



## Association between microRNA-196a2 rs11614913, microRNA-146a rs2910164, and microRNA-423 rs6505162 polymorphisms and esophageal cancer risk: A meta-analysis



Hong-Hai Ji<sup>a</sup>, Hong-Luo<sup>b</sup>, Guang-Lei Huang<sup>b</sup>, Hai-Xin Yin<sup>b</sup>, Ping Xu<sup>b</sup>,  
Si-Yang Luo<sup>b</sup>, Ju-Kun Song<sup>b,\*</sup>

<sup>a</sup> The Second People's Hospital in Guiyang, Guiyang 550002, PR China

<sup>b</sup> Gui Zhou Provincial People's Hospital, Guiyang 550002, PR China

### ARTICLE INFO

#### Article history:

Received 5 August 2014

9 December 2014

Accepted 15 December 2014

Available online 5 January 2015

#### Keywords:

MicroRNA

Meta-analysis

Esophageal cancer

### ABSTRACT

Many observational studies have found that microRNA-196a2 rs11614913, microRNA-146a rs2910164, and microRNA-423 rs6505162 are associated with esophageal cancer risk. However, the results were mixed and inconsistent among these studies. We conducted a meta-analysis to assess the relationship between the polymorphisms of three microRNAs and esophageal cancer susceptibility. We systematically searched the PubMed and EMBASE databases to screen relevant studies. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were used to compute the risk of esophageal cancer. Because of the differences in ethnicities, sources of controls, and genotyping methods, the meta-analysis was conducted using a random-effect model regardless of heterogeneity. To further explore potential heterogeneity, we performed subgroup and sensitivity analyses, and publication bias was also evaluated. A total of 6 case-control studies on microRNA-196a2 rs11614913, 4 studies on microRNA-146a rs2910164, and 4 studies on microRNA-423 rs6505162 were considered eligible in the meta-analysis. No statistical association was found between microRNA-196a2 rs11614913, microRNA-146a rs2910164, and microRNA-423 rs6505162 polymorphisms and esophageal cancer susceptibility in any genetic model. Subgroup and sensitivity analyses showed similar results. In summary, based on the currently limited proof, no association exists between microRNA-196a2 rs11614913, microRNA-146a rs2910164, and microRNA-423 rs6505162 polymorphism and esophageal cancer risk.

\* Corresponding author.

E-mail addresses: jihaihong@163.com (H.-H. Ji), luohong@163.com (Hong-Luo), huangguanglei@163.com (G.-L. Huang), yinxinhai@163.com (H.-X. Yin), xuping@163.com (P. Xu), luosiyang@163.com (S.-Y. Luo), songjukun@163.com (J.-K. Song).

However, the result should be cautiously interpreted because of the heterogeneity among studies. Large, high quality clinical trials are required to verify our findings.

© 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## Contents

Introduction . . . . .	15
Methods . . . . .	16
Search strategy . . . . .	16
Eligibility criteria . . . . .	16
Data extraction . . . . .	16
Quality assessment . . . . .	16
Statistical analysis . . . . .	16
Result . . . . .	17
Study selection . . . . .	17
Study characteristics . . . . .	17
Association between microRNA-196a2 C > T rs11614913 polymorphism and esophageal cancer susceptibility . . . . .	19
Association between microRNA-146a C > G rs2910164 polymorphism and esophageal cancer susceptibility . . . . .	19
Association between microRNA-423 C > A rs6505162 polymorphism and esophageal cancer susceptibility . . . . .	22
Publication bias . . . . .	22
Discussion . . . . .	23
References . . . . .	24

## Introduction

Esophageal cancer, including esophageal squamous cell carcinoma and esophageal adenocarcinoma, is the sixth leading cause of cancer-related death and the eighth most frequently diagnosed cancer in the world (Pennathur et al., 2013). In 2014, 18,170 new cases, which led to approximately 15,450 deaths, were diagnosed (Siegel et al., 2014). The overall 5 year survival rate for patients with esophageal cancer is poor (only 5% to 10%) (Enzinger and Mayer, 2003; Ferlay et al., 2010). Furthermore, pain, disability, and mortality are consequences that incur high cost to society. The abovementioned data highlight the importance of screening patients who are at highest risk and of identifying the potential risk factors for esophageal cancer development.

In recent years, many studies have demonstrated that deregulation of microRNAs may play crucial role in malignant transformation of esophageal cancer (Matsushima et al., 2010). The microRNAs are non-coding RNAs comprising small 18 nucleotides to 22 nucleotides in length; microRNAs are believed to play an important regulatory role in host genome expression at the post-transcriptional level (Calin, 2009). The microRNAs do not directly encode for protein, but they regulate protein expression by binding to the complementary nucleotide sequence in the target messenger RNA (mRNA) molecules. A single microRNA can modulate a number of mRNA transcripts. Furthermore, microRNAs play a major role in maintaining tissue homeostasis by regulating many biological processes, such as cellular proliferation, differentiation, migration, morphogenesis, apoptosis, cell-to-cell communication and inflammation (Kim, 2005; Kim et al., 2009). The human genome may harbor up to 1,000 microRNAs (Berezikov et al., 2005; Tricoli and Jacobson, 2007). MicroRNAs are potential biomarkers that are used for early diagnosis, prognosis, decision-making, and ongoing surveillance (Pritchard et al., 2012).

As of this writing, a number of epidemiological studies have been conducted to examine the association between microRNA-196a2 rs11614913, microRNA-146a rs2910164, and microRNA-423 rs6505162 polymorphisms and esophageal cancer risk. However, the findings of these data were mixed and inconsistent (Guo et al., 2010; Qu et al., 2014; Umar et al., 2013; Wang et al., 2010, 2013, 2014; Wei et al., 2013; Ye et al., 2008; Yin et al., 2013). Given the poor prognosis, any risk factor for the development of esophageal cancer

would substantially affect public health. Therefore, we performed this meta-analysis to assess the association between the three single nucleotide polymorphisms (SNPs) and esophageal cancer susceptibility.

## Methods

### *Search strategy*

To identify all potentially eligible studies, we performed a comprehensive search on PubMed and EMBASE databases, and the search included studies published until June 2014. The following search strategies were used without any limitation: “esophageal cancer OR oesophageal cancer OR esophageal neoplasms OR esophageal squamous cell carcinoma OR esophageal adenocarcinoma” and “microRNA OR miRNA OR microRNAs” and “polymorphism OR polymorphisms”. We also screened the reference lists of eligible articles and review articles for other related studies.

### *Eligibility criteria*

Studies were considered eligible in the meta-analysis if they met the following criteria: (1) an evaluation of the association between microRNA-196a2 rs11614913, microRNA-146a rs2910164, and microRNA-423 rs6505162 polymorphisms and esophageal cancer susceptibility (with full text); (2) a case-control study; (3) and detailed genotype data were available for calculating ORs and the corresponding 95% CIs. Articles were excluded according to the following criteria: these articles were letters, comments, correspondence, conference reports or laboratory studies or they did not contain enough data with which to compute the ORs.

If multiple studies covered the same population, only the most comprehensive study with the largest sample size was included. Two authors (SJK and YXH) independently assessed the inclusion of all retrieved studies and resolved any disagreements through discussion or after consultation with a third author (HGL).

### *Data extraction*

Two authors (SJK and YXH) independently extracted the following relevant information using a standardized data extraction form. The following key points were collected: first author's surname, publication year, country, ethnicity, genotyping methods, source of control group, Minor allele frequency (MAF) in case and control groups, Hardy–Weinberg equilibrium (HWE) in the control group, numbers of cases and controls for each genotype and genotyping method. Disagreements between reviewers regarding data extraction were resolved through discussion.

### *Quality assessment*

We evaluated the methodological quality of each study using the methodological quality assessment scale, which was obtained from a previous publication (Guo et al., 2012; Li et al., 2014). Five major components were judged, as follows: representativeness of cases, source of controls, sample size, quality control of genotyping methods, and HWE. The quality score ranged from 0 to 10. A high score indicated better methodology quality.

### *Statistical analysis*

We used the OR with 95% CI as a common measure for the eligible studies. HWE was examined for each study by Chi-square test in the control groups, and  $P < 0.05$  was considered a significant departure from the HWE. The overall ORs were summarized to assess the strength of the association between the microRNAs 196a2/146a/423 and esophageal cancer susceptibility. The summary ORs were used for allelic, heterozygote, homozygote, dominant, and recessive models. The test of heterogeneity was performed using the Cochrane Q test and the  $I^2$  statistics. A  $p$  value  $< 0.05$  and/or an  $I^2$  statistic of  $> 50\%$  was considered statistically significant. Because of differences in ethnicities, sources of controls, and genotyping methods, a random-effects model was used regardless of heterogeneity. Subgroup analyses were stratified by ethnicity, source of control, and genotyping method. Sensitivity analyses were also performed to evaluate the effect of individual studies on

the pooled ORs by sequential omission of one study in each turn or by exclusion of the study that deviated from the HWE equilibrium. The publication bias was evaluated by using the Begg and Egger tests (Begg and Mazumdar, 1994; Egger et al., 1997). All statistical analyses were conducted by using STATA version 12.0 (Stata Corporation, College Station, Texas, USA).

## Result

### Study selection

Using the outlined search strategy, a total of 88 citations were identified for title and abstract reviews. Of these 88 citations, 44 were not relevant and 18 were duplicates. The full texts of the remaining 26 studies were retrieved for review. Among these 26 studies, 17 studies were excluded because of the following reasons: outcomes were related to prognosis (Christensen et al., 2010; Eng et al., 2013; Faluyi et al., 2013; Wu et al., 2014; Yang et al., 2014; Zhang et al., 2013); no interesting outcome was reported (Shi et al., 2013; Shuto et al., 2009; Wang et al., 2012; Wu et al., 2013; Yang et al., 2013; Zhou et al., 2013), the study was a review article (Buas et al., 2013; David and Meltzer, 2011; Li et al., 2011; Zheng et al., 2011); and the study was a duplicate of another study (Zhang et al., 2010). Finally, 9 studies (6 studies on microRNA-196a2, 4 studies on microRNA-146a, and 4 studies on microRNA-423) were considered eligible in the meta-analysis (Fig. 1).

### Study characteristics

The main data from the abovementioned 9 studies are summarized in the Table 1. The included studies involving 2,071 cases and 2,547 controls for microRNA-196a2 C > T rs11614913 (Qu et al., 2014; Umar et al., 2013; Wang et al., 2010, 2014; Wei et al., 2013; Ye et al., 2008), 1,494 cases and 1,538 controls for microRNA-146a C > G rs2910164 (Guo et al., 2010; Qu et al., 2014; Umar et al., 2013; Wei et al., 2013) and 2,048 cases and 2,995 controls for microRNA-423 C > A rs2910164 (Umar et al., 2013; Wang et al., 2013; Yin et al., 2013) were considered eligible in the meta-analysis. Genotype frequencies of microRNA-196a2 rs11614913, microRNA-146a rs2910164, and microRNA-423 rs6505162 were reported in one study (Umar et al., 2013). In two studies, the genotype frequencies of microRNA-196a2 rs11614913 and microRNA-146a rs2910164 were reported (Qu et al., 2014; Wei et al., 2013). Genotype frequencies of microRNA-423 rs6505162 were presented separately in one study; thus, each of the abovementioned studies were considered separately (Wang et al., 2013). Among the studies on microRNA-196a2 C > T rs11614913, 5 studies used Asians as subjects (Qu et al., 2014; Umar et al., 2013; Wang et al., 2010, 2014; Wei et al., 2013) and

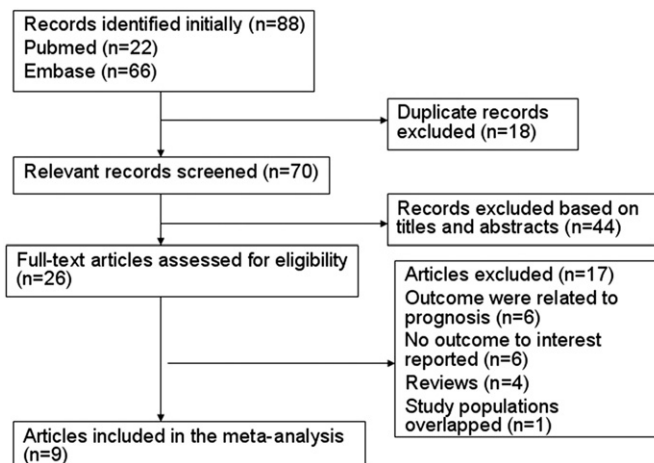


Fig. 1. Flow chart from identification of eligible studies to final inclusion.

**Table 1**  
Characteristic of studies included in the meta-analysis.

Study	Year	Country	Ethnicity	Design	Genotyping methods	Number of cases/controls	MAF value		Genotypes distribution of cases/controls			HWE(P)	Quality
							case	control	microRNA-196a2 C > T rs11614913				
									CC	CT	TT		
Ye Y	2008	USA	Caucasian	HB	SNPlex assay	346/346	0.50	0.43	83/106	141/173	83/59	0.42	7
Wang K	2010	China	Chinese Han	HB	SNaPshot assay	458/489	0.39	0.48	148/128	262/250	48/111	0.60	7
Umar M	2013	India	Indian	HB	PCR-RFLP	289/309	0.29	0.25	146/171	121/122	22/16	0.33	6
<b>Wei J</b>	2013	China	Chinese	HB	SNaPshot assay	380/380	0.46	0.46	65/87	196/170	106/113	0.14	8
Qu Y	2014	China	Chinese Han	PB	PCR-RFLP	381/426	0.40	0.44	126/133	207/211	48/82	0.92	9
Wang N	2014	China	Chinese Han	PB	PCR-LDR	597/597	0.47	0.49	128/145	307/298	162/154	0.97	9
									microRNA-146a C > G rs2910164				
									CC	CG	GG		
Guo H	2010	China	Chinese Han	HB	SNaPshot assay	444/468	0.26	0.32	20/42	190/220	234/206	0.12	6.5
Umar M	2013	India	Indian	HB	PCR-RFLP	289/309	0.26	0.29	24/27	102/127	163/155	0.16	6
Wei J	2013	China	Chinese	HB	SNaPshot assay	380/380	0.43	0.43	117/122	184/181	67/67	0.99	8
Qu Y	2014	China	Chinese Han	PB	PCR-RFLP	381/426	0.43	0.44	116/123	203/228	62/75	0.08	9
									microRNA-423 C > A rs6505162				
									CC	CA	AA		
Umar M	2013	India	Indian	HB	PCR-RFLP	289/309	0.46	0.46	90/96	132/143	67/70	0.23	6
Wang Y	2013	Africa	Black	PB	TaqMan	565/1000	0.23	0.18	16/12	128/184	207/376	0.052	9
Wang Y	2013	Africa	Mix	PB	TaqMan	565/1000	0.30	0.30	14/34	84/188	89/198	0.25	8
Yin J	2013	China	Chinese	HB	PCR-LDR	629/686	0.21	0.19	374/425	197/207	29/19	0.299	8

HB: hospital-based control; PB: population-based control; PCR-LDR: polymerase chain reaction–ligation detection reaction; PCR-RFLP: polymerase chain reaction–restriction fragment length polymorphism; HWE: Hardy–Weinberg equilibrium.

the other one used Caucasians as subjects (Ye et al., 2008). The genotyping distribution was in agreement with the HWE in all studies. All studies on microRNA-146a C > G rs2910164 included Asian subjects (Guo et al., 2010; Qu et al., 2014; Umar et al., 2013; Wei et al., 2013). The genotyping distribution was in agreement with the HWE in most included studies, except for one study, which was conducted by Qu Y (Qu et al., 2014). Among the studies on microRNA-423 C > A rs6505162, two studies involved Asian subjects (Umar et al., 2013; Yin et al., 2013), whereas African subjects were included in the other two studies (Wang et al., 2013). The genotyping distribution was in agreement with the HWE in all included studies. Different genotyping methods were used including polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP), TaqMan, polymerase chain reaction–ligation detection reaction (PCR–LDR), SNPlex assay and SNaPshot assay. According to the results of the quality assessment, all studies were considered of good quality (in the range, 5 to 9).

#### *Association between microRNA-196a2 C > T rs11614913 polymorphism and esophageal cancer susceptibility*

Six studies presented data on microRNA-196a2 C > T rs11614913. As shown in Table 2, the pooled estimates suggested that no statistically significant association was identified in any genetic model, as follows: allele model T vs. C: OR = 0.99, 95% CI 0.83–1.19,  $P = 0.94$ ,  $P_H < 0.001$ ,  $I^2 = 80.0\%$  (Fig. 2); heterozygote model CT vs. CC: ORs = 1.01, 95% CIs 0.97–1.26,  $P = 0.15$ ,  $P_H = 0.392$ ,  $I^2 = 3.8\%$ ; homozygote model TT vs. CC: ORs = 0.99, 95% CIs 0.61–1.60,  $P = 0.96$ ,  $P_H < 0.001$ ,  $I^2 = 6.6\%$ ; dominant model TT/CT vs. CC: OR = 1.08, 95% CI 0.89–1.31,  $P = 0.44$ ,  $P_H = 0.039$ ,  $I^2 = 57.2\%$ ; and recessive model TT vs. CT/CC: ORs = 0.91, 95% CIs 0.60–1.37,  $P = 0.64$ ,  $P_H < 0.001$ ,  $I^2 = 87.1\%$ . Subsequently, we performed subgroup analyses based on ethnicities and sources of control, genotyping methods. For ethnicity, the results obtained using the Asian population were similar to that of the overall comparisons in the pooled eligible studies. No significant association was observed in any genetic models, as follows: allele model T vs. C: ORs = 1.03, 95% CIs 0.83–1.27,  $P = 0.81$ ,  $P_H < 0.001$ ,  $I^2 = 82.4\%$ ; heterozygote model CT vs. CC: ORs = 1.03, 95% CIs 0.83–1.27,  $P = 0.17$ ,  $P_H = 0.279$ ,  $I^2 = 21.3\%$ ; homozygote model TT vs. CC: ORs = 0.87, 95% CIs 0.52–1.46,  $P = 0.60$ ,  $P_H < 0.001$ ,  $I^2 = 85.8\%$ ; dominant model TT/CT vs. CC: ORs = 1.06, 95% CIs 0.84–1.32,  $P = 0.64$ ,  $P_H = 0.028$ ,  $I^2 = 63.1\%$ ; and recessive model TT vs. CT/CC: ORs = 0.79, 95% CIs 0.53–1.18,  $P = 0.25$ ,  $P_H < 0.001$ ,  $I^2 = 83.6\%$ . Among studies involving an American population, only one study reported the related data. Thus, the data were not appropriate for quantitative analysis. In addition, three studies reported the hsa-microRNA-196a2 rs11614913 polymorphism, and the pooled estimates suggested that the association between hsa-microRNA-196a2 rs11614913 polymorphism and esophageal cancer in Chinese Han population was not detected in all genetic models (Table 2). Coincidentally, 2 and 4 studies on esophageal cancer were population- and hospital-based articles, respectively. We did not find any significant association between microRNA-196a2 and esophageal cancer risk in any genetic model in both groups. Subgroup analyses by genotyping methods, similar results were yielded in most genetic models with low to moderate heterogeneity, except for the PCR–RFLP and SNPlex assay method (Table 2). Sensitivity analyses were performed to examine the effect of the individual study on the pooled ORs by sequential omission of one study in each turn. Consistently, the pooled estimates remained non-significant.

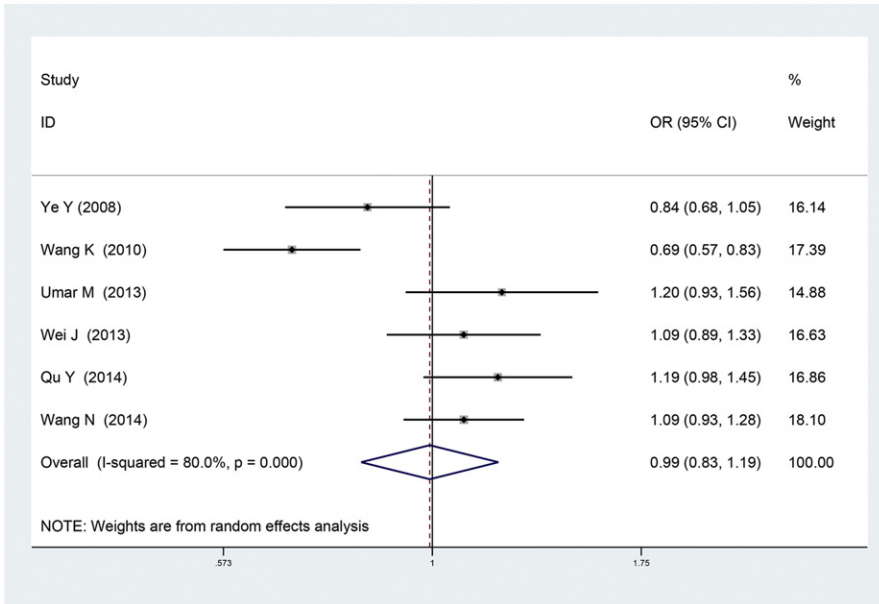
#### *Association between microRNA-146a C > G rs2910164 polymorphism and esophageal cancer susceptibility*

Four studies examined the association between microRNA-146a C > G rs2910164 and esophageal cancer risk. The overall ORs estimates indicated that no statistically significant association was detected in any genetic model, as follows: allele model G vs. C: ORs = 1.12, 95% CIs 0.94–1.33,  $P = 0.21$ ,  $P_H = 0.052$ ,  $I^2 = 61.2\%$  (Fig. 3); heterozygote model CG vs. CC: ORs = 1.08, 95% CIs 0.84–1.39,  $P = 0.53$ ,  $P_H = 0.238$ ,  $I^2 = 29.1\%$ ; homozygote model GG vs. CC: ORs = 1.23, 95% CIs 0.82–1.85,  $P = 0.32$ ,  $P_H = 0.042$ ,  $I^2 = 63.5\%$ ; dominant model GG + CG vs. CC: ORs = 1.15, 95% CIs 0.85–1.55,  $P = 0.37$ ,  $P_H = 0.088$ ,  $I^2 = 54.2\%$ ; and recessive model GG vs. CG + CC: ORs = 1.17, 95% CIs 0.95–1.44,  $P = 0.13$ ,  $P_H = 0.191$ ,  $I^2 = 36.8\%$  (Table 2). Because the ethnicity used in all studies was Asian, hence, the results were identical to that of the overall comparisons of pooled eligible studies. The subgroup analysis was conducted based on the sources of control and genotyping methods. In population-control studies, no significant association was detected in all genetic models. In hospital-based studies, no significant association was detected among most genetic models except for the recessive model (TT/CT + CC) (Table 2). The controls used were healthy subjects, except for one study,

**Table 2**  
Summary of overall ORs in the meta-analysis.

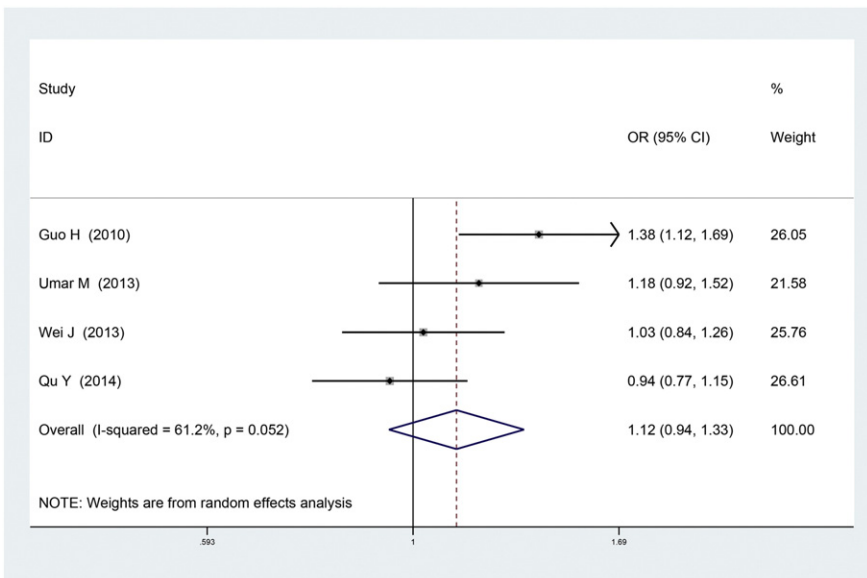
	N	ORs (95%CI)	P <sub>H</sub>	ORs (95%CI)	P <sub>H</sub>	ORs (95%CI)	P <sub>H</sub>	ORs (95%CI)	P <sub>H</sub>	ORs (95%CI)	P <sub>H</sub>
microRNA-196a2		T/C		CT/CC		TT/CC		TT + CT/CC		TT/CT + CC	
Overall	6	0.99(0.83–1.19)	<0.001	1.10(0.97–1.26)	0.392	0.99(0.61– 1.60)	<0.001	1.08(0.89– 1.31)	0.039	0.91(0.60– 1.37)	<0.001
<i>Ethnicity</i>											
Asian	5	1.03(0.83–1.27)	<0.001	1.12(0.95–1.31)	0.279	0.87(0.52– 1.46)	<0.001	1.06(0.84– 1.32)	0.028	0.79(0.53– 1.18)	<0.001
Caucasian	1	0.84(0.68–1.05)	NA	1.04(0.72–1.50)	NA	1.80(1.16– 2.79)	NA	1.23(0.88– 1.73)	NA	1.75(1.20– 2.56)	NA
Chinese Han	3	0.96(0.69–1.33)	<0.001	1.03(0.87– 1.23)	0.481	0.66(0.33– 1.32)	<0.001	0.93(0.71– 1.22)	0.069	0.64(0.35– 1.16)	<0.001
<i>Design</i>											
HB	4	0.93(0.72–1.19)	0.001	1.12(0.90–1.39)	0.180	1.07(0.50– 2.29)	<0.001	1.11(0.82– 1.50)	0.017	0.98(0.50– 1.91)	<0.001
PB	2	1.13(0.99–1.28)	0.485	1.11(0.90–1.36)	0.579	0.87(0.46– 1.66)	0.017	1.05(0.82– 1.33)	0.228	0.81(0.48– 1.39)	0.021
<i>Genotyping methods</i>											
SNaP shot assay	2	0.86(0.55–1.35)	0.001	1.17(0.69–1.96)	0.030	0.69(0.21–2.25)	<0.001	1.02(0.54–1.94)	0.005	0.61(0.27–1.39)	0.001
PCR-RFLP	2	1.20(1.02–1.40)	0.948	1.09(0.87–1.37)	0.622	0.96(0.38–2.45)	0.020	1.05(0.80–1.38)	0.212	0.92(0.38–2.24)	0.020
SNPlex assay	1	0.84(0.68–1.05)	NA	1.04(0.72–1.50)	NA	1.80(1.16–2.80)	NA	1.23(0.88–1.73)	NA	1.75(1.20–2.56)	NA
PCR-LDR	1	1.09(0.93–1.28)	NA	1.17(0.88–1.55)	NA	1.20(0.86–1.65)	NA	1.18(0.90–1.54)	NA	1.05(0.81–1.36)	NA
microRNA-146a		G/C		CG/CC		GG/CC		GG + CG/CC		GG/CG + CC	
Overall	4	1.12(0.94–1.33)	0.052	1.08(0.84–1.39)	0.238	1.23(0.82–1.85)	0.042	1.15(0.85–1.55)	0.088	1.17(0.95–1.44)	0.191
<i>Ethnicity</i>											
Asian	4	1.12(0.94– 1.33)	0.052	1.08(0.84–1.39)	0.238	1.23(0.82–1.85)	0.042	1.15(0.85–1.55)	0.088	1.17(0.95–1.44)	0.191
<i>Design</i>											
HB	3	1.19(0.99–1.42)	0.139	1.18(0.82–1.69)	0.189	1.41(0.85–2.32)	0.063	1.29(0.85–1.95)	0.092	1.27(1.05–1.53)	0.339
PB	1	0.94(0.68–1.30)	NA	0.94(0.69–1.30)	NA	0.88(0.58–1.34)	NA	0.93(0.69–1.26)	NA	0.91(0.63–1.32)	NA
<i>Genotyping methods</i>											
SNaP shot assay	2	1.19(0.89–1.59)	0.047	1.31(0.79–2.21)	0.107	1.54(0.69–3.47)	0.021	1.43(0.73–2.78)	0.033	1.23(0.88–1.71)	0.142
PCR-RFLP	2	1.04(0.84–1.30)	0.168	0.94(0.71–1.24)	0.900	0.97(0.69–1.37)	0.419	0.93(0.73–1.25)	0.693	1.10(0.78–1.54)	0.167
microRNA-423		A/C		CA/CC		AA/CC		AA + CA/CC		AA/CA + CC	
Overall	4	0.98(0.81–1.18)	0.044	1.01(0.83– 1.23)	0.374	0.98(0.59– 1.63)	0.038	0.98(0.74– 1.30)	0.149	1.00(0.75– 1.33)	0.077
<i>Ethnicity</i>											
African	2	0.87(0.65– 1.18)	0.086	0.77(0.38– 1.58)	0.164	0.68(0.26– 1.77)	0.062	0.72(0.30– 1.70)	0.082	0.86(0.64– 1.16)	0.173
Asian	2	1.10(0.94– 1.27)	0.351	1.05(0.86– 1.29)	0.678	1.28(0.77– 2.14)	0.161	1.09(0.90– 1.32)	0.537	1.25(0.78– 2.01)	0.168
<i>Design</i>											
PB	2	0.87(0.65–1.18)	0.086	0.77(0.38– 1.58)	0.164	0.68(0.26– 1.77)	0.062	0.72(0.30– 1.70)	0.082	0.86(0.64– 1.16)	0.173
HB	2	1.10(0.94– 1.27)	0.351	1.05(0.87– 1.29)	0.678	1.28(0.77– 2.14)	0.161	1.09(0.90– 1.32)	0.537	1.25(0.78– 2.01)	0.168
<i>Genotyping methods</i>											
TaqMan	2	0.87(0.65–1.18)	0.086	0.77(0.38–1.58)	0.16	0.68(0.26–1.77)	0.062	0.72(0.30–1.70)	0.082	0.86(0.64–1.16)	0.173
PCR-LDR	1	1.16(0.96–1.42)	NA	1.08(0.85–1.37)	NA	1.73(0.96–3.14)	NA	1.14(0.90–1.43)	NA	1.69(0.94–3.05)	NA
PCR-RFLP	1	1.01(0.80–1.27)	NA	0.99(0.68–1.43)	NA	1.02(0.66–1.59)	NA	1.00(0.71–1.41)	NA	1.03(0.70–1.51)	NA

P<sub>H</sub>:P value for heterogeneity; NA: not available.



**Fig. 2.** Forest plot of allele comparison of microRNA-196a2 rs11614913 for pooled comparison (T versus C).

in which a patient without cancer was included as a control (Wei et al., 2013). When we excluded this study, the pooled estimates among the remaining studies showed that no significant association was observed in any genetic model. As for genotyping methods, similar findings were yielded between SNP shot assay and PCR-RFLP method. The sensitivity analysis was also performed by omitting one study, which deviated from HWE



**Fig. 3.** Forest plot of allele comparison of microRNA-146a rs2910164 for pooled comparison (G versus C).



(Qu et al., 2014). The pooled estimates indicated that no significant association was obtained among the remaining studies in any genetic model.

#### Association between microRNA-423 C > A rs6505162 polymorphism and esophageal cancer susceptibility

Four studies contained data on microRNA-423 C > A rs6505162. No association was found to be significant in any genetic model, as follows: allele model A vs. C: ORs = 0.98, 95% CIs 0.81–1.18,  $P = 0.83$ ,  $P_H = 0.044$ ,  $I^2 = 62.9\%$  (Fig. 4); heterozygote model CA vs. CC: ORs = 1.01, 95% CIs 0.83–1.23,  $P = 0.93$ ,  $P_H = 0.374$ ,  $I^2 = 3.7\%$ ; homozygote model AA vs. CC: ORs = 0.98, 95% CIs 0.59–1.63,  $P = 0.95$ ,  $P_H = 0.038$ ,  $I^2 = 64.4\%$ ; dominant model AA + CA vs. CC: ORs = 0.98, 95% CIs 0.74–1.30,  $P = 0.87$ ,  $P_H = 0.149$ ,  $I^2 = 43.8\%$ ; and recessive model AA vs. CA + CC: ORs = 1.00, 95% CIs 0.75–1.33,  $P = 0.99$ ,  $P_H = 0.077$ ,  $I^2 = 56.3\%$  (Table 2). Subsequently, the subgroup analysis was conducted according to ethnicities and sources of control, genotyping methods. In Asian and African populations, no statistically significant association was found. Moreover, similar results were observed in both population- and hospital-based studies. The stratified analyses by genotyping methods, similar results were yields with low to moderate heterogeneity. Sensitivity analyses were performed to examine the effect of the individual study on the pooled ORs by excluding one study in each turn in every genetic model. Consistently, the pooled estimates remained non-significant.

#### Publication bias

No publication bias for the association between microRNA-196a2 C > T rs11614913 polymorphism and esophageal cancer susceptibility was detected by Begg's funnel plot (allele model G vs. C,  $P = 1.00$ ) or by Egger's regression test (allele model G vs. C,  $P = 0.75$ ) (Fig. 5).

Publication bias was not assessed for the association between microRNA-146a C > G rs2910164 and microRNA-423 C > A rs6505162 and esophageal cancer susceptibility because of the limited number of studies included in each analysis.

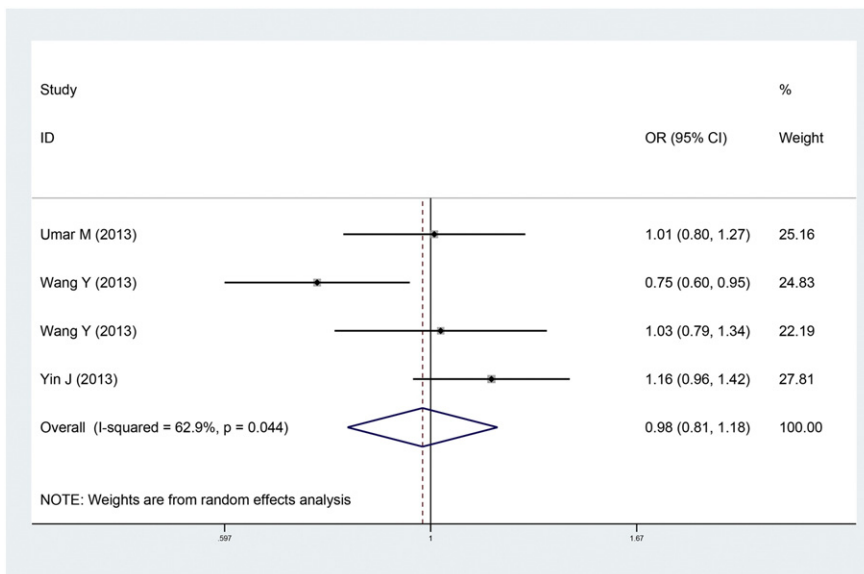


Fig. 4. Forest plot of allele comparison of microRNA-423 rs6505162 for pooled comparison(A versus C).

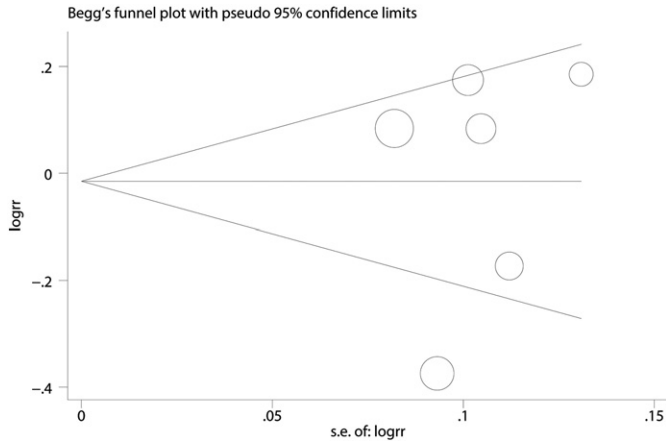


Fig. 5. Begg's funnel plot for publication bias analysis for microRNA-196a2 rs11614913 (T versus C).

## Discussion

To the best of our knowledge, this meta-analysis is the first to evaluate the association between microRNA-196a2 C > T rs11614913, microRNA-146a C > G rs2910164, and microRNA-423 C > A rs6505162 and esophageal cancer risk. In this meta-analysis, 6 eligible case-control studies involving 2,071 cases and 2,547 controls for microRNA-196a2 C > T rs11614913, 4 studies including 1,494 cases and 1,538 controls for microRNA-146a C > G rs2910164, and 4 studies involving 2,048 cases and 2,995 controls for microRNA-423 C > A rs6505162 were analyzed. The major finding of the present meta-analysis suggested that no significant association between microRNA-196a2/146a/423 polymorphisms and esophageal cancer susceptibility was observed in all genetic models.

Similar findings were observed in subgroup analyses, which were conducted according to ethnicities and sources of controls. For the microRNA-196a2 genotype, no significant association was detected in any genetic model involving an Asian population, but an exception was found for the TT/CC genetic model involving a Caucasian population (ORs = 1.75, 95% CIs: 1.20–2.56). Only one study included a Caucasian population. Therefore, because of limited samples, interpreting the results should be done cautiously. When this study was omitted, the summary OR indicated that no association was present in all genetic models in the remaining studies. Among studies on Hsa-microRNA-196a2, three studies demonstrated that Hsa-microRNA-196a2 was associated with increased esophageal cancer risk (Qu et al., 2014; Wang et al., 2010, 2014). However, our pooled estimates suggested that no significant association was found between Hsa-microRNA-196a2 and esophageal cancer susceptibility (Table 2). We enlarged the sample size and strengthened the statistical power, which may have led to this result. However, this finding should be interpreted with caution because of the limited number of studies and the presence of significant heterogeneity. As for genotyping methods, similar results were yielded, but an exception was found for the PCR-RFLP and SNPlex assay methods. No association was found for microRNA-146a in all genetic models. Among these studies, three studies used healthy subjects as controls, whereas one study enrolled patients without cancer as the controls. When this study (Wei et al., 2013) was excluded, no significant association was observed in all genetic models. Stratified by genotyping methods, similar results were found. For studies on microRNA-423, similar results were obtained in all genetic models during subgroup analyses by considering either ethnicities or sources of control. Subgroup by genotyping methods, similar results were found. The three studies discussed the association between microRNA-26a-1 C > T rs7372209 and microRNA-499 T > C rs3746444 and esophageal cancer susceptibility, and the pooled estimates suggested that no significant association was detected (Qu et al., 2014; Wang et al., 2010, 2014). These studies, two of which were on microRNA-26a-1 and microRNA-499, did not conform to HWE expectations. However, in this meta-analysis, microRNA-26a-1 and microRNA-499 were not become our study objects, and the number of eligible studies was relatively small. Hence, results of this meta-analysis were not sufficiently strong to result in a decisive conclusion.

In the meta-analysis, MAF are very different among different population (Table 1). For the microRNAs-196a2/146a genotype, the MAF value in the case and control group in Chinese population are very close to 0.5, while the MAF value in the case and control group in Indian population are very close to 0.5 for the microRNAs-423 genotype. It may be explained that the conflicting results with respect to microRNA-196a2 polymorphisms and esophageal cancer risk between Caucasian and Asian population. The allele flipping between Asian and non-Asian groups is observed. These seemingly contradictory results may be attributed to different ethnicities or carcinogenic mechanism, settings. A genuine allele flipping results from variations in allele frequencies and linkage disequilibrium (LD) that produce different patterns of risk association of a marker allele or haplotype across different ethnic groups. However, a variety of extraneous factors can create conditions for a genuine allele flip. Allelic heterogeneity, locus heterogeneity, environmental exposures, population differences are all examples of factors that can interact to vary the distribution of a measure of association between studies. Assuming no misclassification or selection biases, study design errors or genotyping errors, we have shown that the probability of observing a significant allele flip when the allele flipping is not genuine is negligible. In the meta-analysis, no clear explanation of allele flipping among the three microRNAs between different ethnic groups has emerged.

Our meta-analysis has several strengths. First, this is the first meta-analysis to investigate the association between and esophageal cancer susceptibility and microRNA-196a2, microRNA-146a, and microRNA-423. Second, no publication bias was observed by either Begg's funnel plot or Egger's regression test. Third, all included studies used high quality methods according to the methodological quality assessment. Thus, our results are reliable.

This meta-analysis has several limitations. First, this meta-analysis was based on case-control studies. Although a case-control study is the most appropriate design for exposure causing rare event, this design has inherent limitations, such as selective bias and recall or memory bias. Additionally, some confounding factors (e.g., race, sex, and lifestyle) are difficult to control in case-control studies. Second, substantial heterogeneity is a potential problem in the interpretation of the results of our analysis. This heterogeneity was expected considering the differences in the characteristics of the study designs, population sources, and genotyping methods among the included studies. After observing substantial heterogeneity, we performed subgroup analyses and obtained similar findings with low-moderate heterogeneity. It shows that ethnicity and source of control, genotyping methods may contribute to the origin of heterogeneity. Furthermore, sensitivity analyses were conducted, and similar results were obtained. Third, individual participant data were not available. More precisely adjusted ORs for other covariates, such as age, ethnicity, year, and lifestyle, were not obtained. Fourth, the number of eligible studies for microRNA-146a and microRNA-423 was small, thereby limiting the statistical power. Finally, the number of included studies for Caucasian and African populations limited further analysis because of the shortage of original studies.

The following points should be taken into account in future studies. First, large-scale studies focusing on microRNA-146a, microRNA-423, microRNA-26a-1, microRNA-499, microRNA-124-1, microRNA-34b/c, microRNA-26a-1, microRNA-27a, and microRNA-218 should be included. Second, more studies should aim to investigate the association between microRNA and esophageal cancer susceptibility among the Caucasian, African, and Asian populations or other ethnicities.

In conclusion, the current available evidence suggested that the microRNA-196a2, microRNA-146a, and microRNA-423 are unlikely to have any important effects on the risk of esophageal cancer, except on Caucasian subjects. However, the results should be interpreted cautiously because of the presence of heterogeneity among studies. Studies involving large-scale, well-designed trials and different ethnic groups are required in future analyses.

## References

- Begg, C.B., Mazumdar, M., 1994. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 50, 1088–1101.
- Berezikov, E., Guryev, V., van de Belt, J., Wienholds, E., Plasterk, R.H., Cuppen, E., 2005. Phylogenetic shadowing and computational identification of human microRNA genes. *Cell* 120, 21–24.
- Buas, M.F., Onstad, L., Levine, D., Vaughan, T.L., Whiteman, D.C., 2013. MiRNA-related SNPs and risk of esophageal adenocarcinoma and Barrett's esophagus: Post genome-wide association analysis in the BEACON consortium. *Cancer Res.* 73.
- Calin, G.A., 2009. MicroRNAs and cancer: what we know and what we still have to learn. *Genome med.* 1, 78.
- Christensen, B.C., Avissar-Whiting, M., Ouellet, L.G., Butler, R.A., Nelson, H.H., McClean, M.D., Marsit, C.J., Kelsey, K.T., 2010. Mature microRNA sequence polymorphism in MIR196A2 is associated with risk and prognosis of head and neck cancer. *Clin. Cancer Res.* 16, 3713–3720.

- David, S., Meltzer, S.J., 2011. MicroRNA involvement in esophageal carcinogenesis. *Curr. Opin. Pharmacol.* 11, 612–616.
- Egger, M., Davey Smith, G., Schneider, M., Minder, C., 1997. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 315, 629–634.
- Eng, L., Faluyi, O.O., Qiu, X., Cheng, D., Renouf, D.J., Dodriba, L., Savas, S., Marsh, S., Knox, J.J., Darling, G.E., et al., 2013. Polymorphisms in microRNA (miRNA) pathways and survival in esophageal cancer (EC) patients. *Cancer Res.* 73.
- Enzinger, P.C., Mayer, R.J., 2003. Esophageal cancer. *N. Engl. J. Med.* 349, 2241–2252.
- Faluyi, O.O., Eng, L., Qiu, X., Cheng, D., Renouf, D.J., Marsh, S., Savas, S., Knox, J.J., Darling, G.E., Wong, R., et al., 2013. MicroRNA polymorphisms and esophageal cancer outcome. *J. Clin. Oncol.* 31.
- Ferlay, J., Shin, H.R., Bray, F., Forman, D., Mathers, C., Parkin, D.M., 2010. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int. J. Cancer* 127, 2893–2917 (*Journal international du cancer*).
- Guo, H., Wang, K., Xiong, G., Hu, H., Wang, D., Xu, X., Guan, X., Yang, K., Bai, Y., 2010. A functional variant in microRNA-146a is associated with risk of esophageal squamous cell carcinoma in Chinese Han. *Fam. Cancer* 9, 599–603.
- Guo, J., Jin, M., Zhang, M., Chen, K., 2012. A genetic variant in miR-196a2 increased digestive system cancer risks: a meta-analysis of 15 case-control studies. *PLoS One* 7, e30585.
- Kim, V.N., 2005. MicroRNA biogenesis: coordinated cropping and dicing. *Nat. Rev. Mol. Cell Biol.* 6, 376–385.
- Kim, V.N., Han, J., Siomi, M.C., 2009. Biogenesis of small RNAs in animals. *Nat. Rev. Mol. Cell Biol.* 10, 126–139.
- Li, S.Q., Wang, H.M., Cao, X.F., 2011. Potential clinical insights into microRNAs and their target genes in esophageal carcinoma. *Biomarkers* 16, 629–636.
- Li, K., Tie, H., Hu, N., Chen, H., Yin, X., Peng, C., Wan, J., Huang, W., 2014. Association of two polymorphisms rs2910164 in miRNA-146a and rs3746444 in miRNA-499 with rheumatoid arthritis: A meta-analysis. *Hum. Immunol.* 75, 602–608.
- Matsushima, K., Isomoto, H., Kohno, S., Nakao, K., 2010. MicroRNAs and esophageal squamous cell carcinoma. *Digestion* 82, 138–144.
- Pennathur, A., Gibson, M.K., Jobe, B.A., Luketich, J.D., 2013. Oesophageal carcinoma. *Lancet* 381, 400–412.
- Pritchard, C.C., Cheng, H.H., Tewari, M., 2012. MicroRNA profiling: approaches and considerations. *Nat. Rev. Genet.* 13, 358–369.
- Qu, Y., Qu, H., Luo, M., Wang, P., Song, C., Wang, K., Zhang, J., Dai, L., 2014. MicroRNAs related polymorphisms and genetic susceptibility to esophageal squamous cell carcinoma. *Mol. Gen. Genomics.* 1123–1130.
- Shi, Z.Z., Shang, L., Jiang, Y.Y., Hao, J.J., Zhang, Y., Zhang, T.T., Lin, D.C., Liu, S.G., Wang, B.S., Gong, T., et al., 2013. Consistent and differential genetic aberrations between esophageal dysplasia and squamous cell carcinoma detected by array comparative genomic hybridization. *Clin. Cancer Res.* 19, 5867–5878.
- Shuto, A., Tanaka, F., Mimori, K., Fujita, H., Shirouzu, K., Mori, M., 2009. Upper G.I. cancer: the esophagus and stomach. II. The current status of comprehensive gene analysis of esophageal cancer. *Gan to kagaku ryoho Cancer & chemotherapy* 36, 1435–1441.
- Siegel, R., Ma, J., Zou, Z., Jemal, A., 2014. Cancer statistics, 2014. *CA Cancer J. Clin.* 64, 9–29.
- Tricoli, J.V., Jacobson, J.W., 2007. MicroRNA: potential for cancer detection, diagnosis, and prognosis. *Cancer Res.* 67, 4553–4555.
- Umar, M., Upadhyay, R., Prakash, G., Kumar, S., Ghoshal, U.C., Mittal, B., 2013. Evaluation of common genetic variants in pre-microRNA in susceptibility and prognosis of esophageal cancer. *Mol. Carcinog.* 52, E10–E18.
- Wang, K., Guo, H., Hu, H., Xiong, G., Guan, X., Li, J., Xu, X., Yang, K., Bai, Y., 2010. A functional variation in pre-microRNA-196a is associated with susceptibility of esophageal squamous cell carcinoma risk in Chinese Han. *Biomarkers* 15, 614–618.
- Wang, K., Li, J., Guo, H., Xu, X., Xiong, G., Guan, X., Liu, B., Li, J., Chen, X., Yang, K., et al., 2012. MiR-196a binding-site SNP regulates RAPIA expression contributing to esophageal squamous cell carcinoma risk and metastasis. *Carcinogenesis* 33, 2147–2154.
- Wang, Y., Vogelsang, M., Schafer, G., Matejic, M., Parker, M.L., 2013. MicroRNA polymorphisms and environmental smoke exposure as risk factors for oesophageal squamous cell carcinoma. *PLoS One* 8, e78520.
- Wang, N., Li, Y., Zhou, R.M., Wang, G.Y., Wang, C.M., Chen, Z.F., Liu, W., 2014. Hsa-miR-196a2 functional SNP is associated with the risk of ESCC in individuals under 60 years old. *Biomarkers* 19, 43–48.
- Wei, J., Zheng, L., Liu, S., Yin, J., Wang, L., Wang, X., Shi, Y., Shao, A., Tang, W., Ding, G., et al., 2013. MiR-196a2 rs11614913 T > C polymorphism and risk of esophageal cancer in a Chinese population. *Hum. Immunol.* 74, 1199–1205.
- Wu, H., Zheng, J., Deng, J., Hu, M., You, Y., Li, N., Li, W., Lu, J., Zhou, Y., 2013. A genetic polymorphism in lincRNA-uc0030pf.1 is associated with susceptibility to esophageal squamous cell carcinoma in Chinese populations. *Carcinogenesis* 34, 2908–2917.
- Wu, C., Li, M., Hu, C., Duan, H., 2014. Prognostic role of microRNA polymorphisms in patients with advanced esophageal squamous cell carcinoma receiving platinum-based chemotherapy. *Cancer Chemother. Pharmacol.* 73, 335–341.
- Yang, P.W., Hsieh, C.Y., Huang, Y.T., Huang, Y.C., Chiang, T.H., Lee, J.M., 2013. The hereditary genetic polymorphism of mir-608 predicts the clinical outcome and expression of IL-6 in patients with esophageal squamous cell carcinoma. *Cancer Res.* 73.
- Yang, P.W., Huang, Y.C., Hsieh, C.Y., Hua, K.T., Huang, Y.T., Chiang, T.H., Chen, J.S., Huang, P.M., Hsu, H.H., Kuo, S.W., et al., 2014. Association of miRNA-related genetic polymorphisms and prognosis in patients with esophageal squamous cell carcinoma. *Ann. Surg. Oncol.* 601–609.
- Ye, Y., Wang, K.K., Gu, J., Yang, H., Lin, J., Ajani, J.A., Wu, X., 2008. Genetic variations in microRNA-related genes are novel susceptibility loci for esophageal cancer risk. *Cancer Prev. Res. (Phila)* 1, 460–469.
- Yin, J., Wang, X., Zheng, L., Shi, Y., Wang, L., Shao, A., Tang, W., Ding, G., Liu, C., Liu, R., et al., 2013. Hsa-miR-34b/c rs4938723 T > C and hsa-miR-423 rs6505162 C > A polymorphisms are associated with the risk of esophageal cancer in a Chinese population. *PLoS One* 8, e80570.
- Zhang, T., Zhao, D., Wang, Q., Yu, X., Guo, L., Lu, S.H., 2010. Allele specific regulation of esophageal cancer related gene 2 by miR-1322 in esophageal squamous cell carcinoma. *Cancer Prev. Res.* 3.
- Zhang, T., Zhao, D., Wang, Q., Yu, X., Cui, Y., Guo, L., Lu, S.H., 2013. MicroRNA-1322 regulates ECRG2 allele specifically and acts as a potential biomarker in patients with esophageal squamous cell carcinoma. *Mol. Carcinog.* 52, 581–590.
- Zheng, S., Vuitton, L., Sheyhidin, I., Vuitton, D.A., Zhang, Y., Lu, X., 2011. Northwestern China: a place to learn more on oesophageal cancer. Part two: gene alterations and polymorphisms. *Eur. J. Gastroenterol. Hepatol.* 23, 1087–1099.
- Zhou, L., Zhang, X., Li, Z., Zhou, C., Li, M., Tang, X., Lu, C., Li, H., Yuan, Q., Yang, M., 2013. Association of a genetic variation in a miR-191 binding site in MDM4 with risk of esophageal squamous cell carcinoma. *PLoS One* 8.