

Polymorphism of miRNA and esophageal cancer risk: an updated systemic review and meta-analysis

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Xudong Guo¹
Lei Zhao²
Yi Shen¹
Yi Shao¹
Wenqiang Wei³
Fen Liu¹

¹Department of Epidemiology and Health Statistics, School of Public Health, Beijing Municipal Key Laboratory of Clinical Epidemiology, Capital Medical University, Beijing 100069, People's Republic of China; ²Department of Molecular Physiology and Biophysics, Holden Comprehensive Cancer Center, University of Iowa Carver College of Medicine, Iowa City, IA 52242, USA; ³National Cancer Center/Cancer Hospital, Chinese Academy of Medical Science & Peking Union Medical College, Beijing 100021, People's Republic of China

Background: Accumulating evidence has demonstrated that single nucleotide polymorphisms (SNPs) in microRNAs (miRNAs) (referred to as miR-SNPs) participate in the process of carcinogenesis by altering the expression and structure of mature miRNAs. However, the associations between several previously reported miR-SNPs, including miR-196a2 rs11614913, miR-146a rs2910164, miR-34b/c rs4938723, and miR-423 rs6505162 and the susceptibility of esophageal squamous cell carcinoma (ESCC) remain controversial. We, therefore, performed a comprehensive meta-analysis to systemically evaluate the correlation of genetic polymorphisms in these four miRNAs with the risk of ESCC.

Methods: Relevant studies were searched in PubMed and other electronic databases up to August 2018, supplemented by a manual search of references from retrieved articles. The pooled ORs with 95% CIs were calculated using a random-effects model.

Results: A total of 22 studies from 13 published articles were included in the meta-analysis. All studies have a relatively high score of quality assessment. The pooled analysis indicated that individuals with the variant TT genotype of rs11614913 in miR-196a2 gene have a significantly decreased risk of ESCC compared with CC genotype (OR =0.83, 95% CI: 0.73–0.95). The decreased risk of ESCC was also shown in the recessive model (TT vs CT/CC: OR=0.86, 95% CI: 0.77–0.96) and allele model (T vs C: OR=0.93, 95%CI: 0.87–0.99). The significantly reduced risk of ESCC was also observed in the polymorphisms of the miR-34b/c rs4938723 locus. The similar tendency was presented in the subgroup of Chinese Han population when stratified by ethnicity. However, no significant associations were observed in the miR-146a rs2910164 and miR-423 rs6505162 with the susceptibility of ESCC in any genetic model.

Conclusion: Our results suggested that the polymorphisms of miR-196a and miR-34b/c genes were related to the risk of ESCC, especially among Chinese. The findings of this study, however, need to be confirmed in further researches.

Keywords: microRNA, single nucleotide polymorphism, ESCC, meta-analysis

Introduction

Esophageal cancer is one of the most common malignancies in the world. It ranks the seventh in terms of incidence (572,000 new cases) and the sixth in mortality overall (509,000 deaths) in 2018.¹ Esophageal squamous cell carcinoma (ESCC) accounts for over 90% of all esophageal cancer cases in lower income countries, especially in parts of Asia.¹ The 5-year survival rate of ESCC patients is less than 15%.² However, patients diagnosed at earlier stages have better outcomes than those diagnosed latterly.³ Therefore, to identify and treat ESCC at early stages may continue to be the most effective strategy for improving patient survival. Thus, screening patients who are at high risk and identifying more efficient risk predicting factors for ESCC are urgently needed.

Correspondence: Fen Liu
Department of Epidemiology and Health Statistics, School of Public Health, Beijing Municipal Key Laboratory of Clinical Epidemiology, Capital Medical University, No.10 Xitoutiao, Youcanmenwai Street, Beijing 100069, People's Republic of China
Tel + 86 108 391 1497
Email liufen05@ccmu.edu.cn

Single nucleotide polymorphisms (SNPs) have been found to be associated with the risk of ESCC in different areas and populations, indicating that specific SNPs may affect the susceptibility to ESCC and potentially be used as biomarkers of ESCC.^{4–6} MicroRNAs (miRNA) are involved in a variety of biological and pathologic processes including cell differentiation, proliferation, apoptosis, and metabolism, and are emerging as highly tissue-specific biomarkers with potential clinical applicability for defining cancer types and origins.^{7–10} Furthermore, accumulating evidence showed that specific SNPs in miRNA genes (miR-SNPs) can influence the function of the corresponding miRNA by modulating the transcription of the primary transcript, the pri-miRNA and pre-miRNA processing and maturation, and miRNA–mRNA interactions and thereby affect the risk of developing cancer.^{11,12} Therefore, investigations into the genetic variations of miRNAs involved in ESCC may provide new insights into the pathogenesis and help in finding novel biomarkers of this disease.

Recently, several research groups have reported the relationship of miR-SNPs with the risk of esophageal cancer.^{13–25} However, the results of these studies were controversial and inconclusive. For example, three studies reported that TT genotype in rs11614913/miR-196a2 might contribute to ESCC susceptibility in the Chinese population,^{14,19,23} whereas the other two studies showed different results.^{17,20} For the SNP rs4938723 in miR-34b/c, two articles reported that CC genotype and C allele could decrease the risk of ESCC.^{21,24} However, another two studies found different results.^{18,22} Therefore, we conducted this meta-analysis in order to comprehensively analyze the associations between the SNPs in four ESCC-involving miRNAs, including miR-196a2 rs11614913, miR-146a rs2910164, miR-34b/c rs4938723, and miR-423 rs6505162, and the susceptibility to ESCC and to evaluate their potential as a risk-predicting biomarker for ESCC.

Methods

Search strategy

To identify all potentially eligible studies, we performed a comprehensive literature search in National Library of Medicine (PubMed), ISI Web of Science databases, Wiley, Embase, the Cochrane Library, Google Scholar, Controlled Trials metaRegister, China National Knowledge Infrastructure (CNKI) and Chinese Wanfang databases until August 15th, 2018 with language limited to English. The following keywords were used for literature retrieval: “esophageal cancer

OR esophageal neoplasms OR esophageal squamous cell carcinoma OR esophageal adenocarcinoma” AND “polymorphism or variation OR susceptibility” and “microRNA OR miRNA OR microRNAs”. We also manually screened the references of eligible articles and reviewed articles for other related studies. This study was performed in accordance with the PRISMA statement checklist (S1 PRISMA checklist).

Inclusion and exclusion criteria

Studies were included in the meta-analysis only if they met all of the following criteria: (1) an evaluation of the association between the polymorphisms of miR-196a2 rs11614913, miR-146a rs2910164, miR-34b/c rs4938723 and miR-423 rs6505162 locus and ESCC susceptibility (with full text); (2) a case-control study; (3) detailed genotype data were available for calculating ORs and the corresponding 95% CI; and (4) the genotype distribution of the studies had to be consistent with a Hardy–Weinberg equilibrium (HWE) in the control group ($P>0.05$). Articles were excluded for the reasons indicated in Figure 1. Two authors independently assessed all retrieved studies based on the inclusion and exclusion criteria. Any disagreement was resolved through discussion or consultation with a third author.

Data extraction and quality assessment

The following information from each publication was independently extracted by two authors: the first author’s last name, year of publication, ethnicity, genotyping methods, the source of the control group, sample size and genotype frequency.

The quality of the included studies was evaluated independently by two investigators using the Newcastle-Ottawa scale (NOS).^{26,27} The NOS criterion is divided into three categories: (1) subject selection; (2) the comparability of the subject; (3) exposure. The total score is nine, a study scored from zero to four is classified as low-quality, from five to six is classified as moderate-quality and from seven to nine is considered as a high-quality study. The moderate- and high-quality studies were included in the meta-analysis. Any disagreement was resolved by discussion before any final decision was made.

Statistical analysis

The strength of association between miR-SNPs and ESCC susceptibility was analyzed by the pooled OR with its 95% CI based on different gene models. There are five common genetic models including allele model (W vs V) (W for the

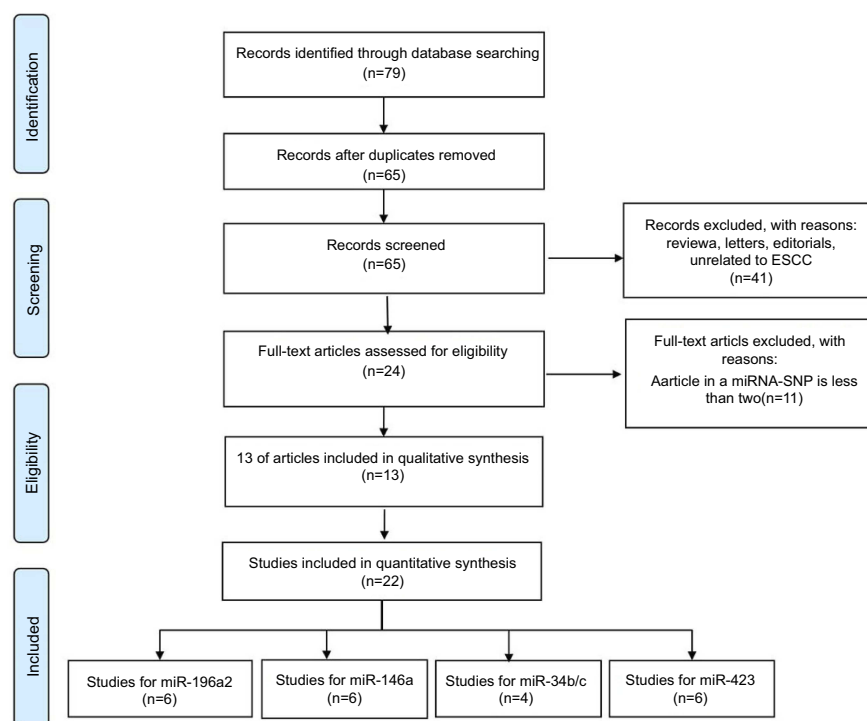


Figure 1 The process of study selection.

Abbreviations: ESCC, esophageal squamous cell carcinoma; SNPs, single nucleotide polymorphisms; miRNAs, microRNAs.

wild allele, V for variation allele), heterozygote model (WV vs WW), homozygote model (VV vs WW), dominant model (WV+VV vs WW), and recessive model (VV vs WW+WW). A χ^2 -based Cochran's Q test and I^2 statistics were used to assess the heterogeneity among studies.^{28,29} If there were significant heterogeneities among studies ($P < 0.1$ or $I^2 > 50\%$), the random effect model was used for calculation of pooled OR value. Otherwise, the fixed effect model was used. The significance of the pooled OR was determined by the Z test, a $P < 0.05$ was considered significant. Moreover, a sensitivity analysis was used to assess the stability of the results. Subgroup analyses were performed to explore the potential heterogeneity among the included studies. Finally, publication bias was investigated by Begg's funnel plot and Egger's linear regression test. All statistical analyses in this meta-analysis were performed with STATA version 14.0 (Stata Corporation, College Station, TX, USA). All P -values were two-sided.

Trial sequential analysis

Trial sequential analysis (TSA) was performed as described by the user manual using the TSA software version 0.9.5.10 Beta (www.ctu.dk/TSA). After adopting a level of significance at 5% for type I error, 20% for type

II error and a relative risk reduction (RRR) assumption of 10%, the required information size was calculated and TSA monitoring boundaries were built.

RNA secondary structure analysis

The RNAfold Web Server was used to analyze the potential secondary structure modification caused by the mutant allele of miRNAs polymorphisms (<http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi>).^{30,31}

Results

Characteristics of the included studies

The literature search process of this meta-analysis is shown in **Figure 1**. Seventy-nine articles were retrieved by the online and manual search, and 65 abstracts were remains after removing duplicate studies. The title and abstract of the studies were then screened, and the full text of remaining articles was read. Finally, thirteen published articles were included in this meta-analysis (**Table 1**).^{13–25}

In total, 22 studies from 13 articles covering 4 types of miR-SNPs were included in this meta-analysis. Among the 22 studies, 17 studies were conducted in China, 3 in India, 1 in Iran and 1 in Africa. All of the studied miR-SNPs fulfilled the HWE ($P > 0.05$).

The quality of the included articles was assessed by the NOS scores (Table S1). Overall, all the included studies had a high quality (Table 1).

The associations between miRNA polymorphisms and ESCC risk

Six studies involving 3505 cases and 4386 controls investigated the association between miR-196a2 rs11614913 T>C variant and ESCC susceptibility;^{14,15,17,19,20,23} The meta-analysis results were summarized in Table 2. The pooled analysis indicated that individuals with the variant TT genotype have a significantly decreased risk of ESCC compared with CC genotype (OR =0.83, 95% CI: 0.73–0.95, $P=0.006$, $I^2=82.4\%$) (Figure 2). The decreased risk of ESCC was also found in the recessive model (TT vs CT/CC: OR=0.86, 95% CI: 0.77–0.96, $P=0.005$, $I^2=81.3\%$) (Figure 3) and allele model (T vs C: OR=0.93, 95%CI: 0.87–0.99, $P=0.021$, $I^2=76.8\%$) (Figure 4)

Four studies focused on miR-34b/c rs4938723 T>C, which included 2161 ESCC and 2447 controls;^{18,21,22,24} The pooled analysis indicated that the polymorphisms of the miR-34b/c rs4938723 locus was correlated with decreased risk of ESCC in the domain model (CC/CT vs. TT, OR=0.87, 95%CI: 0.77–0.98, $P=0.018$, $I^2=3.9\%$) (Figure 5), recessive model (CC vs CT+TT, OR=0.68, 95%CI: 0.56–0.83, $P<0.001$, $I^2=0.0\%$) (Figure 6), allele model (C vs T, OR=0.85, 95%CI: 0.78–0.93, $P<0.001$, $I^2=0.0\%$) (Figure 7) and CC vs.TT (OR=0.65, 95%CI: 0.52–0.79, $P<0.001$, $I^2=0.0\%$) (Figure 8).

For miR-146a rs2910164 C>G polymorphism, six relevant studies with 3120 cases and 4036 controls were included in this meta-analysis.^{13,15,17,19,22,23} We did not find significant associations with ESCC risk in any genetic models, as well as in the subgroup analysis (Table S2).

At last, six studies with 3083 patients and 4483 controls reported the association of miR-423 rs6505162 C>A polymorphism with the risk of ESCC.^{15,16,18,22,23,25} No statistically significant association was observed in the pooled analysis for CC genotype compared with all genetic carriers, and the results are summarized in Table S3. We also did not find a significant association of this miR-SNP with ESCC risk in the subgroup analysis (Table S3).

Subgroup analysis and sensitivity analysis

For miR-196a2 rs11614913, the meta-analysis results indicated the existence of significant heterogeneity in the overall analysis. Therefore, we further carried out different

subgroup analyses according to study population (Chinese/Indian), genotyping method (PCR-LDR/PCR-RFLP/SNPscan/SNaPshot assay), and source of controls (hospital/population). As shown in Table 2, the subgroup analysis performed based on the ethnicity of subjects (Chinese vs Indian) indicated that homozygote variant genotype (TT vs CC: OR=0.81, 95%CI: 0.71–0.93, $P=0.002$), allele model (T vs C: OR=0.91, 95%CI: 0.85–0.97, $P=0.006$), and recessive model (TT vs CT +CC: OR=0.85, 95%CI: 0.76–0.94, $P=0.002$) were all correlated with a decreased ESCC risk in Chinese Han population. In the subgroup analysis based on the source of controls showed that there were significant correlations with a decreased ESCC risk in hospital-based control for allele model (T vs C: OR=0.91, 95%CI: 0.84–0.98, $P=0.012$), homozygote variant genotype (TT vs CC: OR=0.79, 95%CI: 0.68–0.93, $P=0.004$) and recessive model (TT vs CT+CC: OR=0.85, 95%CI: 0.75–0.96, $P=0.008$). The same tendency was found in the subgroup analyses based on the SNaPshot assay genotyping method (Table 2).

Although the heterogeneity analysis result was negative for the miR-34b/c rs4938723, we still performed subgroup analyses based on study population (Chinese Han/Chinese Kazakh) and source of controls (hospital/population) to further explore the potential correlation of miR-34b/c rs4938723 polymorphism with the susceptibility of ESCC. As shown in Table 3, the subgroup analysis results indicated that reduced ESCC risk in Chinese Han population was observed for allele model (C vs T: OR=0.84, 95%CI: 0.76–0.94, $P=0.001$), homozygote variant genotype (CC vs TT: OR=0.59, 95%CI: 0.44–0.80, $P=0.001$), recessive model (CC vs CT+TT: OR=0.65, 95%CI: 0.56–0.83, $P<0.001$) and domain model (CC+CT vs TT: OR=0.86, 95%CI: 0.76–0.98, $P=0.018$). The same tendency was observed in the population-based study for allele model (C vs T: OR=0.83, 95%CI: 0.73–0.94, $P=0.002$) (Table 3).

The sensitivity analysis of miR-196a2 rs11614913 was conducted by sequentially removing one individual study per time to weigh the influence of each study on the overall meta-analysis. No significant change in the heterogeneity was observed for this polymorphism (Figure S1).

Publication bias

We found no evidence of publication bias using the Begg's funnel plots and Egger's test for all genetic models of miR-SNPs included in this study (data not shown).

Table I Characteristics of the included literature in this meta-analysis

First author	Year	Ethnicity	Genotyping methods	Source of control	Sample size cases/controls	Genotypes distribution of cases and controls			NOS
						miR-196a2 rs11614913			
						TT	TC	CC	
Shen et al.	2016	Chinese Han	SNaPshot assay	HB	1400/2185	407/672	698/1121	295/392	7
Wang et al.	2014	Chinese Han	PCR-LDR	PB	597/597	162/154	307/298	128/145	8
Qu et al.	2014	Chinese Han	PCR-RFLP	PB	381/426	48/82	207/211	126/133	8
Umar et al.	2013	Indian	PCR-RFLP	HB	289/309	146/171	121/171	22/16	7
Wei et al.	2013	Chinese Han	SNPscan	HB	380/380	106/113	196/170	65/87	7
Wang et al.	2010	Chinese Han	SNaPshot assay	HB	458/489	148/128	262/250	48/111	7
						miR-146a rs2910164			
						CC	GC	GG	
Shen et al.	2016	Chinese Han	SNaPshot assay	PB	1400/2185	220/345	685/1060	495/780	7
Zhu et al.	2015	Chinese Kazakh	MALDI-TOF MS	HB	248/300	82/99	120/139	36/40	7
Qu et al.	2014	Chinese Han	PCR-RFLP	PB	381/426	116/123	203/228	62/75	8
Umar et al.	2013	Indian	PCR-RFLP	HB	289/309	146/171	121/122	22/16	7
Wei et al.	2013	Chinese Han	SNPscan	HB	380/380	117/122	184/181	67/67	7
Guo et al.	2010	Chinese Han	SNaPshot assay	HB	444/468	234/206	190/220	20/42	7
						miR-34b/c rs4938723			
						TT	TC	CC	
Bulibu et al.	2017	Chinese Han	DHPLC	HB	175/186	64/52	74/81	37/53	8
Zhu et al.	2015	Chinese Kazakh	MALDI-TOF MS	HB	248/300	113/122	99/122	25/30	7
Zhang et al.	2014	Chinese Han	SNaPshot assay	PB	1109/1275	489/569	536/573	84/133	8
Yin et al.	2013	Chinese Han	PCR-LDR	HB	629/686	277/310	278/290	45/73	7
						miR-423 rs6505162			
						CC	CA	AA	
Nariman-Saleh-Fam et al.	2017	Iran Caucasian	PCR-RFLP	HB	200/300	141/110	123/81	36/9	7
Shen et al.	2016	Chinese Han	SNaPshot assay	PB	1400/2185	920/1421	421/680	59/84	7
Zhu et al.	2015	Chinese Kazakh	MALDI-TOF MS	HB	248/300	99/109	122/140	21/31	7
Umar et al.	2013	Indian	PCR-RFLP	HB	289/309	90/96	132/143	67/70	7
Wang et al.	2013	Africa Black	TaqMan	PB	565/1000	16/12	128/184	207/376	8
Yin et al.	2013	Chinese Han	PCR-LDR	HB	629/686	374/425	197/207	29/19	7

Abbreviations: HB, hospital-based; PB, population-based DHPLC, denaturing high-performance liquid chromatography; MALDI-TOF MS, Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry; PCR-LDR, Polymerase Chain Reaction ligase detection reaction; PCR-RFLP, Polymerase Chain Reaction restriction fragment length polymorphism NOS, Newcastle-Ottawa scale.

Trial sequential analysis

The recessive model for miR-196a2 rs11614913 was then subjected to the trial sequential analysis to further strengthen the robustness of its association with ESCC risk. According to TSA result, the required information size was 32610 subjects to demonstrate the issue (Figure S2). Until now, the cumulative Z-curve has not crossed the trial monitoring boundary before reaching the required information size,

indicating that the current evidence is insufficient and more trials need to be included for this assay.

RNA secondary structure analysis

We conducted an RNA secondary structure analysis of the miR-196a2 rs11614913 polymorphisms using the RNAfold Web server. This SNP decreased the minimum free energy (MFE) from -52.02 kcal/mol of the C allele

Table 2 Meta-analysis of the association between miR-196a2 rs11614913 and esophageal cancer risk

miR-196a2	N	T/C		TT/CC		CT/CC		TT+CT/CC		TT/CT+CC	
		OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
Overall	6	0.93 (0.87–0.99)	0.021	0.83 (0.73–0.95)	0.006	1.00 (0.90–1.12)	0.92	0.95 (0.85–1.05)	0.319	0.86 (0.77–0.96)	0.005
Ethnicity											
Chinese Han	5	0.91 (0.85–0.97)	0.006	0.81 (0.71–0.93)	0.002	1.04 (0.84–1.27)	0.738	0.92 (0.82–1.03)	0.143	0.85 (0.76–0.94)	0.002
Indian	1	1.20 (0.93–1.56)	0.157	1.61 (0.82–3.18)	0.170	1.16 (0.83–1.62)	0.380	1.21 (0.88–1.67)	0.238	1.51 (0.78–2.93)	0.225
Genotyping methods											
PCR-LDR	2	0.95 (0.88–1.03)	0.323	0.89 (0.76–1.06)	0.187	0.91 (0.78–1.06)	0.230	0.91 (0.79–1.05)	0.182	0.96 (0.84–1.09)	0.501
PCR-RFLP	2	0.96 (0.82–1.12)	0.617	0.82 (0.57–1.17)	0.264	1.09 (0.87–1.37)	0.448	1.05 (0.80–1.29)	0.700	0.76 (0.55–1.06)	0.106
SNPscan	1	1.09 (0.89–1.34)	0.424	1.26 (0.83–1.90)	0.284	1.54 (1.05–2.26)	0.026	1.42 (0.99–2.04)	0.052	0.92 (0.67–1.26)	0.622
SNaPshot assay	1	0.68 (0.57–0.82)	<0.001	0.37 (0.25–0.57)	<0.001	0.90 (0.67–1.22)	0.511	0.74 (0.56–0.98)	0.038	0.39 (0.27–0.57)	<0.001
Design											
PB	2	0.98 (0.87–0.99)	0.770	0.94 (0.73–1.21)	0.624	1.11 (0.90–1.36)	0.354	1.05 (0.86–1.28)	0.627	0.90 (0.72–1.11)	0.310
HB	4	0.91 (0.84–0.98)	0.012	0.79 (0.68–0.93)	0.004	0.96 (0.84–1.09)	0.488	0.91 (0.81–1.03)	0.140	0.85 (0.75–0.96)	0.008

Abbreviations: HB, hospital-based; PB, population-based; PCR-LDR, Polymerase Chain Reaction ligase detection reaction; PCR-RFLP, Polymerase Chain Reaction restriction fragment length polymorphism.

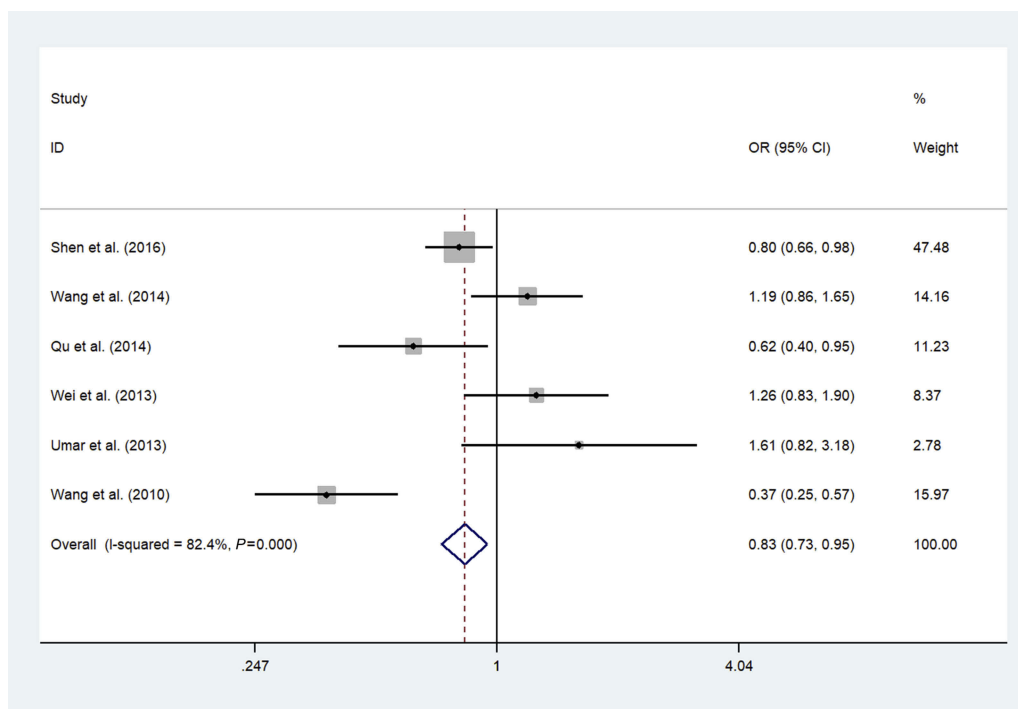


Figure 2 Forest plot shows the odds ratio for the associations between miR-196a2 gene rs11614913 polymorphism and ESCC risk (TT vs CC).

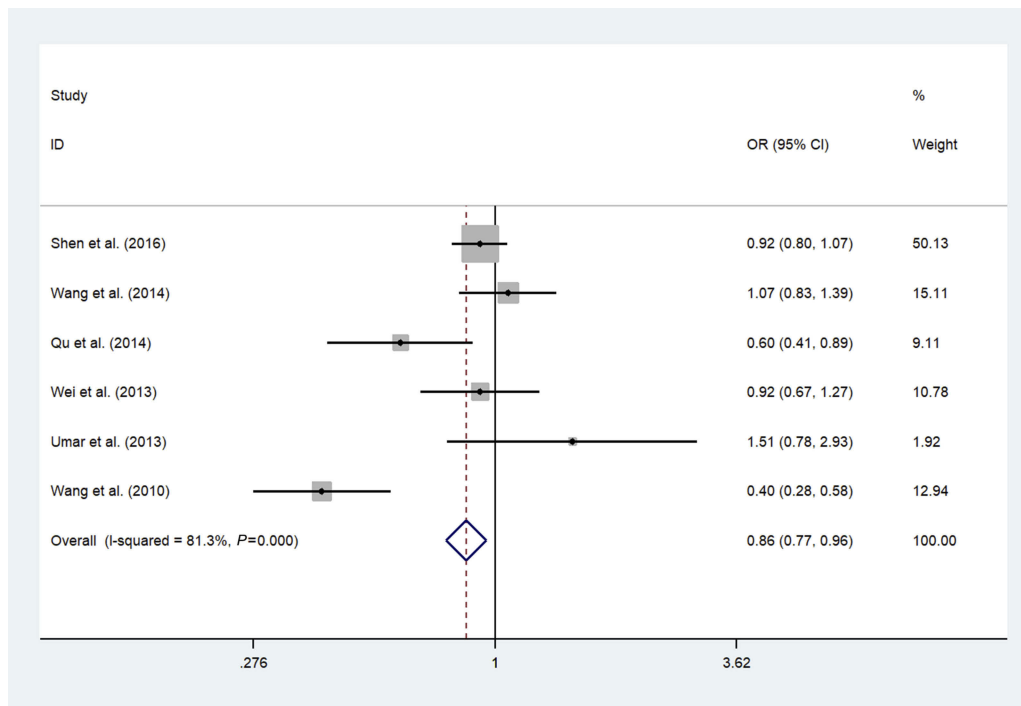


Figure 3 Forest plot shows the odds ratio for the associations between miR-196a2 gene rs11614913 polymorphism recessive model and ESCC risk (TT vs CC+CT).

to -46.52 kcal/mol of the T allele, suggesting a less stable secondary structure for the variant T allele, and

therefore this variant might affect the stability of the secondary structure of miR-196a2.

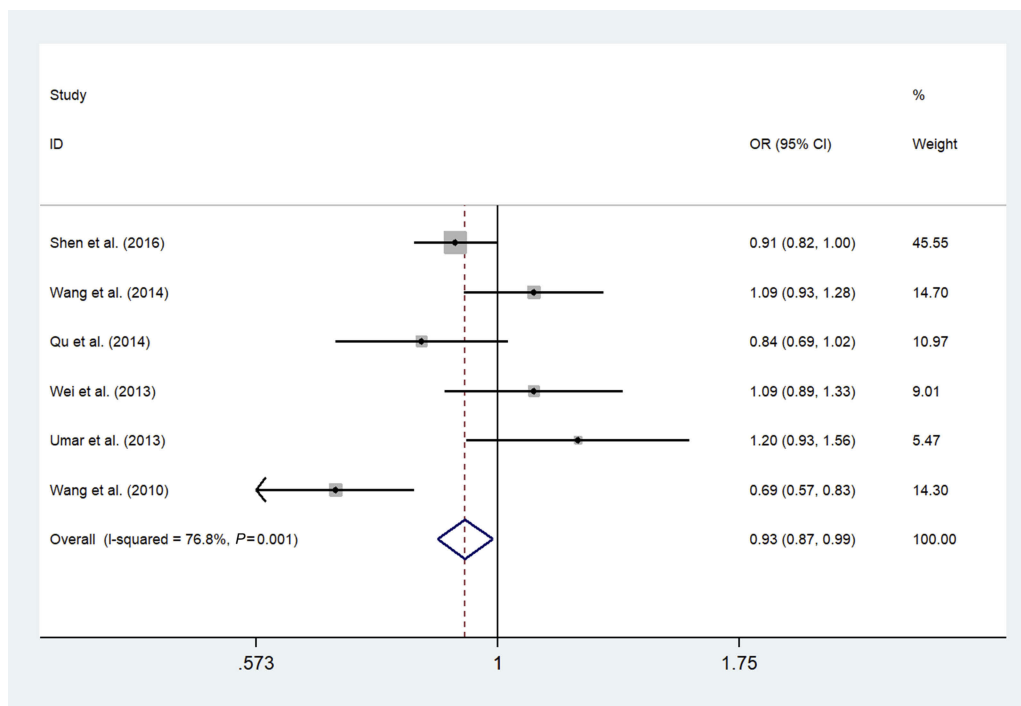


Figure 4 Forest plot shows the odds ratio for the associations between miR-196a2 gene rs11614913 polymorphism allele model and ESCC risk (T vs C).

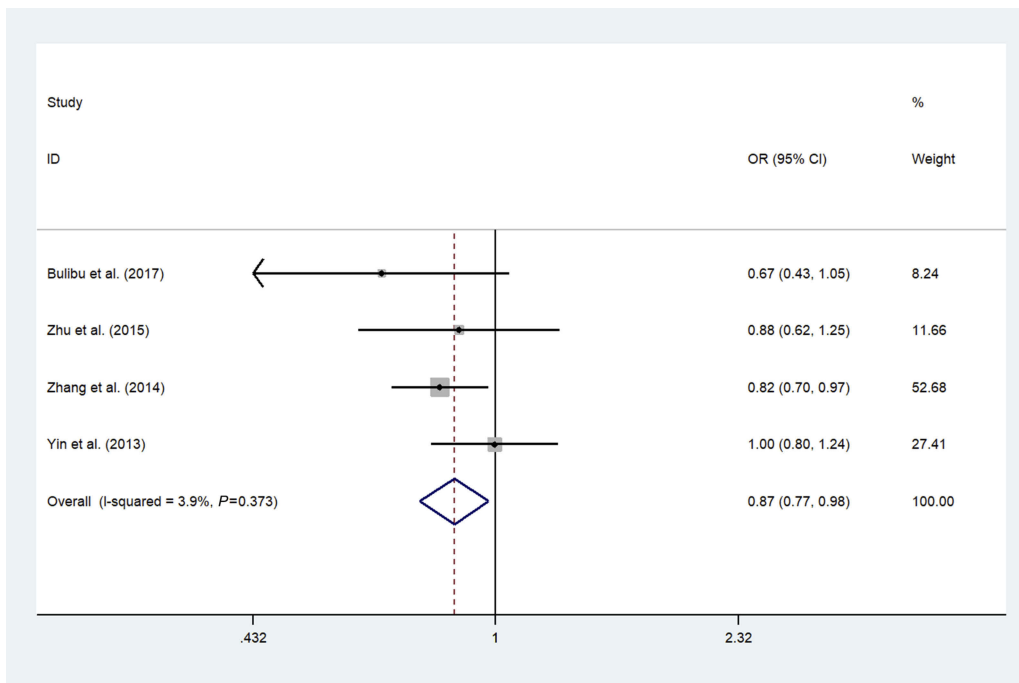


Figure 5 Forest plot shows the odds ratio for the associations between miR-34b/c rs4938723 polymorphism domain model and ESCC risk (CC+CT vs TT).

Discussion

This meta-analysis shows that overall, variant TT genotype and T allele carriers of the rs116149138473 polymorphism of miR-196a2 gene are correlated to reduced risk for ESCC, especially among Chinese population (Table 2 and Figures

2–4). The miR-196a2 polymorphism site rs11614913 is located in the mature sequence of miR-196a-3p (passenger strand), and it could lead to less efficient processing of the miRNA precursor to its mature form and therefore reduce the capacity of mature miR-196a-3p in regulating target

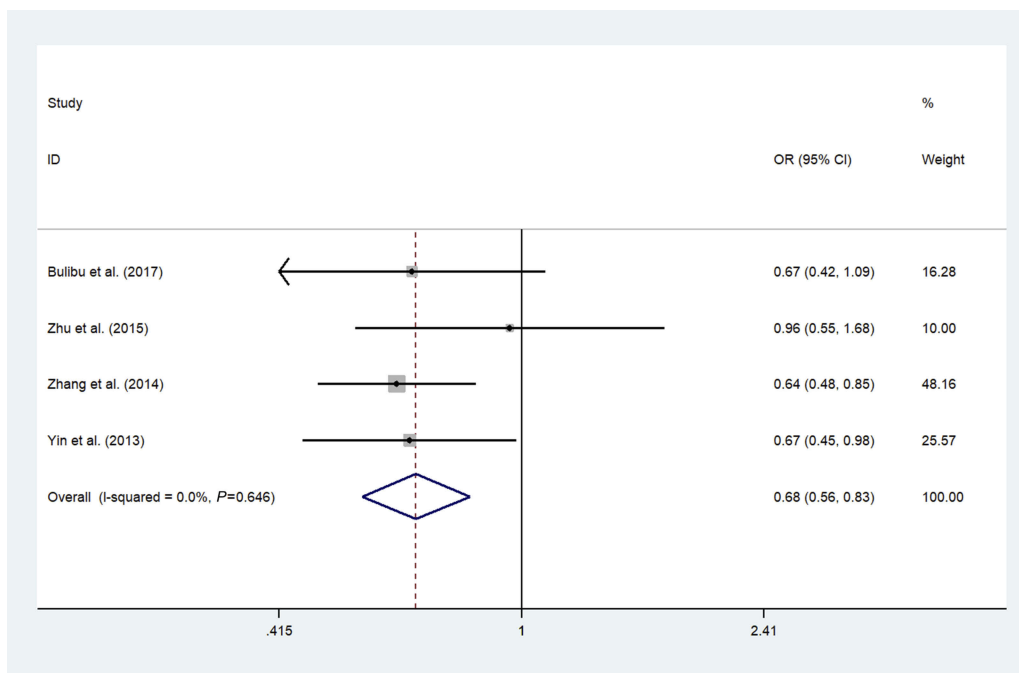


Figure 6 Forest plot shows the odds ratio for the associations between miR-34b/c rs4938723 polymorphism recessive model and ESCC risk (CC vs CT+TT).

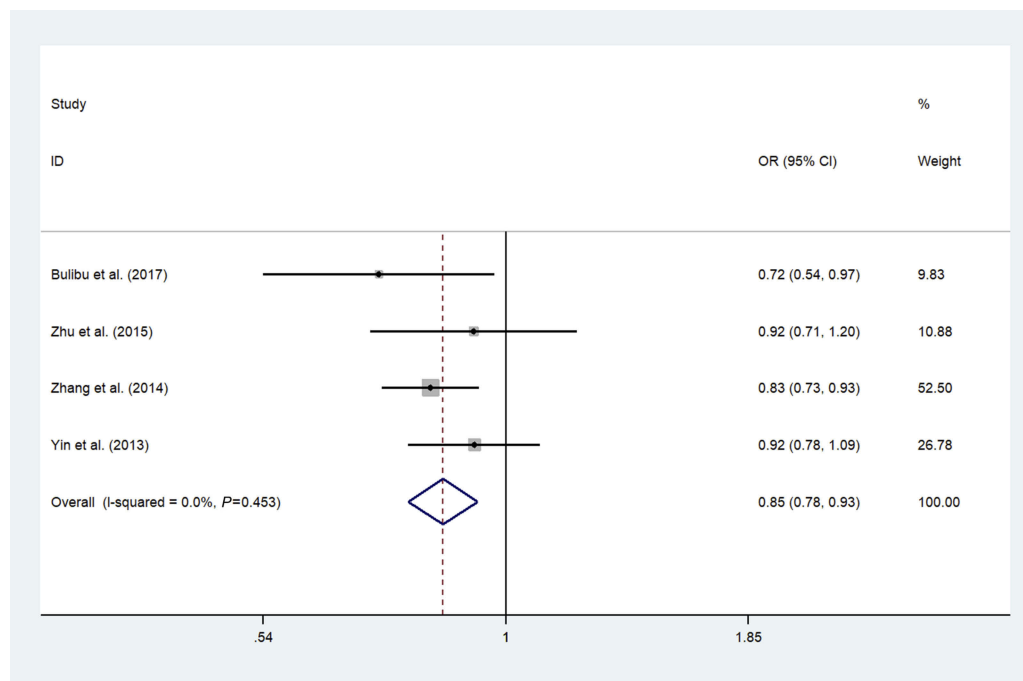


Figure 7 Forest plot shows the odds ratio for the associations between miR-34b/c rs4938723 polymorphism allele model and ESCC risk (C vs T).

genes.³² Another research reported that miR-196a2 rs11614913 not only influences the levels of mature miR-196a but also has an effect on target gene expression.³³ Our further RNA secondary structure analysis suggested that the variant T allele might affect the stability of the secondary structure of miR-196a2.

In the subgroup analyses, we did not find rs116149138473 polymorphism of miR-196a2 gene was associated with the ESCC risk in the population-based studies. Since only two studies were included in this subgroup analysis, the limited sample size may not have enough power to detect the significance. In addition,

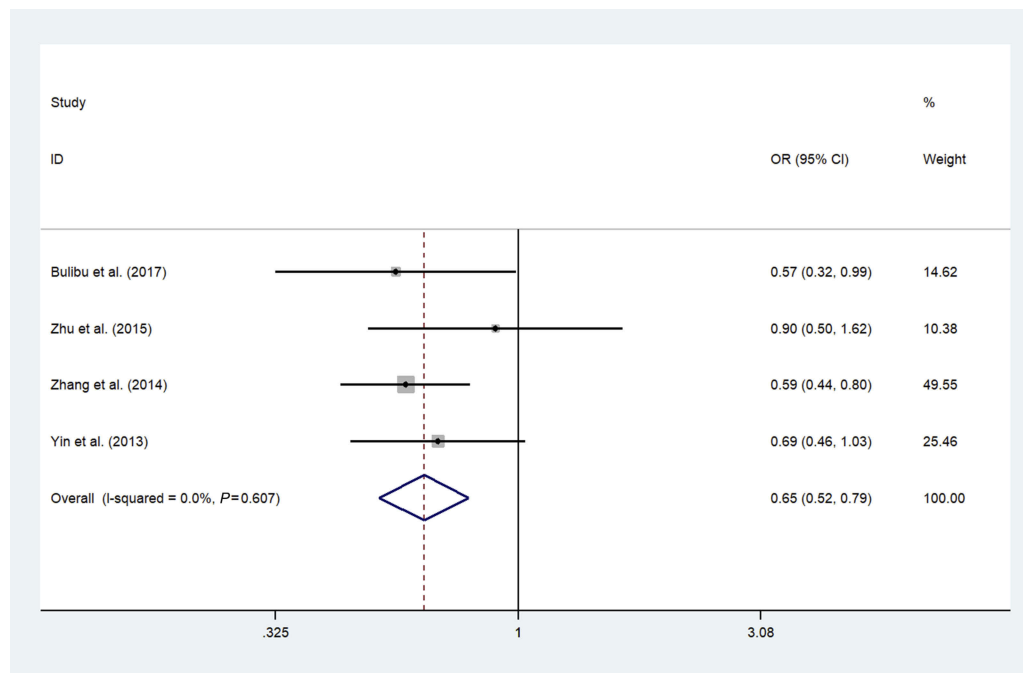


Figure 8 Forest plot shows the odds ratio for the associations between miR-34b/c rs4938723 homozygote mode and ESCC risk (CC vs TT).

Table 3 Meta-analysis of the association between miR-34b/c rs4938723 and esophageal cancer risk

miR-34b/c	N	C/T		CC/TT		CT/TT		CC+CT/TT		CC/CT+TT	
		OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
	4	0.85 (0.78–0.93)	<0.001	0.65 (0.52–0.79)	<0.001	0.92 (0.81–1.04)	0.196	0.87 (0.77–0.98)	0.018	0.68 (0.56–0.83)	<0.001
Ethnicity											
Chinese Han	3	0.84 (0.76–0.94)	0.001	0.62 (0.49–0.77)	<0.001	0.93 (0.78–1.09)	0.382	0.86 (0.76–0.98)	0.024	0.65 (0.52–0.80)	<0.001
Chinese Kazakh	1	0.92 (0.70–1.19)	0.545	0.90 (0.49–1.62)	0.725	0.88 (0.61–1.26)	0.482	0.88 (0.62–1.24)	0.476	0.95 (0.54–1.68)	0.884
Design											
PB	1	0.83 (0.73–0.94)	0.002	0.59 (0.44–0.80)	0.001	0.88 (0.74–1.04)	0.142	0.82 (0.69–0.97)	0.023	0.63 (0.47–0.84)	0.002
HB	3	0.88 (0.77–1.00)	0.059	0.70 (0.52–0.93)	0.014	0.96 (0.79–1.16)	0.691	0.89 (0.73–1.09)	0.288	0.72 (0.55–0.94)	0.018

Abbreviations: HB, hospital-based; PB, population-based.

other specific factors, such as patient stratification, genotyping errors, and environmental exposure, may also contribute to this observed discrepancy.³⁴

Moreover, our meta-analysis found that the CC genotype or C allele of miR-34b/c rs4938723 had significant associations with decreased risk of ESCC, especially among Chinese Han population (Table 3 and Figures 5–8). The miR-34b/c rs4938723 is located in CpG islands in miR-34b/c promoter region and may take part in the epigenetic silencing of miR-34b/c.³⁵ To the best of our knowledge, this study is the first systematic review and meta-analysis to evaluate the association of miR-34b/c rs4938723 with the risk of ESCC. In addition, we also conducted pooled analysis with the other two polymorphisms, miR-146a rs2910164 G>C and miR-423 rs6505162 C>A. However, we didn't find a significant association with ESCC risk in any genetic models, even though they have been reported to be biomarkers of various types of cancer.^{36,37} Further studies need to be done to explore the association of these miR-SNPs with susceptibility in a larger cohort of ESCC patients and verify the association with laboratory experiments.

A meta-analysis by Ji et al has evaluated the association between miR-196a2 rs11614913, miR-146a rs2910164, and miR-423 rs6505162 polymorphisms and esophageal cancer risk.³⁸ The main difference between our systematic reviews is that we included the most updated publication and more miRNA-SNPs, which can provide a more comprehensive understanding of the relationship between miRNA-SNPs and ESCC risk. Furthermore, the advantages of our meta-analysis

are as follows: first, our meta-analysis included only the squamous cell carcinomas cases but not adenocarcinoma,³⁹ because these two major subtypes of esophageal cancer have quite different molecular mechanisms. Second, we performed subgroup analyses to comprehensively investigate the impact of the miR-SNPs on esophageal cancer risk. Third, in addition to the regular meta-analysis, we conducted the TSA and RNA secondary structure analysis to determine if the current evidence is reliable and conclusive and predict the potential biological significance of these miR-SNPs.

Although considerable efforts have been made to discover possible associations between miR-SNPs and ESCC risk, there are still some inherent limitations of this study that may decrease the robustness of the findings of this study. Firstly, gene-environment interactions are of great interest to evaluate the exact roles of genetic polymorphism. However, not all of the reviewed studies analyzed the same environmental factors such as tobacco use and alcohol drinking. Thus, this limitation hindered the evaluation of the potential influence of gene-environment interactions in the association between miR-SNPs and ESCC risk. Secondly, this meta-analysis was based on case-control studies, but this type of study has inherent limitations, such as selection bias and recall bias. Finally, as shown in Table 1, most included studies were from China; therefore, further studies based on Caucasian, African, and other ethnic populations are needed to generalize the conclusion of this study. And these results also suggested that the biological functions of genetic variations in miRNAs

might be influenced by ethnics, ages and environment factors (smoking, drinking). In order to verify this hypothesis, larger sample size and more detailed sample information such as environmental factors are needed in further studies.

Conclusion

In summary, despite these limitations, a systematic review of the association of miR-SNPs with esophageal cancer risk is statistically more powerful than any single study. The present study indicated that the polymorphisms of miR-196a2 rs11614913 and miR-34b/c rs4938723 are associated with decreased risk of ESCC, especially in Chinese people. However, the current data suggested that miR-146a rs2910164 or miR-423 rs6505162 are not related to the susceptibility of ESCC in any genetic model. Moreover, no obvious publication bias was detected in this analysis. Further large-scale prospective studies from different ethnic groups and with gene-environment interactions in the relationship between miR-SNPs and esophageal cancer risks are warranted to confirm our conclusions.

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Author contributions

F Liu and XD Guo designed research; XD Guo, Y Shen, and Y Shao collected and analyzed the data; L Zhao, XD Guo, and F Liu wrote the manuscript; L Zhao and F Liu, WQ Wei contributed to the discussion and revised the manuscript. All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 The Newcastle-Ottawa Scale (NOS) for assessing the quality of studies in the meta-analyses

Studies	Selection				Comparability	Exposure			Total quality score
	Is the case definition adequate?	Representativeness of the cases	Selection of controls	Definition of controls		Comparability of cases and controls	Ascertainment of exposure	Same method of ascertainment for cases and controls	
Nariman-Saleh-Fam et al, 2017 ¹³	*	*		*	**	*	*	*	7
Bulibu et al, 2017 ¹²	*	*	*	*	**	*	*	*	8
Shen et al, 2016 ¹¹	*	*		*	**	*	*	*	7
Zhu et al, 2015 ¹⁰	*	*		*	**	*	*	*	7
Zhang et al, 2014 ⁹	*	*	*	*	**	*	*	*	8
Wang et al, 2014 ⁸	*	*	*	*	**	*	*	*	8
Qu et al, 2014 ⁷	*	*	*	*	**	*	*	*	8
Wang et al, 2013 ⁴	*	*	*	*	**	*	*	*	8
Wei et al, 2013 ⁵	*	*		*	**	*	*	*	7
Yin et al, 2013 ⁶	*	*		*	**	*	*	*	7
Umar et al, 2013 ³	*	*		*	**	*	*	*	7
Guo et al, 2010 ¹	*	*		*	**	*	*	*	7
Wang et al, 2010 ²	*	*		*	**	*	*	*	7

Table S2 Meta-analysis of the association between miR-146a rs2910164 and esophageal cancer risk

miR-146a	N	C/G		CC/GG		CG/CC		GG+CG/CC		GG/CG+CC	
		OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
	6	1.074 (0.965–1.195)	0.193	1.035 (0.924–1.159)	0.554	1.125 (0.892–1.420)	0.319	1.062 (0.915–1.233)	0.426	1.190 (0.445–3.181)	0.729
Ethnicity											
Chinese	4	1.067 (0.923–1.233)	0.381	1.058 (0.894–1.250)	0.513	1.149 (0.826–1.598)	0.410	1.091 (0.877–1.358)	0.433	0.978 (0.320–2.986)	0.969
Han											
Chinese	1	1.040 (0.810–1.336)	0.757	1.042 (0.712–1.526)	0.831	1.087 (0.635–1.859)	0.762	1.052 (0.732–1.513)	0.784	0.428 (0.280–0.655)	0.000
Kazakh											
Indian	1	1.182 (0.917–1.524)	0.198	0.904 (0.492–1.660)	0.744	1.183 (0.654–2.139)	0.578	1.057 (0.595–1.878)	0.850	7.379 (4.610–11.810)	0.000
Design											
PB	1	0.943 (0.774–1.148)	0.557	0.944 (0.688–1.295)	0.722	0.877 (0.575–1.336)	0.540	0.927 (0.685–1.255)	0.625	0.555 (0.394–0.780)	0.001
HB	5	1.103 (0.976–1.247)	0.115	1.049 (0.929–1.185)	0.440	1.195 (0.909–1.572)	0.202	1.105 (0.921–1.327)	0.283	1.389 (0.415–4.653)	0.594

Abbreviations: HB, hospital-based; PB, population-based

Table S3 Meta-analysis of the association between miR-423 rs6505162 and esophageal cancer risk

miR-423	N	A/C		CA/CC		AA/CC		AA+CA/CC		AA/CA+CC	
		OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
	6	1.010 (0.933–1.093)	0.807	0.989 (0.888–1.100)	0.834	1.112 (0.904–1.368)	0.314	1.014 (0.915–1.123)	0.795	1.008 (0.856–1.187)	0.926
Ethnicity											
Chinese	2	1.035 (0.934–1.146)	0.512	0.989 (0.873–1.121)	0.863	1.223 (0.911–1.642)	0.181	1.013 (0.898–1.142)	0.834	1.228 (0.917–1.645)	0.169
Han											
Chinese	1	0.908 (0.704–1.172)	0.460	0.959 (0.666–1.382)	0.824	0.746 (0.402–1.383)	0.352	0.921 (0.648–1.308)	0.645	0.763 (0.426–1.367)	0.364
Kazakh											
Indian	1	1.009 (0.804–1.267)	0.937	0.985 (0.678–1.429)	0.935	1.021 (0.657–1.588)	0.927	0.997 (0.705–1.409)	0.984	1.030 (0.704–1.509)	0.878
Caucasian											
Caucasian	1	1.464 (1.102–1.945)	0.009	1.185 (0.814–1.724)	0.376	3.121 (1.442–6.752)	0.004	1.378 (0.962–1.974)	0.080	2.894 (1.362–6.150)	0.006
Black											
Black	1	0.753 (0.597–0.949)	0.016	0.522 (0.239–1.140)	0.103	0.413 (0.192–0.889)	0.024	0.449 (0.210–0.960)	0.039	0.749 (0.570–0.985)	0.039
Genotyping methods											
PCR-LDR	2	1.035 (0.934–1.146)	0.512	0.989 (0.873–1.121)	0.863	1.223 (0.911–1.642)	0.181	1.013 (0.898–1.142)	0.834	1.228 (0.917–1.645)	0.169
PCR-RFLP											
PCR-RFLP	2	1.169 (0.980–1.395)	0.083	1.079 (0.829–1.406)	0.571	1.399 (0.965–2.030)	0.077	1.165 (0.908–1.495)	0.229	1.319 (0.946–1.837)	0.102
TaqMan											
TaqMan	1	0.753 (0.597–0.949)	0.016	0.522 (0.239–1.140)	0.103	0.413 (0.192–0.889)	0.024	0.449 (0.210–0.960)	0.039	0.749 (0.570–0.985)	0.039
MALDI-TOF MS											
MALDI-TOF MS	1	0.908(0.704–1.172)	0.460	0.959 (0.666–1.382)	0.824	0.746 (0.402–1.383)	0.352	0.921 (0.648–1.308)	0.645	0.763 (0.426–1.367)	0.364

(Continued)

Table S3 (Continued).

miR-423	N	A/C		CA/CC		AA/CC		AA+CA/CC		AA/CA+CC	
		OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
Design											
PB	1	0.753 (0.597–0.949)	0.016	0.522 (0.239–1.140)	0.103	0.413 (0.192–0.889)	0.024	0.449 (0.210–0.960)	0.039	0.749 (0.570–0.985)	0.039
HB	5	1.049 (0.965–1.140)	0.264	1.001 (0.898–1.115)	0.987	1.204 (0.970–1.493)	0.092	1.029 (0.928–1.141)	0.585	1.188 (0.969–1.458)	0.098

Abbreviations: HB, hospital-based; PB, population-based; MALDI-TOF MS, Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry; PCR-LDR, Polymerase Chain Reaction ligase detection reaction; PCR-RFLP, Polymerase Chain Reaction restriction fragment length polymorphism.

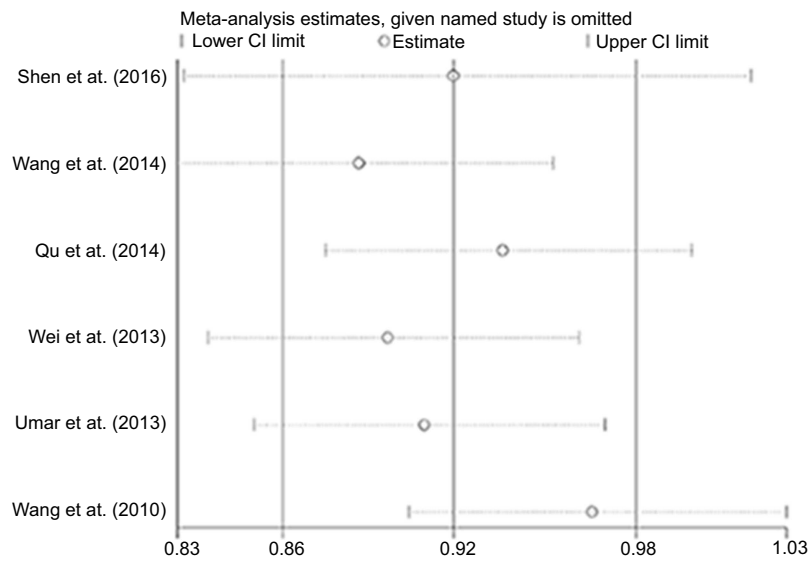


Figure S1 Forest plot of OR for the sensitivity analysis of miR-196a2 rs11614913 polymorphism and ESCC risk.

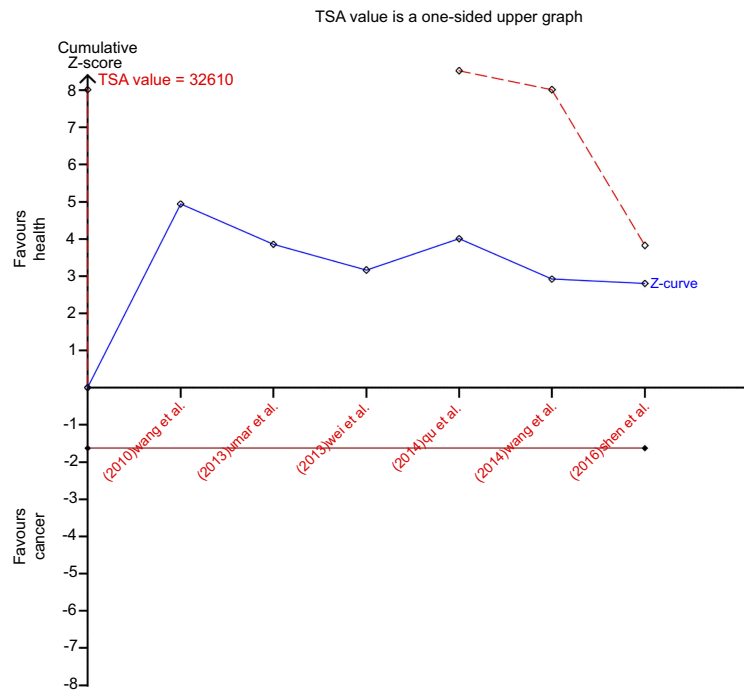


Figure S2 Trial sequential analysis for association between miR-196a2 rs11614913 and ESCC risk in the recessive model.

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