

## Original Article

# Mir-196a-2 C>T polymorphism as a susceptibility factor for colorectal cancer

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**Abstract:** Objective: This study aimed to gain a better insight into the impact of the *mir-196a-2* C>T polymorphism on the susceptibility to colorectal cancer (CRC). Methods: In a meta-analysis of 6 publications with a total of 1,754 cancer cases and 2,430 controls, we summarized the data on the associations between the studied *mir-196a-2* C>T polymorphism and CRC risk and conducted subgroup analyses by ethnicity and control sources. Results: We found no overall association between the *mir-196a-2* C>T polymorphism and CRC risk. But a significant association was found in the stratified analysis according to ethnicity among Asians ( $OR_{CC\ vs.\ TT} = 1.22$ , 95% CI = 1.02-1.45,  $P_{heterogeneity} = 0.718$ ;  $OR_{CC\ vs.\ TC + TT} = 1.22$ , 95% CI = 1.04-1.44,  $P_{heterogeneity} = 0.590$ ;  $OR_{allele\ C\ vs.\ allele\ T} = 1.10$ , 95% CI = 1.01-1.20,  $P_{heterogeneity} = 0.726$ ) rather than Caucasians. Conclusions: Our results suggested that there was no overall risk of CRC in relation to the *mir-196a-2* C>T polymorphism. However, this polymorphism was associated with an increased risk in Asian populations.

**Keywords:** *mir-196a-2* C>T, CRC, polymorphism, susceptibility

## Introduction

Colorectal cancer (CRC) is one of the most common cancers and ranks the third in men and the second in women throughout the world [1]. Despite previous adoption of various treatment methods, including early diagnosis, surgical techniques and chemotherapy, the mortality rate still remains as high as 8.8% in 2008 [2]. Therefore, it is quite necessary to identify the susceptibility factors for CRC so as to facilitate diagnosis and treatment of the disease.

In recent years, numerous evaluations of microRNA expression profiles have implicated that variation of microRNA expression could be applied in CRC diagnosis, prognosis and susceptibility [3]. MicroRNAs consisting of non-coding RNA molecules of 18-25 nucleotides are important regulatory molecules and do not emerge until experiencing numerous stages of maturation [4]. MicroRNAs regulate gene expression at the posttranscriptional level through binding to complementary sequences in the 3'-untranslated region (3'-UTR) [5].

Recently published researches have revealed that mutations and single-nucleotide polymorphisms (SNPs) in microRNA sequence might inhibit microRNA maturation and expression [6, 7].

In the complementary region of mature microRNA in *mir-196a-2* lies an SNP (rs11614913). Many studies have reported the SNP is associated with breast cancer, lung cancer, gastric cancer, head and neck cancer, esophagus squamous cell carcinoma and hepatocellular carcinoma risk, and non-small cell lung cancer [8-17]. Meanwhile, the polymorphism of *mir-196a-2* C>T has also attracted widespread attention and quantities of single case-control studies were carried out to evaluate the association of this polymorphism with the risk of CRC in humans [18-23]. However, these association studies generated inconclusive results. So a meta-analysis that has more statistical power was performed in order to combine all published data so that a more exact estimation could be derived.

## Materials and methods

### Search strategy

To extract all the studies that examined the association of the *mir-196a-2* C>T polymorphism with CRC risk, we searched the electronic databases of the PubMed, EMBASE, and Web of Science with a limitation of the English language. The following terms were used in the search: “mirRNAs” or “microRNAs”, “mirRNA-196a-2” or “microRNA-196a-2” or “*mir-196a-2*” or “196-a-2 C>T” or “rs11614913”, or “rectal cancer” or “colon cancer” or “colorectal cancer”. References of retrieved studies were also manually screened to obtain the relevant original articles. When more than one article had overlapping data, only the most recent study with a larger sample size was selected in the final analysis. If one article included different ethnic populations, each population was retrieved separately according to Asians and Caucasians.

### Inclusion criteria

The eligible studies in this meta-analysis must satisfy the following inclusion criteria: (a) evaluating the association of the *mir-196a-2* C>T polymorphism with CRC risk in humans; (b) using an unrelated case-control design; (c) detailed data for genotype frequency; (d) no deviation from Hardy-Weinberg equilibrium (HWE) in the genotype distribution of the control populations.

### Exclusion criteria

The major exclusion criteria were the following: (a) insufficient information to estimate ORs and 95% CIs; (b) overlapped data, if more than one version of the same study was extracted, only the latest one or the largest one was considered; and (c) letters or editorial, review paper, duplicate data.

### Data extraction

Two authors independently conducted the computerized search and extracted the following essential data: first author, publication year, country of study, racial background of the study population, source of controls, genotyping methods, number of cases and controls, distribution of genotypes in both case and control groups. Disagreements were resolved through

discussion among the authors or through consultation with a senior reviewer to reach a consensus.

### Statistical analysis

Odds ratios (ORs) with the corresponding 95% confidence intervals (CIs) were pooled to measure the strength of the association between the *mir-196a-2* C>T polymorphism and CRC risk. We assessed the risk of CRC in the contrast models of CC vs. TT, CC + TC vs. TT, CC vs. TC + TT, allele C vs. allele, and TC vs. TT. The stratified analysis according to ethnicity was conducted to estimate ethnic-specific ORs. In addition, the subgroup analysis by control source was also carried out. We performed a chi-square-based Q statistic test to assess the between-study heterogeneity [24], and  $P < 0.05$  indicates significant heterogeneity. When there was an obvious indication of heterogeneity, the fixed-effects model using Mantel and Haenszel's method was employed [25]; otherwise the random-effects model using the DerSimonian and Laird's method was performed [26]. The significance of the summary OR was evaluated by the Z test and  $P < 0.05$  was considered significant. HWE was tested by the chi-square-test for goodness of fit.

Besides, to examine influence from the individual comparisons on our findings, sensitivity analysis was performed by respectively omitting the included studies, one at a time, and recalculating the ORs with 95% CIs.

Publication bias of the included studies was tested by Begg's funnel plot and Egger's test [27]. A symmetric funnel plot suggests no publication bias, and there does exist significant bias in the meta-analysis if the funnel plot is asymmetric. All statistical analyses were performed using the Stata software (version 12.0, Stata Corp LP, College Station, TX, USA). All  $P$ -values were two-sided and  $P < 0.05$  was considered statistically significant.

## Results

### Study characteristics

We conducted a careful search and initially extracted seventy-six publications in total, of which sixty-five irrelevant papers were excluded. After further examination, we included six qualified studies in our final meta-analysis. Of

**Table 1.** Main characteristics of all studies included in the meta-analysis

First author (ref no.)	Country of study	Ethnicity	Source of control	Genotyping method	HWE
Zhan [18]	China	Asian	Hospital	PCR-RFLP	0.849
Min [19]	South Korea	Asian	Population	PCR-RFLP	0.633
Chen [20]	China	Asian	Hospital	PCR-LDR	0.788
Zhu [21]	China	Asian	Hospital	TaqMan	0.789
Hezova [22]	Czech	Caucasian	Hospital	TaqMan	0.291
Vinci [23]	Italy	Caucasian	Hospital	RT-PCR	0.087

PCR: polymerase chain reaction; PCR-RFLP: PCR-restriction fragment length polymorphism; PCR-LDR: PCR-ligation detection reaction; RT-PCR: real time-PCR; TaqMan: TaqManSNP; HWE: Hardy-Weinberg equilibrium.

the selected publications, four were conducted on Asians [18-21] and two were on Caucasians [22, 23]. Moreover, the majority of the selected publications were based on hospital populations [18, 20-23]. The essential information such as first author, year of publication, country of study, genotype detection methods, the sample sizes, and the distribution of the genotypes and alleles were displayed in **Table 1**. The genotype distribution of the all control groups in the studies was consistent with HWE.

#### Meta-analysis results

The main results of the meta-analysis and heterogeneity were listed in **Table 2**. There was no significant heterogeneity across the studies, thus the fixed-effects model was applied for the combined ORs in this meta-analysis. For the overall data including 1,754 cases and 2,430 controls, no obvious association of *mir-196a-2* C>T polymorphism with CRC risk was indicated under the contrast models (OR<sub>CC vs. TT</sub> = 1.14, 95% CI = 0.98-1.33,  $P_{\text{heterogeneity}} = 0.651$ ; OR<sub>CC + TC vs. TT</sub> = 1.04, 95% CI = 0.95-1.15,  $P_{\text{heterogeneity}} = 0.949$ ; OR<sub>CC vs. TC + TT</sub> = 1.13, 95% CI = 0.99-1.30,  $P_{\text{heterogeneity}} = 0.352$ ; OR<sub>allele C vs. allele T</sub> = 1.07, 95% CI = 0.99-1.15,  $P_{\text{heterogeneity}} = 0.627$ ; OR<sub>TC vs. TT</sub> = 1.04, 95% CI = 0.93-1.17,  $P_{\text{heterogeneity}} = 0.930$ ) (**Figure 1**).

Given the possible impact of the confounding factors on the overall results, we further conducted subgroup analyses. In the subgroup analysis according to ethnicity, significantly increased risk of CRC was found in Asians rather than Caucasians under the CC vs. TT model (OR<sub>CC vs. TT</sub> = 1.22, 95% CI = 1.02-1.45,  $P_{\text{heterogeneity}} = 0.718$ ), CC vs. TC + TT model (OR<sub>CC vs. TC + TT</sub> = 1.22, 95% CI = 1.04-1.44,  $P_{\text{heterogeneity}} = 0.590$ ), and allele model (OR<sub>allele C vs. allele T</sub> = 1.10, 95% CI = 1.01-1.20,  $P_{\text{heterogeneity}} = 0.726$ ). In the mean-

while, the population-based studies showed 1.35-fold risk in the CC vs. TC + TT model (OR<sub>CC vs. TC + TT</sub> = 1.35, 95% CI = 1.01-1.81).

#### Sensitivity analysis

Sensitivity analysis was carried out to assess the stability and credibility of the meta-analysis. The deletion of the single studies did not affect the statistical significance of the results (data not shown), suggesting the reliability of our results.

#### Publication bias

We adopted Begg's funnel plots and Egger's test to assess the publication bias. The seemingly symmetrical funnel plots for the overall data indicated there was no publication bias. This was further confirmed in the Egger's test, suggesting the stable results of our meta-analyses (CC + TC vs. TT: Begg's test:  $P = 1.000$ ; Egger's test:  $P = 0.958$ ) (**Figure 2**).

#### Discussion

An increasing body of evidence supports that genetic polymorphisms play a crucial role in determining the risk of cancer, and association studies have been devoted to searching susceptibility genes involved in cancer [28]. Nevertheless, the association studies lack statistical power due to small sample size and different study designs, resulting in apparently contradictory findings [29]. Meta-analysis is a powerful way to estimate the genetic susceptibility to cancer risk by pooling the individual data into one dataset, thus enhancing the statistical power of the results [30]. Therefore, we conducted a meta-analysis to gain a more precise estimation of the effects of *mir-196a-2* C>T polymorphism on the risk of CRC.

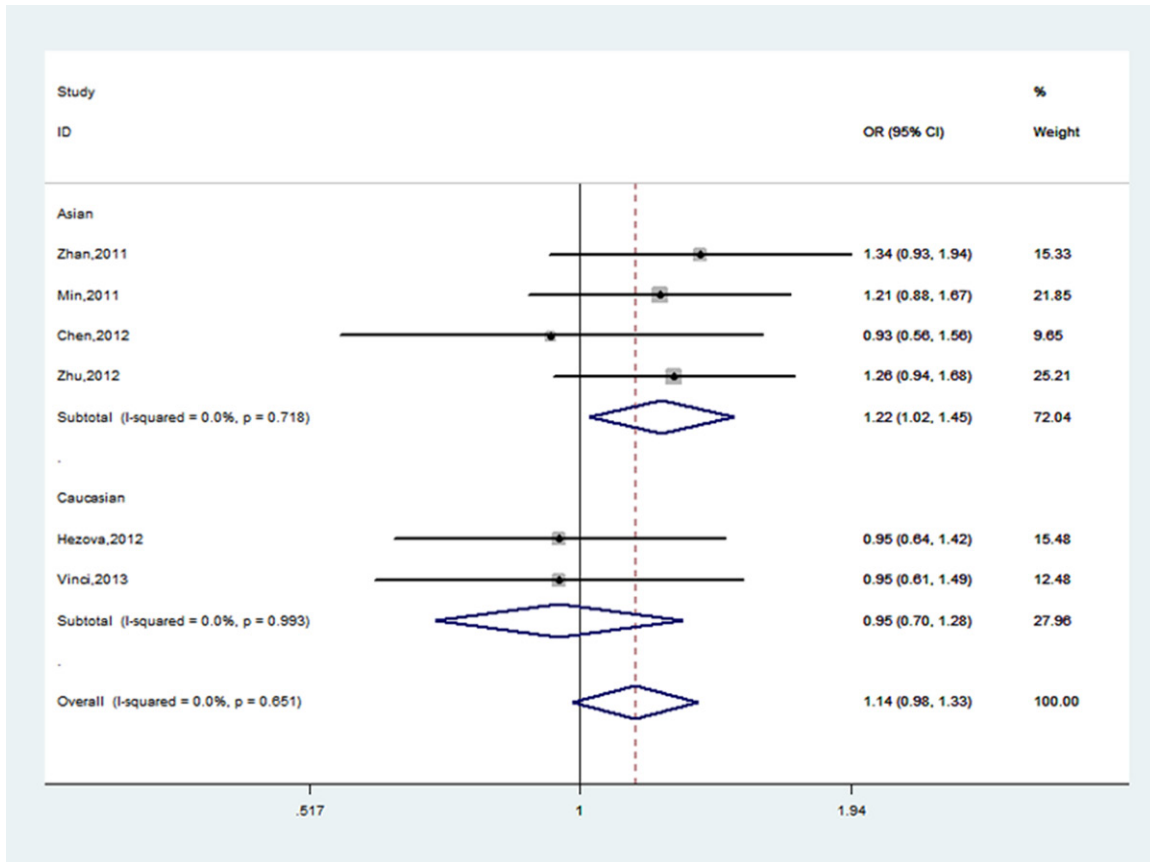
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**Table 2.** Meta-analysis of the association between the *mir-196a-2* C>T polymorphism and CRC risk

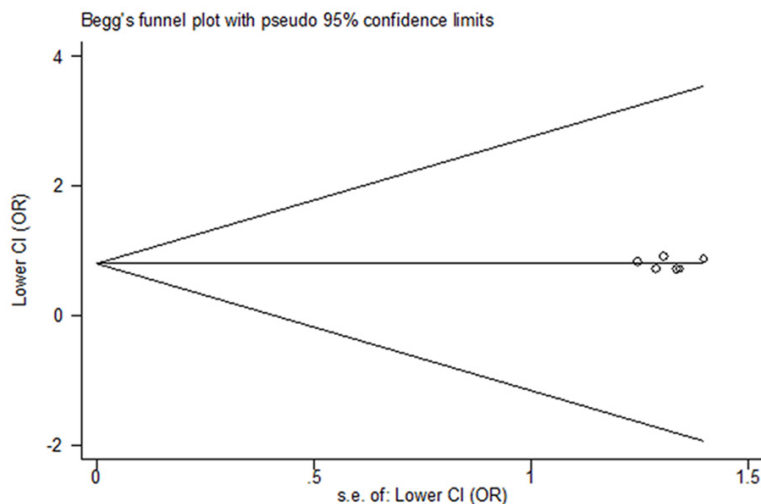
	CC vs. TT		CC + TC vs. TT		CC vs. TC + TT		Allele C vs. Allele T		TC vs. TT	
	OR (95% CI)	$P_h$	OR (95% CI)	$P_h$	OR (95% CI)	$P_h$	OR (95% CI)	$P_h$	OR (95% CI)	$P_h$
Ethnicity										
Asian	1.22 (1.02, 1.45)	0.718	1.06 (0.95, 1.18)	0.880	1.22 (1.04, 1.44)	0.590	1.10 (1.01, 1.20)	0.726	1.05 (0.93, 1.20)	0.808
Caucasian	0.95 (0.70, 1.28)	0.939	0.98 (0.79, 1.20)	0.933	0.93 (0.71, 1.21)	0.462	0.96 (0.82, 1.13)	0.759	0.96 (0.73, 1.27)	0.847
Source of control										
Hospital	1.12 (0.95, 1.33)	0.535	1.05 (0.94, 1.17)	0.894	1.08 (0.92, 1.26)	0.427	1.06 (0.97, 1.15)	0.502	1.06 (0.93, 1.21)	0.911
Population	1.21 (0.88, 1.67)		1.02 (0.84, 1.24)		1.35 (1.01, 1.81)		1.09 (0.93, 1.28)		0.98 (0.77, 1.24)	
Total	1.14 (0.98, 1.33)	0.651	1.04 (0.95, 1.15)	0.949	1.13 (0.99, 1.30)	0.352	1.07 (0.99, 1.15)	0.627	1.04 (0.93, 1.17)	0.930

Abbreviations:  $P_h$ :  $P$ -value of heterogeneity test; CI: confidence interval; OR, odds ratio.

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**Figure 1.** ORs of overall colorectal cancer (CRC) risks associated with the *mir-196a-2* C>T polymorphism under CC vs. TT model by fixed effects for each of the 18 included studies. For each study, the estimates of OR and its 95% CI were plotted with a box and a horizontal line. ♦: Pooled OR and its 95% CI.



**Figure 2.** Funnel plot analysis of the publication bias for the *mir-196a-2* C>T polymorphism (CC + TC vs. TT).

The polymorphism of *mir-196a-2* C>T have been extensively studied with respect to its role in the susceptibility to cancers, especially CRC.

Zhan et al. suggested that the polymorphism of *mir-196a-2* C>T was significantly associated with CRC risk [18, 19, 21]. Conversely, Chen et al. found no CRC risk in relation to this polymorphism [20, 22]. Additionally, previous meta-analyses also generated inconsistent results. He et al. demonstrated that *mir-196a-2* C>T polymorphism had significant association with a decreased cancer risk, in particular with a decreased risk for CRC [31]. But Guo et al. found that *mir-196a-2* C>T polymorphism increased the susceptibility to CRC [32]. However, the results of our study revealed no association of *mir-196a-2* C>T polymorphism with CRC risk. The different sample sizes may explain these contradictory

findings, but more relevant studies required to be conducted for further confirmation of the association.

In the subgroup analysis by ethnicity, we observed the C allele of the *mir-196a-2* C>T polymorphism, compared with the T allele, significantly increased the risk of CRC in Asian populations. But no association was indicated in Caucasian populations. Our finding was supported by Wang et al. [33]. This suggested that the *mir-196a-2* C>T polymorphism may have ethnic-specific susceptibility to CRC risk. The risk factors such as different genetic backgrounds and lifestyles differently influence the susceptibility to the disease.

The subgroup analysis according to source of controls indicated significant increased CRC risk in the population-based subgroup instead of the hospital-based group. The possible explanation is that selection bias may exist, for hospital-based groups may not always represent the general population. So selection of representative control groups with matching demographic characteristics was quite essential for the future association studies.

The results should be interpreted with caution because of the potential limitations in this study. First, only the published articles in English were included in this meta-analysis, and this may have brought bias to our findings. Second, despite no obvious publication bias implicated in this meta-analysis, we could not exclude the possibility that our significant results might be due to chance, because the undetectable publication bias may have an impact on our analysis. Finally, we failed to evaluate the gene-gene and gene-environment interaction because of insufficient original data in the individual studies.

In conclusion, our meta-analysis did not provide statistical evidence for the association between the *mir-196a-2* C>T polymorphism and the overall CRC susceptibility. But we did find a significant association of the C allele of this polymorphism with the increased susceptibility to CRC in Asians. However, gene-gene and gene-environment interaction are needed to be considered in the future genetic association studies to confirm these findings.

### Disclosure of conflict of interest

None.

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