNAs) are an abundant class of endogenous small non-coding RNAs of 20-25 nucleotides in length that function as negative gene regulators. MiRNAs play roles in most biological processes, as well as diverse human diseases including cancer. Recently, many studies investigated the association between SNPs in miR-146a rs2910164, miR-196a2 rs11614913, miR-149 rs229283, miR-499 rs3746444 and colorectal cancer (CRC), which results have been inconclusive. **Methodology/Principal Findings:** PubMed, EMBASE, CNKI databases were searched with the last search updated on November 5, 2013. For miR-196a2 rs11614913, a significantly decreased risk of CRC development was observed under three genetic models (dominant model: OR = 0.848, 95% CI: 0.735–0.979, P = 0.025; recessive model: OR = 0.838, 95% CI: 0.721–0.974, P = 0.021; homozygous model: OR = 0.754, 95% CI: 0.627–0.907, P = 0.003). In the subgroup analyses, miR-196a2\*<sup>T</sup> variant was associated with a significantly decreased susceptibility of CRC (allele model: OR = 0.839, 95% CI: 0.749–0.940, P = 0.000; dominant model: OR = 0.770, 95% CI: 0.653–0.980, P = 0.002; recessive model: OR = 0.802, 95% CI: 0.685–0.939, P = 0.006; homozygous model: OR = 0.695, 95% CI: 0.570–0.847, P = 0.000). As for miR-149 rs2292832, the two genetic models (recessive model: OR = 1.199, 95% CI 1.028–1.398, P = 0.021; heterozygous model: OR = 1.226, 95% CI 1.039–1.447, P = 0.013) demonstrated increased susceptibility to CRC. On subgroup analysis, significantly increased susceptibility of CRC was found in the genetic models (recessive model: OR = 1.180, 95% CI 1.008–1.382, P = 0.040; heterozygous model: OR = 1.202, 95% CI 1.013–1.425, P = 0.013) in the Asian group. **Conclusions:** These findings supported that the miR-196a2 rs11614913 and miR-149 rs2292832 polymorphisms may contribute to susceptibility to CRC.

**Keywords:** MicroRNA - polymorphism - colorectal cancer - meta-analysis

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### Introduction

Colorectal cancer (CRC) is the fourth most common cause of death from cancer and the most common malignancy of the gastrointestinal tract. About 608,000 deaths are estimated worldwide, accounting for 8% of all cancer deaths (Ferlay et al., 2010). The five-year survival rate of early-stage CRC is 90%, but only 10% if distant metastases are present (Levin et al., 2008). Though the recent statistics reveals the CRC incidence rates have rapidly declined in the United States due to increasing in screening that can detect and allow the removal of precancerous polyps, it is still the third common cancer in both males and females in the United States (Siegel et al., 2013). Therefore, it is of great value to develop the early and non-invasive diagnostic biomarkers for patients with CRC.

MicroRNAs (miRNAs) are an abundant class of 20–25 nucleotides single-stranded non-coding RNAs (Bartel 2009; Brodersen et al., 2009). They can regulate gene expression by translational repression or mRNA degradation of the targets with the nucleotides 2–7 of the mature miRNA sequence, which is called the ‘seed region’ (Brodersen and Voinnet 2009; Fabian et al., 2010). MiRNAs involve in the most critical functions in biological processes, including cell cycle, differentiation, development, metabolism and tumor development and progression (Krol et al., 2010; Xiao et al., 2009); (Croce 2009; Garzon et al., 2009; Visone et al., 2009). Single nucleotide polymorphisms (SNPs) may influence the biogenesis and functions of their host miRNAs, consequently contributing to cancer susceptibility (Chen et al., 2008; Landi et al., 2008; Mishra et al., 2009).

Recently, several studies have been conducted to

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**Table 1. Characteristics of the Included Studies in this Meta-analysis**

First author	Year	Country	Ethnicity	Genotyping method	Number		MicroRNA gene	SNPs	Quality scores
					Case	Control			
Vinci	2013	Italy	Caucasian	PCR-HRMA	160	178	miR-146a, miR-196a2, miR-499, miR-149	rs11614913 (C > T), rs2910164 (G > C), rs3746444 (A > G), rs2292832 (T > C)	28
Lv -1	2013	China	Asian	PCR-RFLP	331	513	miR-146a	rs2910164 (G > C)	25
Lv -2	2013	China	Asian	PCR-RFLP	347	531	miR-196a2	rs11614913 (C > T)	25
Lv -3	2013	China	Asian	PCR-RFLP	347	459	miR-149	rs2292832 (C > T)	25
Hu -1	2013	China	Asian	PCR-RFLP	200	373	miR-146a	rs2910164 (G > C)	26
Hu -2	2013	China	Asian	PCR-RFLP	211	373	miR-149	rs2292832 (T > C)	26
Chae	2013	Korea	Asian	PCR-RFLP	399	568	miR-499	rs3746444 (T > C)	27
Ma	2013	China	Asian	TaqMan	1147	1203	miR-146a	rs2910164 (G > C)	26
Zhu	2012	China	Asian	TaqMan	573	588	miR-196a2	rs11614913 (C > T)	26
Min	2012	Korea	Asian	PCR-RFLP	446	502	miR-146a, miR-196a2, miR-499, miR-149	rs2910164 (G > C), rs11614913 (C > T), rs3746444 (A > G), rs2292832 (T > C)	25
Chen	2012	China	Asian	PCR-LDR	126	407	miR-196a2	rs11614913 (C > T)	24
Hozova	2012	Czech	Caucasian	TaqMan	197	212	miR-146a, miR-196a2	rs11614913 (C > T), rs2910164 (G > C)	27
Zhan	2011	China	Asian	PCR-RFLP	252	543	miR-196a2	rs11614913 (C > T)	24
Zhang	2011	China	Asian	PCR-RFLP	443	435	miR-149	rs2292832 (C > T)	25

investigated the association between four SNPs in miRNAs (rs2910164 SNP in miR-146a, rs11614913 SNP in miR-196a2, rs2292832 SNP in miR-149, rs3746444 SNP in miR-499) and the susceptibility of colorectal cancer (CRC) in different populations, but the results remain contradicting (Zhan et al., 2011; Chen et al., 2012; Hezova et al., 2012; Zhang et al., 2012; Zhu et al., 2012; Chae et al., 2013; Hu et al., 2013; Ma et al., 2013; Meili et al., 2013; Min et al., 2012; Vinci et al., 2013). Therefore, we carried out this meta-analysis to assess the association of the four SNPs in miRNAs and CRC susceptibility.

## Materials and Methods

### Identification of Eligible Studies

PubMed, Excerpta Medica Database (EMBASE), Chinese Biomedical Literature Database (CBM) and Chinese National Knowledge Infrastructure (CNKI) databases were searched with the last search updated on November 5, 2013. The following search terms were analyzed: “microRNA OR mir OR miRNA”, “colorectal OR colon OR rectum” “cancer OR tumour OR tumor OR neoplasm OR carcinoma”, “gene OR allele OR polymorphism OR variation”, “miR-196a2 OR rs11614913”, “miR-146a OR rs2910164”, “miR-149 OR rs2292832”, “miR-499 OR rs3746444”. Searching was done without restriction on language or publication years. We evaluated all potentially associated publications to retrieve the most eligible studies. References of the retrieved articles were manually screened to identify other relevant publications.

### Inclusion and Exclusion Criteria

Eligible studies had to meet the following criteria: (1) evaluation of the associations between four SNPs in miRNAs and CRC susceptibility, including rs2910164 SNP in miR-146a, rs11614913 SNP in miR-196a2, rs2292832 SNP in miR-149 and rs3746444 SNP in miR-499; (2) independent case-control design studies for human; (3) sufficient genotype data were showed to calculate the OR with 95 % CI; (4) only full-text

manuscripts were included. The exclusion criteria were: (1) pure cell studies, non-CRC studies; (2) repeated or overlapped studies; (3) abstract, comment, editorial, and review; (4) no sufficient data were reported; (5) articles with obvious mistakes.

### Data extraction

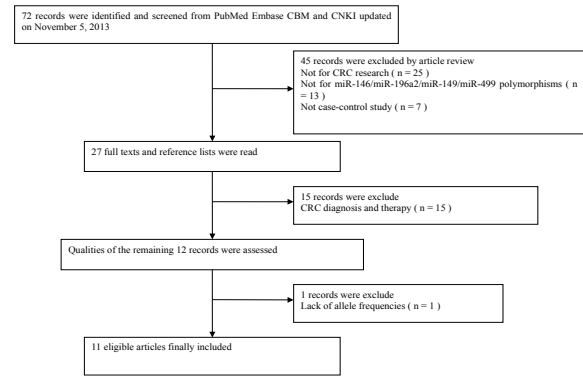
Two reviews (Wei Du and Xuelei Ma) extracted all data independently complied with the inclusion criteria listed above. Discrepancies were adjudicated by discussion with other investigators (Weiqi Kong and Jiayun Yu) until consensus was achieved on all items. From each study, we extracted the following characteristics: (1) first author's name; (2) publication year; (3) country origin; (4) ethnicity; (5) genotyping methods; (6) numbers of cases and controls; (7) allele and genotype frequencies for cases and controls; (8) *P* value for Hardy-Weinberg equilibrium (HWE) of controls. (Table 1)

### Statistical Analysis

The departure of frequencies of SNPs in miRNAs (rs2910164 SNP in miR-146a, rs11614913 SNP in miR-196a2, rs2292832 SNP in miR-149 and rs3746444 SNP in miR-499) from HWE in the control population was evaluated using the goodness-of-fit chi-square test. *P*-value <0.05 was considered significant disequilibrium. ORs and 95% CIs were evaluated to assess the strength of association between SNPs in miRNAs and susceptibility to CRC based on genotype frequencies in cases and controls. The pooled ORs were performed for allele comparison, dominant and recessive models, homozygote comparison and heterozygote comparison, respectively. The significance of the pooled ORs was determined by the Z-test, and *P*-value <0.05 was considered statistically significant. The heterogeneity among the studies was checked by the Chi square-test based Q-test (Cochran 1954; Higgins et al., 2002). When the existence of heterogeneity was detected (*P*-value <0.10 for the Q-test,  $I^2 >50\%$ ), the random-effects model (DerSimonian and Laird method) was chosen (DerSimonian et al., 1986). Otherwise, the fixed-effects model (the Mantel-Haenszel

**Table 2. Meta-analysis Results for the Four Polymorphisms and CRC Risk**

SNPs	2 allele vs 1 allele (Allele model)			1/2 + 2/2 vs 1/1 (Dominant model)			2/2 vs 1/1 + 1/2 (Recessive model)			2/2 vs 1/1 (Homozygous model)			2/2 vs 1/2 (Heterozygous model)			
	OR	95%CI	Z	P	OR	95%CI	Z	P	OR	95%CI	Z	P	OR	95%CI	Z	P
<b>miR-146a rs2910164 G &gt; C</b>																
Overall	0.996	0.852-1.164	0.05	0.959 <sup>a</sup>	0.997	0.806-1.235	0.03	0.980 <sup>a</sup>	0.997	0.717-1.386	0.02	0.986 <sup>a</sup>	1.009	0.714-1.386	0.03	0.974 <sup>a</sup>
Asians	0.969	0.803-1.169	0.33	0.743 <sup>a</sup>	0.981	0.738-1.305	0.13	0.897 <sup>a</sup>	0.915	0.630-1.329	0.46	0.652 <sup>a</sup>	0.927	0.623-1.380	0.49	0.624 <sup>a</sup>
Caucasians	1.103	0.872-1.396	0.82	0.412 <sup>a</sup>	1.049	0.785-1.401	0.32	0.749 <sup>a</sup>	1.489	0.838-2.648	1.36	0.175 <sup>a</sup>	1.497	0.816-2.745	1.3	0.192 <sup>a</sup>
<b>miR-196a2 rs11614913 C &gt; T</b>																
Overall	0.91	0.794-1.043	1.35	0.177 <sup>a</sup>	0.848	0.735-0.979	3.25	0.025	0.838	0.721-0.974	2.31	0.021	0.889	0.759-1.042	1.45	0.147
Asians	0.839	0.749-0.940	3.04	0.000 <sup>a</sup>	0.77	0.653-0.980	3.1	0.002	0.802	0.685-0.939	2.74	0.006	0.857	0.726-1.013	1.8	0.071
Caucasians	1.128	0.910-1.398	1.1	0.272 <sup>a</sup>	1.142	0.854-1.528	0.9	0.371	1.285	0.786-2.101	1	0.318	1.254	0.751-2.095	0.87	0.386
<b>miR-149 rs2292832 C &gt; T</b>																
Overall	1.103	0.985-1.236	1.7	0.089	0.996	0.802-1.238	0.03	0.974	1.199	1.028-1.398	2.32	0.021	1.226	1.039-1.447	2.41	0.013
Asians	1.105	0.979-1.248	1.62	0.106	1.01	0.782-1.299	0.08	0.939	1.18	1.008-1.382	2.06	0.04	1.202	1.013-1.425	2.11	0.035
Caucasian	1.091	0.788-1.510	0.53	0.599	0.958	0.625-1.469	0.19	0.846	1.59	0.816-3.098	1.36	0.173	1.749	0.856-3.574	1.53	0.125
<b>miR-499 rs3746444 A &gt; G</b>																
Overall	1.112	0.941-1.314	1.25	0.213	1.05	0.862-1.279	0.49	0.625	1.408	0.657-3.015	0.88	0.379 <sup>a</sup>	1.474	0.544-3.991	0.76	0.445 <sup>a</sup>
Asians	1.034	0.853-1.255	0.34	0.732	1.054	0.845-1.315	0.47	0.641	0.935	0.496-1.763	0.21	0.835 <sup>a</sup>	0.894	0.464-1.721	0.34	0.737 <sup>a</sup>
Caucasian	1.383	0.989-1.934	1.9	0.058	1.036	0.672-1.598	0.16	0.872	2.652	1.420-4.953	3.06	0.002 <sup>a</sup>	3.603	1.747-7.433	3.47	0.001 <sup>a</sup>

**Figure 1. Flow Diagram of Study Identification with Criteria for Inclusion and Exclusion in the Meta-Analysis**

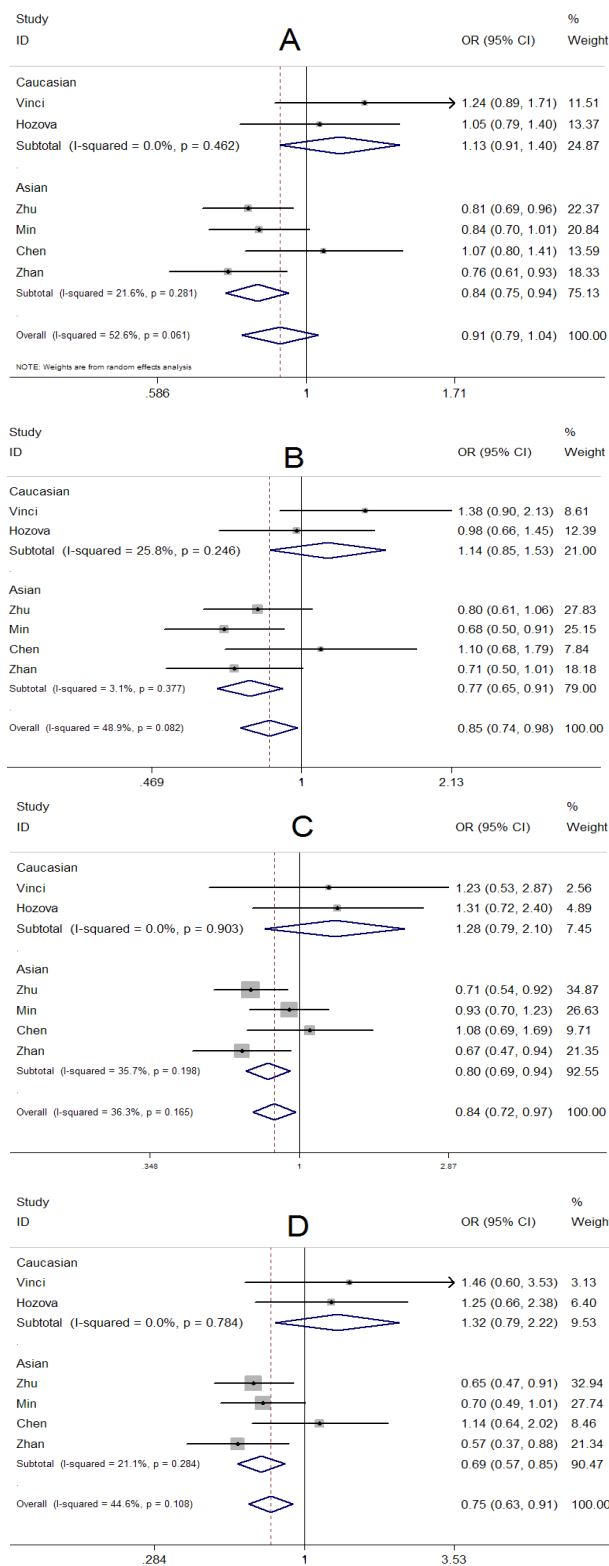
method) was employed (Mantel et al., 2004). Publication bias of literature was assessed with Begg's funnel plots and Egger's test. A *P*-value of Egger's test less than 0.05 was considered representative of statistically significant publication bias (Egger et al., 1997). In the Begg's funnel plot, the standard error of logarithm (Log) for OR was plotted against its OR, and Log OR was plotted versus standard error of Log OR for each enrolled study (Begg et al., 1994). All of the statistical tests were performed with STATA software version 11.0 (STATA Corporation, College Station, TX, USA).

## Results

### Characteristics of studies

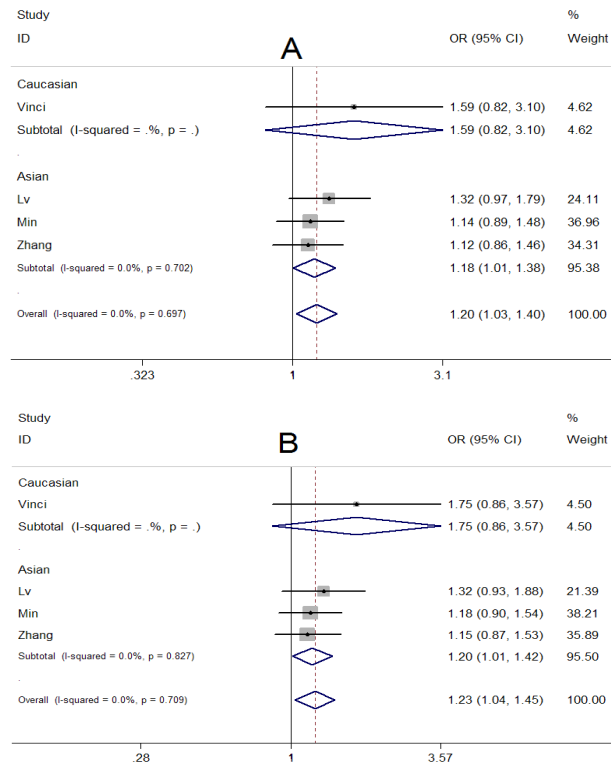
Eligible studies were selected according to the inclusion and exclusion criteria (Figure 1). Seventy-two eligible studies were retrieved from the PubMed, EMBASE, CBM and CNKI databases, according to the inclusion and exclusion criteria. Forty-five records were excluded for improper article titles and abstracts, including 25 records that did not explore CRC, 13 records that did not focus on SNPs in miRNAs (rs2910164 SNP in miR-146a, rs11614913 SNP in miR-196a2, rs2292832 SNP in miR-149 and rs3746444 SNP in miR-499) and 7 records that were not case-control study. Then, 27 full texts and related reference lists were read. Fifteen records were excluded for assessing CRC diagnosis and therapy studies and 1 for lacking of enough genotype frequencies (Lv et al., 2013). In Lv's study (Lv et al., 2013), no specific frequencies of each genotype was presented for the rs3746444 SNP in miR-499, so it was excluded in the analysis for the rs3746444 SNP in miR-499. Finally, 11 case-control studies were analyzed in this meta-analysis on evaluating the relationship between the four polymorphisms (miR-146a, miR-196a2, miR-149, and miR-499) in miRNA genes and CRC risk. There were nine studies on Asian population and two on European population.

Characteristics of eligible studies are presented in Table 1. These included studies were published from year 2011 to 2013 in different countries (Italy, China, Korea and Czech). Ethnicity was categorized as Asian population and Caucasian population. Several genotyping methods



**Figure 2. Forest Plot of ORs for the Association of Mir-196A2 Rs11614913 Polymorphism with CRC Risk is Illustrated in Subgroup Analysis by Ethnicity (A: Allele model; B: Dominant model; C: Recessive model; D: Homozygous model)**

were employed in the studies including polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), polymerase chainreaction-ligation detection reaction (PCR-LDR), PCR-high-resolution melting analysis (PCR-HRMA) and Sequencing Taqman. The distribution of genotypes in the controls did not deviate



**Figure 3. Forest Plot of ORs for the Association of Mir-149 Rs2292832 Polymorphism with CRC Risk is Illustrated in Subgroup Analysis by Ethnicity (A: Recessive model; B: Heterozygous model)**

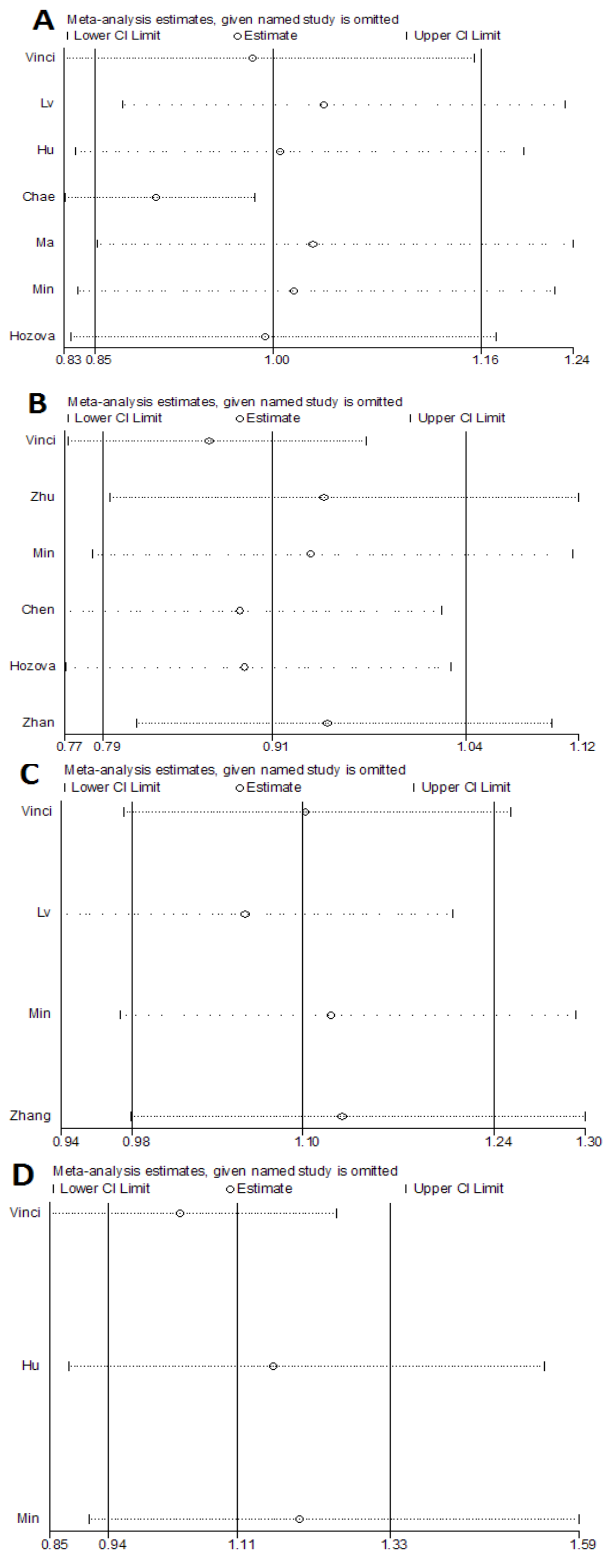
from HWE ( $P > 0.05$ ) in most of the studies, except for Lv's study (Lv et al., 2013).

**Meta-analysis Results**

**For miR-146a rs2910164:** The association between miR-146a rs2910164 polymorphism and CRC susceptibility was assessed in seven studies among which five studies were in Asian population and two in Caucasian populations. Since heterogeneity was observed in all genetic models, so the random effects model was used to pool the results.

When pooling all eligible studies into meta-analysis, no significant CRC risk was observed for five genotype models with relatively large heterogeneity. Then, we perform subgroup analysis for miR-146a rs2910164 polymorphisms by racial decent, no significance risk association were observed in Asians or Caucasians (Table 2).

**For miR-196a2 rs11614913:** When seven case-control studies involving 2091 cases and 3061 controls were pooled into this meta-analysis, no significant statistical heterogeneity was identified in any of the genetic models, therefore fixed-effects model was used in all these models. However, no significant association between rs11614913 SNP in miR-196a2 and CRC susceptibility was observed. After excluding the Lv's study (Lv et al., 2013), the data in which was significantly departed from HWE ( $P = 0.0000$ ), and mixing the remain studies, the heterogeneities of enrolled studies were reduced and the genotypic results were more credible. The meta-analysis results of association between rs11614913 SNP in miR-196a2 and CRC susceptibility was shown in Table 2.



**Figure 4. Sensitivity Analysis of the Summary Odds Ratio Coefficients of the four Functional Polymorphisms is Illustrated Under the Allele Model.** Results were computed by omitting each study in turn. The two ends of the dotted lines represent the 95% CI (A: miR-146a; B: miR-196a-2; C: miR-149; D: miR-499)

For miR-196a2 rs11614913, a significantly decreased risk of CRC development was observed under four genetic models (dominant model: OR = 0.848, 95%CI: 0.735–0.979,  $P = 0.025$ ; recessive model: OR = 0.838, 95%CI: 0.721–0.974,  $P = 0.021$ ; homozygous model: OR = 0.754, 95%CI: 0.627–0.907,  $P = 0.003$ , Table 2). Then, subgroup

analysis for miR-196a2 rs11614913 polymorphisms according to different ethnicities was performed. We found that, in the Asian group, miR-196a2\*T variant was associated with a significantly decreased susceptibility of CRC (allele model: OR = 0.839, 95%CI: 0.749–0.940,  $P = 0.000$ ; dominant model: OR = 0.770, 95%CI: 0.653–0.980,  $P = 0.002$ ; recessive model: OR = 0.802, 95%CI: 0.685–0.939,  $P = 0.006$ ; homozygous model: OR = 0.695, 95%CI: 0.570–0.847,  $P = 0.000$ , Table 2). As for the Caucasian group, none of genetic models demonstrates significant association between miR-196a2 rs11614913 polymorphism and susceptibility of CRC (Figure 2).

**For miR-149 rs2292832:** The association between miR-149 rs2292832 polymorphism and susceptibility to CRC was analyzed in four independent studies with 1396 cases and 1574 controls. Results of the meta-analysis are shown in Table 2. No significant statistical heterogeneity was identified in any of the genetic models, therefore fixed-effects model was used in all these models.

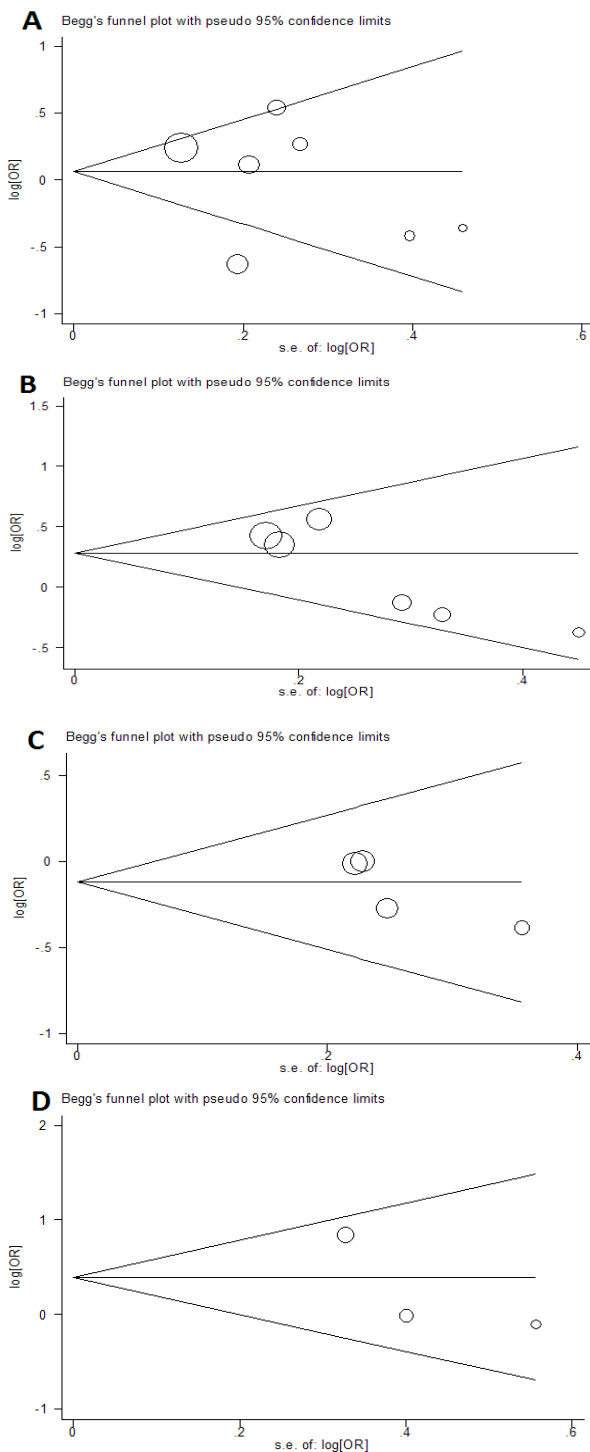
By pooling eligible data, we found that two genetic models (recessive model: OR = 1.199, 95% CI 1.028–1.398,  $P = 0.021$ ; heterozygous model: OR = 1.226, 95% CI 1.039–1.447,  $P = 0.013$ ) increased susceptibility to CRC. Similarly, in subgroup analysis according to different ethnicities, significantly decreased susceptibility of CRC was found in the genetic models (recessive model: OR = 1.180, 95% CI 1.008–1.382,  $P = 0.040$ ; heterozygous model: OR = 1.202, 95% CI 1.013–1.425,  $P = 0.013$ ) in the Asian group (Table 2). After deleting the Lv's study (Lv et al., 2013), in which the distribution of miR-149 rs2292832 genotypes in controls deviated from the HWE ( $P < 0.05$ ) and the included population was mixed, no significant association between SNP rs3746444 and susceptibility to CRC was showed in any genetic model (Figure 3).

**For miR-499 rs3746444:** Three studies involving 817 cases and 1,053 controls were evaluated for the association between miR-499 rs3746444 polymorphism and CRC susceptibility. Significant statistical heterogeneity was identified in the comparison of GG vs. GA/AA, so that random-effects model was used in this models, Fixed-effects model was used in other models. However, for rs3746444, none of the genetic models produced significant association between rs3746444 polymorphism and CRC susceptibility. The results are summarized in Table 2.

Similarly, subgroup analysis showed no significant association between SNP rs3746444 and susceptibility to CRC in Asian population (Table 2).

#### Sensitivity analysis

Sensitivity analysis assessed the influence of each individual study on the overall pooled ORs by deleting individual studies in turn. For the miR-146a rs2910164, the study from Chae (Chae et al., 2013) showed significant effect on the pooled OR. After exclusion of this study in the allele comparison of C vs G, the heterogeneity test turned to be negative, and pooled OR changed from 0.996 (95% CI 0.852–1.164,  $P = 0.959$ ) to 0.898 (95% CI 0.830–0.971,  $P = 0.007$ ) (Figure 4), indicating the study from Chae may have a major influence on the estimation of the potential association under the allele model.



**Figure 5. Begg's Funnel Plot of Publication Bias in the Selection of Studies on the four Functional Polymorphisms is Illustrated under the Allele Model** (A: miR-146a; B: miR-196a-2; C: miR-149; D: miR-499). Each point represents a separate study by the indicated association. Log[OR], natural logarithm of OR. Horizontal line, mean magnitude of the effect

#### Publication Bias

Begg's funnel plot and Egger's test were performed to assess the publication bias of enrolled literatures. The shapes of the funnel plots for miR-146a rs2910164, miR-196a2 rs11614913, miR-149 rs2292832 and miR-499 rs3746444 under the homozygous model seemed approximately symmetrical (Figure 5). Egger's test also showed no statistically significant evidence of publication

bias for homozygous model (miR-146a rs2910164:  $t = 0.64$ ,  $P = 0.548$ ; miR-196a2 rs11614913:  $t = 1.16$ ,  $P = 0.311$ ; miR-149 rs2292832:  $t = 1.96$ ,  $P = 0.189$ ; miR-499 rs3746444:  $t = -1.39$ ,  $P = 0.398$ ).

#### Discussion

MiRNAs play a pervasive role in the diverse biological processes, partly because a single miRNA may broadly regulate several target genes (Krol et al., 2010; Leung et al., 2010). The dysfunction of miRNAs could have profound effect on the expression of numerous genes, which could possibly contribute to cancer susceptibility (Calin et al., 2006; Croce 2009; Garzon et al., 2009; Mishra and Bertino 2009; Visone et al., 2009). MiR-polymorphisms (SNPs) reside at or near a miRNA binding site of a functional gene, influencing its expression by interfering with miRNA function. Thus SNPs in miRNAs is regarded as a key factor in oncogenesis (Chen et al., 2008; Landi et al., 2008; Mishra and Bertino 2009).

Recently, much effort has been made towards investigating the role of SNPs in miRNAs and their impacts on susceptibility to CRC. Four functional SNPs, miR-146a rs2910164 (Hezova et al., 2012; Chae et al., 2013; Hu et al., 2013; Lv et al., 2013; Ma et al., 2013; Min et al., 2012; Vinci et al., 2013), miR-196a2 rs11614913 (Zhan et al., 2011; Chen et al., 2012a; Hezova et al., 2012; Lv et al., 2013; Min et al., 2012; Zhu et al., 2012; Vinci et al., 2013), miR-149 rs2292832 (Min et al., 2012; Zhang et al., 2012; Lv et al., 2013; Vinci et al., 2013) and miR-499 rs3746444 (Min et al., 2012; Cheng et al., 2013; Lv et al., 2013; Vinci et al., 2013), are indicated to be associated with susceptibility to CRC. However, the results of these studies differ from each other. Therefore, this meta-analysis has been carried out to assess the potential associations of the four functional SNPs in miRNAs with CRC susceptibility.

MiR-146a rs2910164 is located in the stem region opposite to the mature miR-146a sequence. This C > G polymorphism results in a change from C: U pair to G: U mismatch in the stem structure of miR-146a precursor. Several studies have been investigated the association of miR-146a rs2910164 polymorphism and risk of various cancer (Jazdzewski et al., 2008; Hu et al., 2009; Catucci et al., 2010; Okubo et al., 2010; Zeng et al., 2010; Garcia et al., 2011; Alshatwi et al., 2012; Hezova et al., 2012; Min et al., 2012; Zhou et al., 2012; Chae et al., 2013; Hu et al., 2013; Lv et al., 2013; Ma et al., 2013; Marino et al., 2013; Wei et al., 2013; Vinci et al., 2013). Our results did not support a genetic association between miR-146a rs2910164 and susceptibility to CRC. Neither allele frequency nor genotype distribution was significantly associated with susceptibility to CRC. Considering the potential relationship between the gene polymorphisms in different ethnic groups and the CRC risk, we further made the subgroup analysis on Asian population and Caucasian population to explore the association between rs2910164 and the risk of CRC. No significant result was showed in the subgroup analysis, either.

However, considering the important biological function of miR-146a in tumorigenesis (Bhaumik et al.,

2008; Hurst et al., 2009; Li et al., 2010), the conclusion that miR-146a rs2910164 polymorphism has no impact on CRC risk is premature due to limited number of studies included in this study.

In the current meta-analysis, we systematically summarized all available up-to-date case-control studies on the association between miR-196a2 rs11614913 SNP and CRC susceptibility. A total of 6 eligible case-control studies (1800 cases and 2329 controls) were included in this updated meta-analysis (Zhan et al., 2011; Chen et al., 2012; Hezova et al., 2012; Min et al., 2012; Zhu et al., 2012; Vinci et al., 2013), including four studies of Asians (Zhan et al., 2011; Chen et al., 2012; Min et al., 2012; Zhu et al., 2012) and two study of Europeans populations (Hezova et al., 2012; Vinci et al., 2013) (Table 1). Our study suggested that the presence of T allele significantly decreased the susceptibility of CRC with the comparison to C allele. Moreover, the rs11614913 polymorphism was found to be associated with a significantly decreased susceptibility of CRC in the TT homozygote and TT/CT genotype as opposed to the CC homozygote and TT homozygote to the TC/CC genotype (Table 2). In the subgroup of ethnicity, we detected significant association between the rs11614913 polymorphism and increased susceptibility of CRC in Asians (Table 2). But no genetic model was found to have any significant association with susceptibility of CRC in Caucasians. The result suggests that genetic background and different environment may contribute to the different impact of rs11614913 SNP in miR-196a2 on susceptibility of CRC.

Our result is consistent with previous meta-analyses (Shi et al., 2013; Wang et al., 2013) reported the association between miR-196a2 rs11614913 and CRC susceptibility. The result is biologically plausible as the mechanism of promoting the development of CRC by rs11614913 SNP in miR-196a2 being focused. The Hoffman's (Hoffman et al., 2009) study indicated that rs11614913 SNP in miR-196a2 produced less efficient miRNA precursor to its mature form and diminished capacity to regulate target genes. Mueller's (Mueller et al., 2011) study showed that stable transfection with an miR-196a expression plasmid led to strong down-regulation of HOX-C8 expression in melanoma cells. Niinuma's (Niinuma et al., 2012) study showed that miR-196a genes were located within the HOX gene clusters and HOTAIR gene were coordinately upregulated in GISTs which overexpressed miR-196a. Schimanski's (Schimanski et al., 2009) study indicated that HoxA7, HoxB8, HoxC8 and HoxD8 were restricted by miR-196a in a dose-dependent and gene-specific manner in CRC and high levels of miR-196a activated the AKT signaling pathway by increased phosphorylation of AKT.

Another SNP shown to have potential relationship to the risk for CRC is miR-149 rs2292832. Several molecular epidemiological studies have investigated the association between the miR-149 rs2292832 polymorphism and cancer (Ding et al., 2013; Dominguez 2013), and the association of miR-149 rs2292832 polymorphism with cancer risks (Hu et al., 2009; Tian et al., 2009; Tu et al., 2012; Wang et al., 2012; Hong et al., 2013) have been conducted. But the conclusions are inconsistent. Our study

suggested that the miR-149 rs2292832 polymorphism was associated with a significantly increased susceptibility of CRC in the TT homozygote as opposed to the TC heterozygote and TC/CC genotype (Table 2). In the subgroup of ethnicity, significant association between the miR-149 rs2292832 polymorphism and increased susceptibility of CRC in Asians was detected, too (Table 2). But no genetic model was found to have any significant association with susceptibility of CRC in Caucasians. This is in accordance with a previous study (Xu et al., 2013) demonstrating that miR-149 was epigenetically silenced in CRC and down-regulation of miR-149 was associated with hypermethylation of the neighbouring CpG island (CGI). They also identified mRNA for Specificity Protein 1 (SP1, Sp1), a potential oncogenic protein, as a target of miR-149. Furthermore, another study (Lin et al., 2010) showed the target genes of miR-149, Akt1, and E2F1, are involved in promoting cell growth and cell cycle progression (Lin et al., 2010).

Only three studies have looked at the association between miR-499 rs3746444 and CRC. The functional miR-499 rs3746444 is located in the stem region opposite to the mature miR-499 sequence (Akkiz et al., 2011). Previous meta-analysis found miR-499 rs3746444 polymorphism (A>G) is low-penetrant risk factor for cancer a development among Asians and may contribute to breast cancer susceptibility (Chen et al., 2012) and miR-499 rs3746444 G allele was a risk factor in Chinese population, and the association varied from different cancer types (Xu et al., 2013). However, no significant correlation between the miR-499 rs3746444 and susceptibility of CRC development in all genetic models is found by us.

However, considering the limited studies and small population sizes included in our meta-analysis, our results should be interpreted with caution.

Limitations: This is the first meta-analysis evaluating the potential association of four functional SNPs in miRNAs (rs2910164 SNP in miR-146a, rs11614913 SNP in miR-196a2, rs2292832 SNP in miR-149, rs3746444 SNP in miR-499) and the susceptibility of CRC. Furthermore, the association between rs2910164 SNP in miR-146a, rs2292832 SNP in miR-149, rs3746444 SNP in miR-499 and CRC risk were studied in a meta-analysis for the first time. However, several limitations of our meta-analysis should be noticed. First, a more precise analysis stratified by other covariates such as age, sex, family history, environmental factors and lifestyle could not be performed due to lack of individual data. Second, so far there was no study of other populations except Asian and Caucasians. Third, the interactions of gene-gene and gene-environment were not considered, which might alter the associations between miRNA gene polymorphisms and cancer. Fourth, studies with no statistically significant results often have less chance for publication. It is still difficult to rule out potential publication bias in the meta-analysis.

In conclusion, our meta-analysis suggests that the miR-196a2 rs11614913 T allele is associated with decreased CRC susceptibility. And TT homozygote in the miR-149 rs2292832 polymorphism was associated with a

significantly increased susceptibility of CRC as opposed to the TC heterozygote and CC/CT genotype. Well-designed studies with larger sample size and more ethnic groups should be considered to further clarify the association.

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