Int J Clin Exp Med 2019;12(4):3810-3820
www.ijcem.com /ISSN:1940-5901/IJCEM0077539

Original Article

Lack of association between MicroRNA-146a rs2910164 C > G polymorphism and risk of gastric carcinoma: a case-control study and a meta-analysis

Jing Lin[1,2,4*], Wansong Lin[3,4*], Bin Lan[5*], Shuping Chen[3,4], Weiliang Wu[6], Jianyong Ruan[6], Chao Liu[7], Weifeng Tang[8], Yu Chen[1,2,4], Zengqing Guo[1,2,4]

[1]Department of Medical Oncology, [2]Cancer Bio-immunotherapy Center, [3]Laboratory of Immuno-Oncology, Fujian Cancer Hospital & Fujian Medical University Cancer Hospital, Fuzhou, Fujian Province, People's Republic of China; [4]Fujian Provincial Key Laboratory of Translational Cancer Medicine, Fuzhou, Fujian Province, People's Republic of China; [5]Key Laboratory of Systems Biomedicine (Ministry of Education), Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, Shanghai, People's Republic of China; [6]Department of Implantology II, School and Hospital of Stomatology, Fujian Medical University, Fuzhou, Fujian Province, People's Republic of China; [7]Department of Cardiothoracic Surgery, Affiliated People's Hospital of Jiangsu University, Zhenjiang, Jiangsu Province, People's Republic of China; [8]Department of Thoracic Surgery, Fujian Medical University Union Hospital, Fuzhou, Fujian Province, People's Republic of China. [*]Equal contributors.

Received April 7, 2018; Accepted October 30, 2018; Epub April 15, 2019; Published April 30, 2019

Abstract: MicroRNAs (miRNAs) may be important molecular biomarkers and therapeutic targets of gastric carcinoma (GC). However, the relationship between the miRNA-146a rs2910164 C > G polymorphism and risk of GC was not clear. In the present study, we conducted a case-control study and then performed an updated meta-analysis to get a more precise evaluation. In total, 490 GC patients and 1,476 cancer-free subjects were enrolled. The SNPscan™ genotyping assay was used to genotype the miRNA-146a rs2910164 C > G polymorphism. We found there was no significant difference in genotype distribution of the miR-146a rs2910164 C > G polymorphism among GC patients and controls. After pooling all enrolled studies, we also found null association of the miR-146a rs2910164 C > G polymorphism with GC risk under all genetic models, even in different ethnicities. There was no publication bias for those genetic models. Results of heterogeneity analysis indicated that a large sample size study, Asian populations and low quality studies might contribute to the major source of heterogeneity. Sensitivity analysis suggested that our findings were stable. In conclusion, this case-control study in an eastern Chinese Han population, along with a meta-analysis, failed to identify a relationship between the miRNA-146a rs2910164 C > G polymorphismand GC risk, even across different ethnicities. Nevertheless, for practical reasons, more well-designed prospective studies with a larger sample size and detailed environmental risk factors are needed to confirm or refute these findings.

Keywords: MiRNA-146a, polymorphism, gastric carcinoma, susceptibility

Introduction

Gastric carcinoma (GC) is the fifth most common form of malignancy, causing the third highest malignancy-related mortality rate worldwide. The GC rate varies in different countries. The GC incidence rates are highest in Eastern Asia, South American, and Central-eastern Europe [1]. In China, approximately 679,100 new GC cases were diagnosed together with 498,000 GC-related deaths in 2015 [2]. Regional variations may be due to differences in lifestyle and genetic background, as well as the prevalence of *H. pylori* infection. It is reported that chronic infection with *H. pylori* may contribute to the risk to GC. However, other risk factors involving genetic factors may also contribute to the development of GC.

A microRNA (miRNA) is a small single-stranded non-coding RNA molecule (19-25 nt) found in animals, plants and some viruses [3, 4]. MiRNA contain about 22 nucleotides and is implicated in gene expression by RNA silencing and post-transcriptional regulation [3]. MiRNA function is silenced via the following processes: (a) cleav-

ing the mRNA strand, (b) shortening poly A tail of mRNA, and (c) decreasing the efficient translation of mRNA [5]. A number of investigations demonstrated that miRNAs play an important role in complex biological processes (e.g. apoptosis, cell differentiation, and proliferation) [6-9]. Previous studies reported that a number of miRNAs act on cancer-related genomic areas, and might influence the development and prognosis of cancers [10] Chronic lymphocytic leukemia (CLL) was the first human malignancy known to be correlated with miRNA deregulation [11]. MiRNA profiling could assess whether CLL patients had more or less aggressive form of the disease. The expression level of miRNA has also been used to evaluate cancer prognosis [12, 13]. Recently, a study suggested that miRNA might be a diagnostic biomarker of GC [14]. In addition, Shin and colleges showed that miRNAs might be important molecular biomarkers and therapeutic targets of GC [15].

A single nucleotide polymorphism (SNP) is a variation which occurs at a specific position in the genome. SNPs may underlie differences in risk to human disease. SNPs may fall within the different regions in a gene, such as non-coding areas, coding sequences, and intergenic sections. However, SNPs in non-coding regions are more frequent than in coding sequences. It was reported that miRNAs polymorphisms could alter expression and function [16], and confer susceptibility to malignancies [17, 18]. Results of previous case-control studies showed that miR-146a rs2910164 C > G polymorphisms might confer GC susceptibility. Recently, several meta-analyses demonstrated that these miRNA polymorphisms might influence the risk of GC [19-22]. However, due to the limited included publications and sample sizes, the results were inconsistent. Thus, considering the potential role of miR-146a rs2910164 C > G polymorphisms for GC risk; we conducted a case-control study with a related large sample size and then performed an updated meta-analysis to get a more precise evaluation.

Materials and methods

Subjects

All participants were recruited from a hospital-based case-control study, which was carried out at the Affiliated Union Hospital of Fujian Medical University and the Affiliated People's Hospital of Jiangsu University in Eastern China. This study consisted of 490 incident GC patients who were diagnosed at these two hospitals between May 2013 and June 2016. At the same time, 1,476 cancer-free subjects who participated in physical examination in the hospitals mentioned above were included as controls. The controls were matched to GC patients by age and sex. Information on age, sex, smoking status and alcohol use was obtained by using a questionnaire. Study participants' height and weight were measured, and then body mass index (BMI) was calculatedby body mass divided by the square of the body height. BMI was expressed in units of kg/m^2. Experienced doctors obtained the relevant information (e.g. demographic variables and risk factors) by face-to-face interview. Each participant signed a written consent and donated a blood sample. For the GC group, all information and the blood sample was obtained right after the diagnosis of pathology and before any treatment. The present study protocol was approved by the Institutional Review Board of Fujian Medical University and Jiangsu University.

DNA extraction and genotyping

We used Promega DNA Purification Kit (Madison, USA) to extract genomic DNA from blood samples, which were donated by participants. The SNPscan™ genotyping assay (GeneskyBiotechologies Inc., Shanghai, China) was used to obtain the genotyping of the miR-146a rs2910164 C > G polymorphism. Seventy-nine (4%) samples were randomly selected and analysis of the genotyping was repeated. Results of quality control showed that the obtained genotypes were reliable.

Statistical analysis

Continuous variables (e.g. age and BMI) were expressed as mean ± standard deviation (SD). And these continuous variables were compared by using Student's *t*-test. Categorical variables (e.g. age, sex, smoking status, alcohol use, and BMI) were expressed as proportions. And they were compared with the Chi-squared test (χ^2-test). The HWE in the control group was assessed using the χ^2-test with an online HWE calculator (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) [23-26]. We carried out a multivariate logis-

Table 1. Distribution of selected demographic variables and risk factors in gastric carcinoma patients and controls

Variable	Overall Cases (n=490)	Overall Controls (n=1,476)	P^a
	n (%)	n (%)	
Age (years)	60.65±11.43	61.30±9.60	0.220
Age (years)			0.597
< 61	221 (45.10)	686 (46.48)	
≥ 61	269 (54.90)	790 (53.52)	
Sex			0.891
Male	331 (67.55)	1,002 (67.89)	
Female	159 (32.45)	474 (32.11)	
Smoking status			0.001
Never	309 (63.06)	1,051 (71.21)	
Ever	181 (36.94)	425 (28.79)	
Alcohol use			< 0.001
Never	374 (76.33)	1,319 (89.36)	
Ever	116 (23.67)	157 (10.64)	
BMI (kg/m^2)	22.41 (±3.12)	23.95 (±3.05)	< 0.001
BMI (kg/m^2)			
< 24	356 (72.65)	761 (51.56)	< 0.001
≥ 24	134 (27.35)	715 (48.44)	

[a]Two-sided χ^2 test and Student's t-test.

tic regression analysis to explore the relationship of miR-146a rs2910164 C > G polymorphisms with GC, with adjustment for GC risk factors including age, sex, smoking status, alcohol use, and BMI. A P value < 0.05 (two sided) was considered as significant. All data analysis was performed using SAS 9.4 (SAS Institute, Cary, NC, USA).

Meta-analysis

We searched PubMed and EMBASE (updated to November 27, 2017) with the terms 'miR-146a or rs2910164' and 'gastric cancer or stomach cancer or gastric carcinoma' and 'polymorphism or SNP or variant' for relevant publications. Only English language studies and human studies were included in our study. And additional publications were searched by checking all references of included studies or reviews on this issue. In the present meta-analysis, the major inclusion criteria were: (a) evaluation of the miR-146a rs2910164 C > G polymorphisms and GC risk, (b) being designed as a case-control study and (c) the genotype frequency of cases and controls was available.

Two investigators (J. Lin and C. Liu) independently extracted the useful data. Any disagreements were resolved by a detailed discussion until a consensus between these two authors was reached on each item. The following information was selected and collected: surname of the first author, publication year, country, ethnicity, sample size, genotyping method, and genotype frequency in GC patients and controls. Ethnicities were defined as Asian and Caucasians.

We analyzed HWE in controls using an online HWE calculator mentioned above. The crude odds ratios (ORs) with their 95% confidence intervals (CIs) were applied to determine the strength of the relationship between miR-146a rs2910164 C > G polymorphisms and GC risk. The Chi-square based Cochran's Q-test and I^2 test [27] were used to analyze the heterogeneity between the included studies. If I^2 > 50% or P < 0.1, which means a significant heterogeneity among studies, random-effects model (the Der-Simonian and Laird method) was used to calculate the pooled ORs and Cis [28, 29]. Otherwise, a fixed-effects model (the Mantel-Haenszel method) [30] was selected. In addition, the sources of heterogeneity were assessed by a stratified meta-analysis by ethnicity, sample size, and quality score. Begg's test and Egger's linear regression test were used to estimate the potential publication bias [31]. A P < 0.1 means a significant bias of publication. One-way sensitivity analysis was performed to determine the stability of the findings. We used the Newcastle-Ottawa Quality Assessment Scale to assess the quality score of the studies enrolled in the meta-analysis [32, 33]. If scores were ≥ 7 stars, the study was accepted as high-quality [32, 33]. In this meta-analysis, we analyzed the data by using the STATA 12.0 software (Stata Corporation, College Station, Texas).

Results

Baseline characteristics

Our study consisted of 490 (331 males and 159 females 27-88 years old with a mean age of 60.65±11.43 years) GC patients and 1,476

Table 2. PrimaryinformationformiR-146a rs2910164 C > Gpolymorphism

Genotyped SNPs	Chromosome	ChrPos (NCBI Build 37)	Region	MAF[a] for Chinese in database	MAF in our controls (n=1,476)	*P* value for HWE[b] test in our controls	Genotyping method	Genotyping value (%)
MiR-146a rs2910164 C > G	5	159912418	UTR	0.43	0.37	0.792	SNPscan	99.59

[a]MAF: minor allele frequency; [b]HWE: Hardy-Weinberg equilibrium.

Table 3. Logistic regression analyses of associations between miR-146a rs2910164 C > G polymorphisms and gastric cancer risk

Genotype	GC Cases (n=490)		Controls (n=1,476)		Crude OR (95% CI)	*P*	Adjusted OR[a] (95% CI)	*P*
	n	%	n	%				
MiR-146a rs2910164 C > G								
CC	182	37.45	583	39.61	1.00		1.00	
CG	223	45.88	683	46.40	1.03 (0.82-1.29)	0.793	0.98 (0.78-1.23)	0.852
GG	81	16.67	206	13.99	1.24 (0.91-1.68)	0.166	1.27 (0.93-1.75)	0.134
CG+GG	304	62.55	889	60.39	1.10 (0.89-1.35)	0.398	1.06 (0.85-1.324)	0.608
CC+CG	405	83.33	1,266	86.01	1.00		1.00	
GG	81	16.67	206	13.99	1.23 (0.93-1.63)	0.149	1.30 (0.97-1.74)	0.079
G allele	385	39.61	1,095	37.19				

[a]Adjusted for age, sex, smoking and drinking status.

(1,002 males and 474 females 25-83 years old with a mean age of 61.30±9.60 years) controls recruited in the Affiliated Union Hospital of Fujian Medical University and the Affiliated People's Hospital of Jiangsu University in the eastern China (**Table 1**). The controls were fully-matched with GC cases by age and gender (*P*= 0.597 and 0.891, respectively). The minor allelic frequency (MAF) valuein controls was 0.37, which was similar to the value of the Chinese patients. The success rate of genotyping for this SNP was 99.59% (**Table 2**). **Table 3** summarizes the genotype distribution of themiR-146a rs2910164 C > G polymorphisms. The *P* value of the Hardy-Weinberg Equilibrium (HWE) in controlsfor this SNP was 0.792 (**Table 2**).

Association of miR-146a rs2910164 C > G polymorphisms with GC risk

The frequencies of rs2910164 CC, CG, and GG genotypes were 37.45%, 45.88%, and 16.67% in the 490 GC patients and 39.61%, 46.40%, and 13.99% in the 1,476 controls, respectively. We found no difference in genotype distribution of the miR-146a rs2910164 C > G polymorphism among GC patients and controls (**Table 3**). In addition, after adjustments for age, sex, smoking, drinking and BMI, there was no significant difference in the genotype distribution of the miR-146a rs2910164 C > G polymorphism between GC patients and controls (**Table 3**).

Meta-analysis of miR-146a rs2910164 C > G polymorphism with GC risk

We carried out a meta-analysis to precisely assess the association of the miR-146a rs29-10164 C > G polymorphisms with GC risk. A total of fifty abstracts were retrieved from Pubmed and EMBASE databases. Here, we summarize the selecting process in **Figure 1**. The extracted detailed characteristics and the miR-146a rs2910164 C > G genotypes of enrolled studies are presented in **Table 4**. Finally, sixteen studies [19, 34-48] plus our study encompassing a total of 7,633 GC patients and 11,263 controls were analyzed, with twelve studies focusing on Asians [19, 34-43] and five on Caucasians [44-48]. And six studies with large sample size (≥ 1000) [34, 36, 37, 42, 43] and eleven study with small sample size (< 1000) [19, 35, 38-41, 44-48] were included. Quality scores of these enrolled case-control studies ranged from 5 to 8 (mean: 6.65) out of a maximal score of 10 with Newcastle-Ottawa Quality Assessment Scale method (**Table 5**). Frequencies of the miR-146a rs2910164 G allele varied widely, and they were much higher in Caucasians than in Asians for both patients (62.00% versus 45.42%) and controls (52.16% versus 42.84%).

After pooling all enrolled studies, we found a null association of the miR-146a rs2910164 C > G polymorphism with GC risk under all genetic models (G *vs.* C: OR=1.04; 95% CI: 0.97-1.13;

Table 4. Characteristics and genotypes of the studies in meta-analysis

Study	Year	Ethnicity	Country	Sample size (case/control)	Genotyping method	Case			Control			HWE
						CC	CG	GG	CC	CG	GG	
Rogoveanu et al.	2017	Caucasians	Romanian	142/288	Taqman	8	48	86	19	109	160	0.94
Yadegari et al.	2016	Caucasians	Iran	120/120	PCR-RFLP	73	38	9	81	34	5	0.56
Gu et al.	2016	Asians	China	186/186	PCR-RFLP	29	91	66	25	90	70	0.64
Jiang et al.	2016	Asians	China	898/992	MassARRAY-TOF MS	303	441	154	325	457	207	0.05
Xia et al.	2016	Asians	China	1,125/1,196	Taqman	397	536	192	420	577	199	0.97
Soleimani et al.	2016	Caucasians	Iran	130/130	PCR-RFLP	13	42	75	10	40	80	0.13
Parlayan et al.	2014	Asians	Japan	160/524	Taqman	61	79	20	216	237	71	0.64
Pu et al.	2014	Asians	China	220/530	PCR-RFLP	65	96	36	143	274	96	0.08
Kupcinskas et al.	2014	Caucasians	German, Lithuania and Latvia	363/351	Taqman	16	94	252	16	108	223	0.53
Dikeakos et al.	2014	Caucasians	Greece	163/480	PCR-RFLP	105	45	13	307	149	24	0.29
Ahn et al.	2013	Asians	Korea	461/447	PCR-RFLP	159	231	71	164	221	62	0.36
Zhou et al.	2012	Asians	China	1,686/1,895	Taqman	286	822	578	393	951	551	0.64
Ma et al.	2012	Asians	China	86/42	PCR-RFLP	14	44	20	14	19	6	0.92
Okubo et al.	2010	Asians	Japan	552/697	PCR-RFLP	236	243	73	254	322	121	0.28
Hishida et al.	2011	Asians	Japan	583/1,637	PCR-CTPP	230	271	82	633	775	229	0.74
Zeng et al.	2010	Asians	China	304/304	PCR-RFLP	89	153	62	119	132	53	0.12
Our study	2017	Asians	China	490/1,476	SNPscan	182	223	81	583	683	206	0.79

HWE: Hardy-Weinberg equilibrium; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; PCR-CTPP: polymerase chain reaction with confronting two-pair primers; MALDI-TOF MS: Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry.

Table 5. Quality assessment of the included studies in meta-analysis

Study	Year	Selection				Comparability of the cases and controls	Exposure			Total Stars
		Adequate case definition	Representativeness of the cases	Selection of the controls	Definition of Controls		Ascertainment of exposure	Same ascertainment method for cases and controls	Non-Response rate	
Rogoveanu et al.	2017	★	★	-	★	★★	★	-	-	6
Yadegari et al.	2016	★	★	-	★	★★		-	-	5
Gu et al.	2016	★	★	-	★	★★	★	★	-	7
Jiang et al.	2016	★	★	-	★	★★	★	★	-	7
Xia et al.	2016	★	★	★	★	★★	★	★	-	8
Soleimani et al.	2016	★	★	-	★	★★	–	★	-	6
parlayan et al.	2014	★	★	-	★	★★	★	★	-	7
Pu et al.	2014	★	★	-	★	★★	★	★	-	7
Kupcinskas et al.	2014	★	★	-	★	★★	★	★	-	7
Dikeakos et al.	2014	★	★	-	★	★	★	–	-	5
Ahn et al.	2013	★	★	-	★	★★	★	★	-	7
Zhou et al.	2012	★	★	-	★	★★	★	★	-	7
Ma et al.	2012	★	★	-	–	★	★	★	-	5
Okubo et al.	2010	★	★	-	★	–	★	★	-	5
Hishida et al.	2011	★	★	-	★	★★	★★	★	-	8
Zeng et al.	2010	★	★	-	★	★★	★★	★	-	8
Our study	2017	★	★	-	★	★★	★★	★	-	8

P=0.271; GG *vs.* CC: OR=1.08; 95% CI: 0.92-1.26; *P*=0.364; GG/CG *vs.* CC: OR=1.05; 95% CI: 0.95-1.16; *P*=0.385 and GG *vs.* CG/CC: OR=1.06; 95% CI: 0.95-1.18; *P*=0.305; **Figure 2**), even in different ethnicity (**Table 6**).

There was no publication bias in any of thegeneticmodels (G *vs.* C: Begg's test *P*=0.434, Egger's test *P*=0.786; GG *vs.* CC: Begg's test *P*=0.387, Egger's test *P*=0.841; GG/CG *vs.* CC: Begg's test *P*=0.773, Egger's test *P*=0.660; GG *vs.* CG/CC: Begg's test *P*=0.650, Egger's test *P*=0.939; **Figure 3**). To account for major sources of the heterogeneity, we performed a subgroup analysis according to quality score, ethnicity and sample size (**Table 6**). We found that a large sample size study, Asian population and a low quality study might contribute to the major source of heterogeneity. One-way sensitivity analysis was harnessed to evaluate the stability of our findings by excluding an individual study in turn. Results of the sensitivity analysis suggested that our findings were stable (**Figure 4**).

Discussion

GC is a common malignancy and has various inherited and environmental determinants. It is reported that *H. pylori* may be a major etiologic agent in the development of GC. However, *H. pylori* infection alone may not contribute to overall susceptibility to GC. Host inherited factors may also be vital in GC risk. MiRNA, animportant mediator for RNA silencing and post-transcriptional regulation, may play a pivotal role in the development and prognosis of cancers [10]. Considering their importance, SNPs in MiRNA may influence the susceptibility of GC. The effect of MiRNA polymorphisms involved in risk to GC has promoted increasing interest in the past decade. With the increase of inherited investigations, it is clearly advantageous to combine the available data to obtain a reliable assessment. Although several previous studies have regarded the miR-146a rs2910164 C > G polymorphism as a promising candidate for GC risk, our case-control study conducted in an eastern Chinese Han population, along with an extensive pooled-analysis, could notidentify this association, even indifferent ethnicities. To the best of the authors' knowledge, this meta-analysis is a most comprehensive exploration into the relationship between the miR-146a rs2910164 C > G polymorphismand GC risk.

Results of our case-control study found no association between the miR-146a rs2910164 C > G polymorphism with GC risk. Considering the fact that most of common SNPs may make a low penetrance susceptibility to the develop-

Table 6. Results of the meta-analysis from different comparative genetic models

	No. of studies	G vs. C				GG vs. CC				GG+CG vs. CC				GG vs. CG+CC			
		OR (95% CI)	*P*	I^2	*P* (Q-test)	OR (95% CI)	*P*	I^2	*P* (Q-test)	OR (95% CI)	*P*	I^2	*P* (Q-test)	OR (95% CI)	*P*	I^2	*P* (Q-test)
Total	17	1.04 (0.97-1.13)	0.271	58.4%	0.001	1.08 (0.92-1.26)	0.364	54.9%	0.003	1.05 (0.95-1.16)	0.385	44.4%	0.026	1.06 (0.95-1.18)	0.305	42.0%	0.036
Ethnicity																	
Asians	12	1.03 (0.94-1.12)	0.539	68.1%	< 0.001	1.05 (0.88-1.26)	0.583	65.9%	0.001	1.05 (0.93-1.18)	0.457	59.5%	0.004	1.02 (0.90-1.16)	0.772	51.7%	0.019
Caucasians	5	1.13 (0.971.31)	0.118	0.0%	0.561	1.24 (0.86-1.79)	0.257	0.0%	0.618	1.05 (0.82-1.36)	0.680	0.0%	0.810	1.22 (0.99-1.50)	0.065	0.0%	0.503
Sample size																	
≥ 1000	6	1.00 (0.89-1.12)	0.992	79.6%	< 0.001	1.00 (0.79-1.27)	0.975	79.1%	< 0.001	1.01 (0.88-1.15)	0.914	0.015	64.6%	1.00 (0.83-1.21)	0.999	74.9%	0.001
< 1000	11	1.09 (1.00-1.09)	0.046	21.04%	0.239	1.16 (0.96-1.19)	0.117	8.5%	0.363	1.11 (0.97-1.26)	0.133	0.191	26.6%	1.13 (0.98-1.29)	0.092	0.0%	0.710
Quality scores																	
< 7.0	6	1.07 (0.85-1.34)	0.575	64.6%	0.015	1.21 (0.72-2.03)	0.481	62.9%	0.019	1.04 (0.77-1.41)	0.797	51.3%	0.068	1.10 (0.79-1.52)	0.582	50.0%	0.075
≥ 7.0	11	1.05 (0.98-1.13)	0.185	53.4%	0.018	1.09 (0.92-1.26)	0.283	49.5%	0.031	1.07 (0.98-1.11)	0.076	36.7%	0.105	1.07 (0.96-1.20)	0.236	39.5%	0.086

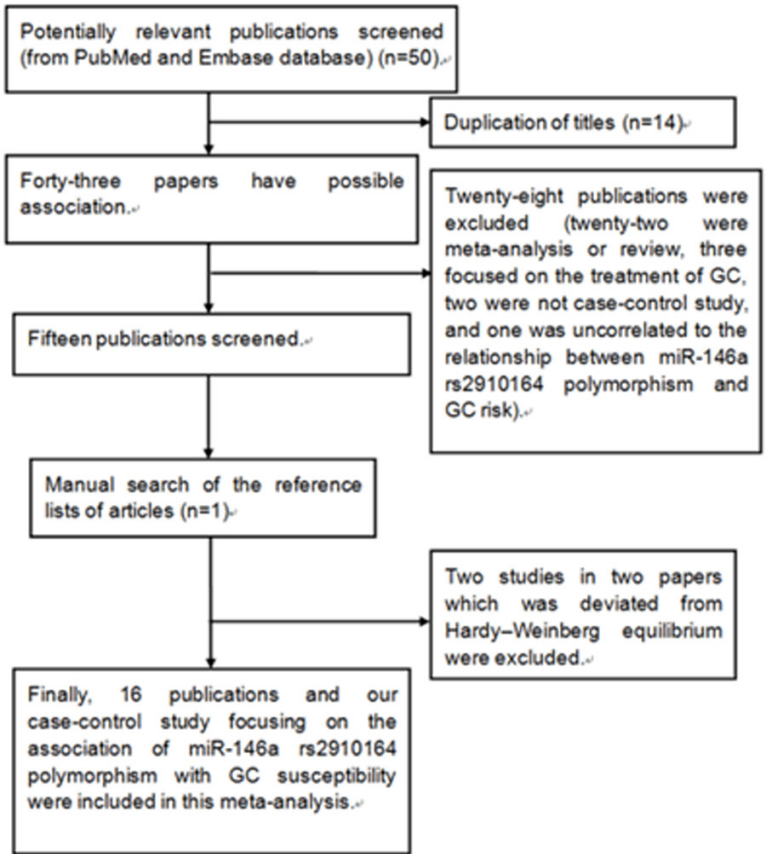

Figure 1. Flow diagram of the meta-analysis of the association between miR-146a rs2910164 C > G polymorphism and GC risk.

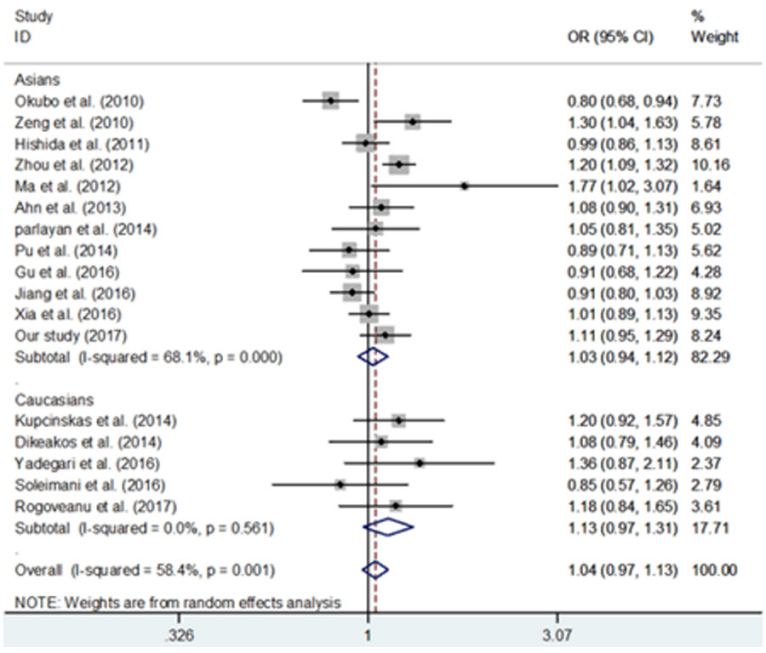

Figure 2. Meta-analysis of the association between miR-146a rs2910164 C > G polymorphism and GC risk (G vs. C, random-effects model).

ment of GC, the current study urges the necessity to obtain a more precise evaluation between the miR-146a rs2910164 C > G polymorphism and GC. In total, 17 case-control studies comprising 7,633 GC patients and 11,263 controls were recruited in our meta-analysis. Several individual studies have reported a positive association between the miR-146a rs2910164 C > G with GC risk [19, 35, 37]. In contrast, as listed in **Table 6**, no significant risk was found in all the genetic models, even in different ethnicities. However, it is worth noting that there was a tendency towards an increased GC risk in Caucasians (P=0.068, **Table 6**). Considering only five studies enrolled Caucasians and all of them were designed with a small sample size (< 1000 subjects); the indication of a possible susceptibility that was found reinforced additional investigations to confirm or refute these results, especially in some populations. Even though, this case-control study along with a meta-analysis could not identify a possible significant effect of the miR-146a rs2910164 G allele in gastric carcinogenesis, and it was possible that the potential role of the miR-146a rs2910164 C > G polymorphism was masked by other factors.

Several merits of this study should be considered. First, the present study was the largest synthesis focusing on the correlation between the-miR-146a rs2910164 C > G polymorphism and GC risk. Second, the results of our case-control study were similar to the findings of the sub-

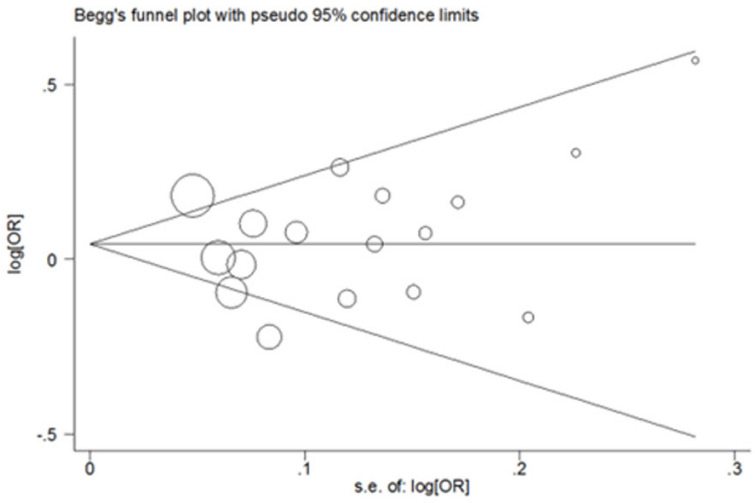

Figure 3. Begg's funnel plot of meta-analysis of the association between miR-146a rs2910164 C > G polymorphism and GC risk (G vs. C compare genetic model, random-effects model).

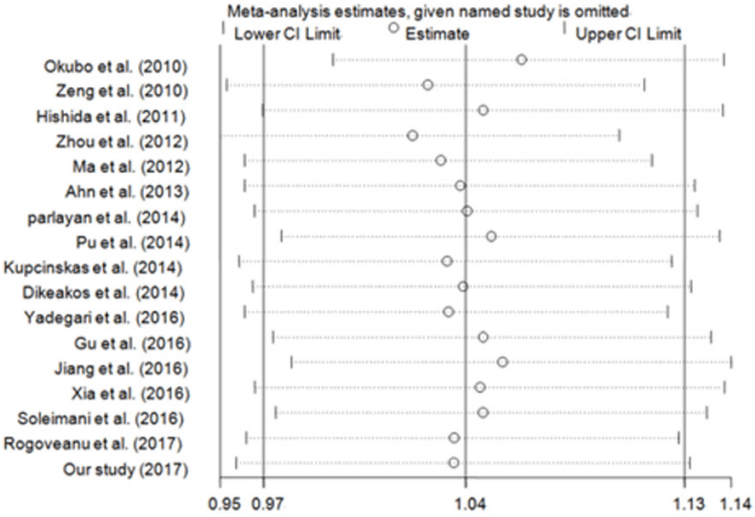

Figure 4. Sensitivity analysis of the influence of G vs. C comparison (random-effects estimates for miR-146a rs2910164 C > G polymorphism).

sequent meta-analysis. Third, in meta-analysis, there was no significant publication bias in the genetic models.

In our study, some limitations should be acknowledged. Firstly, only the miRNA-146a rs2910164 C > G polymorphism was selected for exploring the potential association of this SNP with risk of GC, other important miRNA SNPs were not considered. Secondly, bias might have occurred because only published studies were enrolled. Thirdly, there was significant heterogeneity among the enrolled publications; therefore our conclusions should be interpreted with caution. Finally, data on *H. pylori* infection were not available in some publications, and this important risk factor was not considered in our study.

In summary, the case-control study in an eastern Chinese Han population, along with an extensive meta-analysis, failed to identify a relationship between the miRNA-146a rs2910164 C > G and GC risk, even across different ethnicities. Nevertheless, for practical reasons, more well-designed prospective studies with larger sample sizes and detailed environmental risk factors are needed to confirm or refute these findings.

Acknowledgements

We appreciate all subjects who participated in this study. We wish to thank Dr. Yan Liu (Genesky Biotechnologies Inc., Shanghai, China) for technical support. The project was supported by the National Natural Science Foundation of China (Grant No. U17-05282), Natural Science Foundation of Fujian Province (Grant No. 2017J01259, 2018J01267), Fujian provincial health and family planning research talent training program (Grant No. 2015-CX-7, 2018-ZQN-13, 2016-1-11, 2018-1-13), Joint Funds for the innovation of science and Technology, Fujian province (Grant No. 2017Y9077), and the National Clinical Key Specialty Construction Program.

Disclosure of conflict of interest

None.

Address correspondence to: Zengqing Guo and Yu Chen, Department of Medical Oncology, Fujian

Provincial Cancer Hospital, No. 420, Fuma Road, Fuzhou 350014, Fujian Province, People's Republic of China. Tel: 008613905918836; E-mail: gzq_005@126.com (ZQG); Tel: 008613859089836; E-mail: chenyu1980@fjmu.edu.cn (YC)

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[2] Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-132.

[3] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.

[4] Ambros V. The functions of animal microRNAs. Nature 2004; 431: 350-355.

[5] Fabian MR, Sonenberg N and Filipowicz W. Regulation of mRNA translation and stability by microRNAs. Annu Rev Biochem 2010; 79: 351-379.

[6] Abreu FB, Liu X and Tsongalis GJ. miRNA analysis in pancreatic cancer: the Dartmouth experience. Clin Chem Lab Med 2017; 55: 755-762.

[7] Mizuguchi Y, Takizawa T, Yoshida H and Uchida E. Dysregulated miRNA in progression of hepatocellular carcinoma: a systematic review. Hepatol Res 2016; 46: 391-406.

[8] Guo Z, Zhou H and Zhang W. [Progress in research on genetic variations in miRNA regulatory pathway]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2015; 32: 109-112.

[9] Di Leva G, Cheung DG, and Croce CM. miRNA clusters as therapeutic targets for hormone-resistant breast cancer. Expert Rev Endocrinol Metab 2015; 10: 607-617.

[10] Chen CZ. MicroRNAs as oncogenes and tumor suppressors. N Engl J Med 2005; 353: 1768-1771.

[11] Musilova K and Mraz M. MicroRNAs in B-cell lymphomas: how a complex biology gets more complex. Leukemia 2015; 29: 1004-1017.

[12] Vosa U, Vooder T, Kolde R, Fischer K, Valk K, Tonisson N, Roosipuu R, Vilo J, Metspalu A and Annilo T. Identification of miR-374a as a prognostic marker for survival in patients with early-stage nonsmall cell lung cancer. Genes Chromosomes Cancer 2011; 50: 812-822.

[13] Akcakaya P, Ekelund S, Kolosenko I, Caramuta S, Ozata DM, Xie H, Lindforss U, Olivecrona H and Lui WO. miR-185 and miR-133b deregulation is associated with overall survival and metastasis in colorectal cancer. Int J Oncol 2011; 39: 311-318.

[14] Li C, Li JF, Cai Q, Qiu QQ, Yan M, Liu BY and Zhu ZG. MiRNA-199a-3p: a potential circulating diagnostic biomarker for early gastric cancer. J Surg Oncol 2013; 108: 89-92.

[15] Shin VY and Chu KM. MiRNA as potential biomarkers and therapeutic targets for gastric cancer. World J Gastroenterol 2014; 20: 10432-10439.

[16] Lv H, Pei J, Liu H, Wang H and Liu J. A polymorphism site in the premiR34a coding region reduces miR34a expression and promotes osteosarcoma cell proliferation and migration. Mol Med Rep 2014; 10: 2912-2916.

[17] Xu B, Feng NH, Li PC, Tao J, Wu D, Zhang ZD, Tong N, Wang JF, Song NH, Zhang W, Hua LX and Wu HF. A functional polymorphism in Pre-miR-146a gene is associated with prostate cancer risk and mature miR-146a expression in vivo. Prostate 2010; 70: 467-472.

[18] Qi P, Wang L, Zhou B, Yao WJ, Xu S, Zhou Y and Xie ZB. Associations of miRNA polymorphisms and expression levels with breast cancer risk in the Chinese population. Genet Mol Res 2015; 14: 6289-6296.

[19] Xie WQ and Wang XF. MiR-146a rs2910164 polymorphism increases the risk of digestive system cancer: a meta-analysis. Clin Res Hepatol Gastroenterol 2017; 41: 93-102.

[20] Chen ZF, Ma LL and Xue HB. Common polymorphisms of the microRNA genes (miR-146a and miR-196a-2) and gastric cancer risk: an updated meta-analysis. Genet Mol Res 2015; 14: 8589-8601.

[21] Nikolic ZZ, Savic Pavicevic DL, Vucic NL, Romac SP and Brajuskovic GN. Association between a genetic variant in the hsa-miR-146a gene and cancer risk: an updated meta-analysis. Public Health Genomics 2015; 18: 283-298.

[22] Sun Y and Li M. Genetic polymorphism of miR-146a is associated with gastric cancer risk: a meta-analysis. Eur J Cancer Care (Engl) 2017; 26.

[23] Tang W, Wang Y, Jiang H, Liu P, Liu C, Gu H, Chen S and Kang M. Programmed death-1 (PD-1) rs2227981 C > T polymorphism is associated with cancer susceptibility: a meta-analysis. Int J Clin Exp Med 2015; 8: 22278-22285.

[24] Tang W, Wang Y, Chen S, Lin J, Chen B, Yu S, Chen Y, Gu H and Kang M. Investigation of cytotoxic T-lymphocyte antigen 4 polymorphisms in gastric cardia adenocarcinoma. Scand J Immunol 2016; 83: 212-218.

[25] Tang W, Wang Y, Chen Y, Gu H, Chen S and Kang M. Polymorphisms in the intercellular adhesion molecule 1 gene and cancer risk: a meta-analysis. Int J Clin Exp Med 2015; 8: 11996-12008.

[26] Tang W, Qiu H, Jiang H, Sun B, Wang L, Yin J and Gu H. Lack of association between cytotoxic T-lymphocyte antigen 4 (CTLA-4) -1722T/

C (rs733618) polymorphism and cancer risk: from a case-control study to a meta-analysis. PLoS One 2014; 9: e94039.
[27] Higgins JP and Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002; 21: 1539-1558.
[28] Higgins JP, Thompson SG, Deeks JJ and Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003; 327: 557-560.
[29] DerSimonian R and Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986; 7: 177-188.
[30] Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 1959; 22: 719-748.
[31] Egger M, Davey Smith G, Schneider M and Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997; 315: 629-634.
[32] Wang W, Shao Y, Tang S, Cheng X, Lian H and Qin C. Peroxisome proliferator-activated receptor-gamma (PPARgamma) Pro12Ala polymorphism and colorectal cancer (CRC) risk. Int J Clin Exp Med 2015; 8: 4066-4072.
[33] Wang Y, Huang Q, Liu J, Wang Y, Zheng G, Lin L, Yu H, Tang W and Huang Z. Vascular endothelial growth factor A polymorphisms are associated with increased risk of coronary heart disease: a meta-analysis. Oncotarget 2017; 8: 30539-30551.
[34] Okubo M, Tahara T, Shibata T, Yamashita H, Nakamura M, Yoshioka D, Yonemura J, Ishizuka T, Arisawa T and Hirata I. Association between common genetic variants in pre-microRNAs and gastric cancer risk in Japanese population. Helicobacter 2010; 15: 524-531.
[35] Zeng Y, Sun QM, Liu NN, Dong GH, Chen J, Yang L and Wang B. Correlation between pre-miR-146a C/G polymorphism and gastric cancer risk in Chinese population. World J Gastroenterol 2010; 16: 3578-3583.
[36] Hishida A, Matsuo K, Goto Y, Naito M, Wakai K, Tajima K and Hamajima N. Combined effect of miR-146a rs2910164 G/C polymorphism and Toll-like receptor 4 +3725 G/C polymorphism on the risk of severe gastric atrophy in Japanese. Dig Dis Sci 2011; 56: 1131-1137.
[37] Zhou F, Zhu H, Luo D, Wang M, Dong X, Hong Y, Lu B, Zhou Y, Zhou J, Zhang Z and Gong W. A functional polymorphism in Pre-miR-146a is associated with susceptibility to gastric cancer in a Chinese population. DNA Cell Biol 2012; 31: 1290-1295.
[38] Ahn DH, Rah H, Choi YK, Jeon YJ, Min KT, Kwack K, Hong SP, Hwang SG and Kim NK. Association of the miR-146a C > G, miR-149 T > C, miR-196a2 T > C, and miR-499 A > G polymorphisms with gastric cancer risk and survival in the Korean population. Mol Carcinog 2013; 52 Suppl 1: E39-51.
[39] Parlayan C, Ikeda S, Sato N, Sawabe M, Muramatsu M and Arai T. Association analysis of single nucleotide polymorphisms in miR-146a and miR-196a2 on the prevalence of cancer in elderly Japanese: a case-control study. Asian Pac J Cancer Prev 2014; 15: 2101-2107.
[40] Pu JY, Dong W, Zhang L, Liang WB, Yang Y and Lv ML. No association between single nucleotide polymorphisms in pre-mirnas and the risk of gastric cancer in Chinese population. Iran J Basic Med Sci 2014; 17: 128-133.
[41] Gu JY and Tu L. Investigating the role of polymorphisms in miR-146a, -149, and -196a2 in the development of gastric cancer. Genet Mol Res 2016; 15.
[42] Jiang J, Jia ZF, Cao DH, Wu YH, Sun ZW and Cao XY. Association of the miR-146a rs2910164 polymorphism with gastric cancer susceptibility and prognosis. Future Oncol 2016; 12: 2215-2226.
[43] Xia ZG, Yin HF, Long Y, Cheng L, Yu LJ, Guo WJ, Zhu XD, Li J, Wang YN, Yang YJ, Wang JC, Jin L, Qiu LX and Wei Y. Genetic variant of miR-146a rs2910164 C > G and gastric cancer susceptibility. Oncotarget 2016; 7: 34316-34321.
[44] Kupcinskas J, Wex T, Link A, Leja M, Bruzaite I, Steponaitiene R, Juzenas S, Gyvyte U, Ivanauskas A, Ancans G, Petrenkiene V, Skieceviciene J, Kupcinskas L and Malfertheiner P. Gene polymorphisms of micrornas in Helicobacter pylori-induced high risk atrophic gastritis and gastric cancer. PLoS One 2014; 9: e87467.
[45] Dikeakos P, Theodoropoulos G, Rizos S, Tzanakis N, Zografos G and Gazouli M. Association of the miR-146a C > G, miR-149 T > C, and miR-196a2 T > C polymorphisms with gastric cancer risk and survival in the Greek population. Mol Biol Rep 2014; 41: 1075-1080.
[46] Yadegari ZS, Akrami H, Hosseini SV and Erfani N. miR-146a gene polymorphism and susceptibility to gastric cancer. Br J Biomed Sci 2016; 73: 201-203.
[47] Soleimani A, Ghanadi K, Noormohammadi Z and Irani S. The correlation between miR-146a C/G polymorphism and UHRF1 gene expression level in gastric tumor. J Dig Dis 2016; 17: 169-174.
[48] Rogoveanu I, Burada F, Cucu MG, Vere CC, Ioana M and Cimpeanu RA. Association of microRNA polymorphisms with the risk of gastric cancer in a Romanian Population. J Gastrointestin Liver Dis 2017; 26: 231-238.