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The Association between miR-196a2 rs11614913 Polymorphism and Digestive System Cancer Risk: A Meta-Analysis of 34 Studies

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Abstract

Background: MicroRNAs (miRNAs) negatively regulate the gene expression and act as tumor suppressors or oncogenes in carcinogenesis. The association between single nucleotide polymorphism (SNP) in miR-196a2 rs11614913 and the susceptibility of digestive system cancers was inconsistent in previous studies. **Methods:** A standardized search of PubMed, Embase, and Cochrane library databases for publications on miR-196a2 rs11614913 polymorphism and digestive system cancer risk was performed. Then the genotype data were analyzed in a meta-analysis. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to evaluate the association. Test of heterogeneity, sensitivity analysis and assessment of publication bias were conducted in the present meta-analysis by STATA software 12.0. **Results:** An updated meta-analysis based on 34 independent case-control studies consisting of 13,013 cases and 16,046 controls was performed to address this association. There was a remarkable association between miR-196a2 rs11614913 polymorphism and overall digestive system cancer risk, especially in Asian populations. Moreover, subgroup analysis revealed that variant C allele increased risk of colorectal carcinoma, gastric cancer and hepatocellular carcinoma (HCC), compared with wild T allele. **Conclusions:** There was a remarkable association between miR-196a2 rs11614913 polymorphism and overall digestive system cancer risk, especially in Asian populations.

Keywords

miR-196a2, rs11614913, Polymorphism, Digestive System Cancers, Meta-Analysis

[*]These authors contributed equally to this work.

1. Introduction

MicroRNAs (miRNAs) are a kind of evolutionarily highly conserved, non-coding, single-stranded RNAs with the length of about 22 nucleotides (nt). MiRNAs play crucial roles in a board range of biological processes, including cell differentiation, proliferation, apoptosis and carcinogenesis [1]. The miRNAs regulate the expression of approximately 10% - 30% of all human genes through post-transcriptional mechanism pairing to 3'-untranslated region (3'UTR) of target messenger RNA (mRNA), leading to mRNA degradation or translational repression [2]. Accumulating studies have demonstrated that this novel kind of gene regulators got involved in cancer-related process [3]. The loss and gain of function of some specific miRNAs were also considered to be crucial events in diverse cancers [4]. Although the precise mechanism of how miRNAs mediate carcinogenesis still remains ambiguous, genetic alterations of miRNAs are supposed to be a key event [5].

Single-nucleotide polymorphisms (SNPs) are defined as variation of a single nucleotide (A, T, C, or G) in DNA sequence that occurs at least 1% in certain populations. As the most common type of variation in the human genome, SNPs can influence the population diversity, disease susceptibility, and individual response to medicine by affecting sequence coding and splicing [6]. SNPs in miRNA-coding genes may affect processing and binding ability of miRNAs by altering the secondary structure of miRNA precursors, resulting in aberrant expression of a series of target genes and contributing to cancer susceptibility [7]. Hu *et al.* [8] first reported that SNP miR196a2 rs11614913 was associated to non-small cell lung cancer survival and the CC genotype presented a significant correlation with mature has-mir-196a expression but not with changes in levels of its precursor, suggesting that the process of the pre-miRNA to its mature form was enhanced. Hoffman *et al.* [9] found that rs11614913 not only has an impact on the level of mature miR-196a, but also influences the expression of target genes. Numerous epidemiological studies have demonstrated the association of SNPs in miRNAs with the development and progression of cancer [7] [10]. Furthermore, the association between hsa-miR-196a2 rs11614913 polymorphism and cancer risk has been analyzed in several studies, but the conclusions of these studies remain inconsistent due to heterogeneity of the cancer subtype, limited sample size, and differences in the ethnicity of patients. To reduce the potential between-study heterogeneity which might derive from various cancers in diverse systems and improve the efficiency of meta-analysis on digestive cancers, we only focused on digestive system cancers. Therefore, we conducted this meta-analysis to derive a more precise estimation of the association of miR-196a2 rs11614913 with digestive system cancer risk.

2. Materials and Method

2.1. Publication Search

To identify all potentially eligible studies, we conducted a comprehensive literature search in PubMed, Embase, and Cochrane library databases between 2000 and Sep-

tember 2016 (last updated on September 21st, 2016) with the following search strategy: "miR-196a2 or microRNA-196a2 or rs11614913"; "SNP or polymorphism or mutation or variant or allele" and "cancer or tumor or carcinoma or neoplasm". The references of retrieved articles were also screened to search other potentially related articles. Studies containing two or more case-control groups were considered as two or more independent studies.

2.2. Selection Criteria

Eligible studies were selected according to the following explicit inclusion criteria: I) evaluation of miR-196a2 polymorphism and digestive system cancer risk; II) independent case-control studies for human; III) sufficient published data for calculating odds ratios (ORs) with their 95% confidence intervals (95% CIs); IV) cases with carcinomas were diagnosed by histopathology; V) published in English or Chinese.

2.3. Data Extraction Methodological Assessment

Two investigators (Zhao and Zhou) independently extracted information from eligible studies using a standardized data collection protocol: first author's name, year of publication, country of origin, ethnicity, cancer type, genotyping method, source of control groups, whether verified Hardy-Weinberg equilibrium (**Table 1**), C allele frequency in controls, and genotype frequency distribution. If original genotype frequency data was unavailable in relevant articles, a request for additional data was sent to the corresponding author. Disagreements were resolved by discussion between the two investigators.

The same authors evaluated the methodological quality of the included studies using the Newcastle-Ottawa Scale (NOS) criteria, independently [11]. The NOS criteria are scored based on three aspects: 1) subject selection: 0 - 4, 2) comparability of subject: 0 - 2, and 3) clinical outcome: 0 - 3. Total NOS scores ranged from 0 to 9, with scores ≥ 7 indicating good quality.

2.4. Statistical Analysis

Hardy-Weinberg equilibrium (HWE) in control subjects was tested by the Chi-square goodness-of-fit test. It was considered to be a state of disequilibrium with P-value less than 0.05. Crude ORs and 95% CIs were used to assess the strength of the association between the miR-196a2 rs11614913 polymorphism and digestive system cancer risk. The pooled ORs were calculated for homozygote comparison (CC vs.TT), heterozygote comparison (CC vs. CT), dominant model (CC + CT vs.TT), recessive model (CC vs. CT + TT) and allele model (C vs. T), respectively. Subgroup analyses were also conducted by cancer type, ethnicity (Caucasian and Asian), source of control (population-based and hospital-based), and HWE in controls. The significance of the pooled OR was determined by the Z test in which P value < 0.05 was considered statistically significant.

We used chi-square-based Q-test and the I^2 index to determine the heterogeneity

Table 1. Characteristics of included studies.

First Author	Year	Country	Ethnicity	Cancer Type	Genotyping Method	Source of Control	Sample Size (Case/Control)	HWE (P value)	Quality score
Christensen [12]	2010	USA	Caucasian	OSCC	Taqman	PB	269/555	0.36	8
Christensen [12]	2010	USA	Caucasian	PSCC	Taqman	PB	123/555	0.36	8
Li [13]	2010	China	Asian	HCC	PCR-RFLP	HB	310/222	0.40	7
Liu [14]	2010	USA	Caucasian	OSCC	PCR-RFLP	HB	326/1130	0.74	8
Liu [14]	2010	USA	Caucasian	PSCC	PCR-RFLP	HB	566/1130	0.74	8
Okubo [15]	2010	Japan	Asian	Gastric cancer	PCR-RFLP	HB	552/697	0.51	9
Peng [16]	2010	China	Asian	Gastric cancer	PCR-RFLP	HB	213/213	0.94	7
Qi [17]	2010	China	Asian	HCC	PCR-LDR	HB	361/590	0.40	7
Srinastava [18]	2010	India	Asian	Gallbladder cancer	PCR-RFLP	PB	230/230	0.07	7
Wang [19]	2010	China	Asian	ESCC	SNaPshot	PB	458/489	0.60	9
Akkız [20]	2011	Turkey	Caucasian	HCC	PCR-RFLP	HB	185/185	0.49	7
Zhan [21]	2011	China	Asian	CRC	PCR-RFLP	HB	252/543	0.85	7
Zhang [22]	2011	China	Asian	HCC	PIRA-PCR	HB	934/1622	0.52	9
Chen [23]	2012	China	Asian	CRC	PCR-LDR	HB	126/407	0.79	7
Chu [24]	2012	China	Asian	OSCC	PCR-RFLP	HB	470/425	0.69	8
Hezova [25]	2012	Czech	Caucasian	CRC	Taqman	HB	197/212	0.29	7
Kim [26]	2012	Korea	Asian	HCC	PCR-RFLP	PB	286/201	0.36	7
Min [27]	2012	Korea	Asian	CRC	PCR-RFLP	HB	446/502	0.63	8
Zhu [28]	2012	China	Asian	CRC	Taqman	HB	573/588	0.79	8
Ahn [29]	2013	Korea	Asian	Gastric cancer	PCR-RFLP	HB	461/447	0.32	8
Dikeakos [30]	2013	Greece	Caucasian	Gastric cancer	PCR-RFLP	HB	163/480	0.85	7
Vinci [31]	2013	Italy	Caucasian	CRC	HRMA	NR	160/178	0.09	7
Wang [32]	2013	China	Asian	ESCC	PCR-LDR	HB	597/597	0.97	8
Wei [33]	2013	China	Asian	ESCC	MassARRAY	HB	367/370	0.14	7
Chu [34]	2014	China	Asian	HCC	PCR-RFLP	HB	188/377	0.99	7
Kou [35]	2014	China	Asian	HCC	PCR-RFLP	PB	271/532	0.00	8
Kupcinskas [36]	2014	Lithuania	Caucasian	Gastric cancer	MassARRAY	HB	363/351	0.16	7
Pu [37]	2014	China	Asian	Gastric cancer	PCR-RFLP	HB	220/530	0.00	8
Qi [38]	2014	China	Asian	HCC	HRMA	PB	314/407	0.16	7
Zhang [39]	2014	China	Asian	HCC	MassARRAY	HB	1000/1000	0.24	8
Zhou [40]	2014	China	Asian	HCC	PCR-RFLP	PB	266/281	0.02	8
Li [41]	2015	China	Asian	HCC	PCR-RFLP	HB	266/266	0.69	7
Shen [42]	2015	China	Asian	ESCC	SNaPshot	HB	1400/2185	0.04	8
Sushma [43]	2015	India	Asian	OSCC	PCR-RFLP	PB	100/102	0.00	6

OSCC: oral squamous cell carcinoma, PSCC: pharynx squamous cell carcinoma, HCC: hepatocellular carcinoma, ESCC: esophageal squamous cell carcinoma, CRC: colorectal cancer, PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism, PCR-LDR: polymerase chain reaction-ligation detection reaction, PIRA-PCR: primer-introduced restriction analysis-polymerase chain reaction, HRMA: high-resolution melting analysis, PB: population-based, HB: hospital-based, NR: not reported, HWE: Hardy-Weinberg equilibrium of controls.

among different studies. The Galbraith plot was used to detect the potential sources of heterogeneity. The random-effect model was used when heterogeneity was considered significant (P-value < 0.10 and/or I^2 index > 50%); otherwise, the fixed-effects model was conducted. In addition, potential publication bias was evaluated using Begg's funnel plot, Begg's test and Egger's test. Asymmetric funnel-shaped plots or P < 0.05 was considered the existence of publication bias. We conducted one-way sensitivity analysis to assess the stability of the results. One single study was excluded each time to reflect the influence of the individual data set to the pooled ORs.

All statistical analyses were carried out with STATA software version 12.0 (STATA Corp, College Station, TX). All the P values were two-sided.

3. Results

3.1. Studies Characteristic

In total, 32 eligible studies [12]-[43] including 34 data sets were collected according to the inclusion criteria, with 13013 cases and 16046 controls (**Figure 1**). Characteristics of these studies were shown in **Table 1**. Among Christensen *et al.* and Liu *et al.*'s studies [12] [14] on head and neck squamous cell carcinoma (HNSCC, which included oral, pharyngeal and laryngeal cancers), oral and pharyngeal cancers in digestive system were included, while laryngeal cancer in respiratory system was not used. Oral cancer and pharyngeal cancer were considered as separate groups and calculated independently.

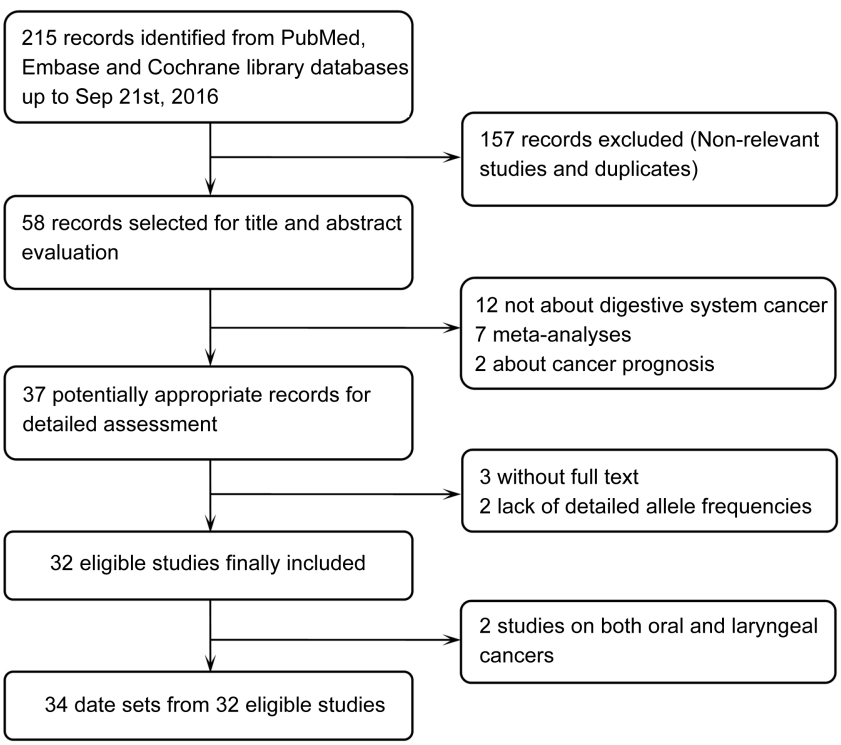

Figure 1. Flow chart of study selection.

Of all the including studies, 4 were on oral squamous cell carcinoma (OSCC), 2 were on pharyngeal squamous cell carcinoma (PSCC), 11 were on hepatocellular carcinoma (HCC), 6 were on gastric cancer (GC), 1 was on gallbladder cancer (GBC), 4 were on esophageal squamous cell carcinoma (ESCC), and 6 were on colorectal carcinoma (CRC). The subjects of 25 studies were Asian and the other 9 studies were Caucasian. Matching for age and sex, controls of 25 studies were hospital-based and 9 studies were population-based. Genotype distribution of controls in most studies was in agreement with HWE (Table 1).

3.2. Quantitative Synthesis

The association strength of miR-196a2 rs11614913 polymorphism and the susceptibility for digestive system cancers is presented in Table 2.

Significant association between miR-196a2 rs11614913 polymorphism and the risk of digestive system cancers were observed in all comparisons (CC vs.TT: OR = 1.317, 95%CI 1.119 - 1.1.549; CT vs.TT: OR = 1.163, 95%CI 1.057 - 1.279; CC + CT vs. TT: OR = 1.219, 95%CI 1.095 - 1.357; CC vs. CT + TT: OR = 1.164, 95%CI 1.013 - 1.337; C vs. T: OR = 1.139, 95%CI 1.047 - 1.240).

Cancer types, source of controls, ethnicity and HWE in controls were taken into consideration for subgroup analysis (Table 2). The forest plot of dominant model (CC + CT vs. TT) in different cancer types is shown in Figure 2. In the stratified analysis by cancer type, remarkable association was detected in Colorectal carcinoma (CC vs. TT: OR = 1.325, 95%CI 1.102 - 1.594; CC + CT vs. TT: OR = 1.193, 95%CI 1.027 - 1.386), Gastric carcinoma (CT vs. TT: OR = 1.189, 95%CI 1.017 - 1.389; CC + CT vs. TT: OR = 1.475, 95%CI 1.007 - 2.162) and HCC (CC vs. TT: OR = 1.302, 95%CI 1.019 - 1.663; C vs. T: OR = 1.130 95%CI 1.004 - 1.272). However, no association was found in other types of cancers. In subgroup analysis by ethnicity, significant increased risk was found in Asians (CC vs. TT: OR = 1.253, 95%CI 1.081 - 1.451; CT vs. TT: OR = 1.140 95%CI 1.032 - 1.260; CC + CT vs.TT: OR = 1.179, 95%CI 1.067 - 1.304; C vs. T: OR = 1.109 95%CI 1.035 - 1.188), but not in Caucasians. In subgroup analysis according to source of control, significant increased risk was observed in both hospital-based studies (CC vs. TT: OR = 1.232, 95%CI 1.026 - 1.478; CT vs. TT: OR = 1.028 95%CI 1.017 - 1.201; CC + CT vs. TT: OR = 1.150, 95%CI 1.033 - 1.280; C vs. T: OR = 1.112 95%CI 1.007 - 1.228) and population-based studies (CC vs. TT: OR = 1.630, 95%CI 1.148 - 2.314; CC + CT vs.TT: OR = 1.480 95%CI 1.104-1.983; C vs. T: OR = 1.225, 95%CI 1.049 - 1.431). When stratified by HWE status, significant increased risk was found in studies consistent with HWE (CC vs. TT: OR = 1.252, 95%CI 1.041 - 1.505; CT vs.TT: OR = 1.171 95%CI 1.049 - 1.307; CC + CT vs. TT: OR = 1.203, 95%CI 1.063 - 1.361; C vs. T: OR = 1.111 95%CI 1.009 - 1.224), as well as studies not (CC vs. TT: OR = 1.317 95%CI 1.119 - 1.549; CC + CT vs. TT: OR = 1.179, 95%CI 1.114 - 1.243; CC vs. CT + TT: OR = 1.501 95%CI 1.169 - 1.927; C vs. T: OR = 1.304 95%CI 1.089 - 1.562).

3.3. Evaluation of Heterogeneity

Heterogeneity between studies was observed in overall comparisons (data not shown).

Table 2. Meta-Analysis of miR-196a2 rs11614913 polymorphism and digestive system cancer risk.

Stratification	No. of studies	Homozygote Model (CC vs. TT)			Heterozygote Model (CT vs. TT)			Dominant Model (CC + CT vs. TT)			Recessive Model (CC vs. CT + TT)			Allelic Model (C vs. T)		
		OR (95%CI)	*P*	P_Q	OR (95%CI)	*P*	P_Q	OR (95%CI)	*P*	P_Q	OR (95%CI)	*P*	P_Q	OR (95%CI)	*P*	P_Q
Total	34	**1.317 (1.119 - 1.549)**	**0.001**	0.000	**1.163 (1.057 - 1.279)**	**0.002**	0.000	**1.219 (1.095 - 1.357)**	**0.000**	0.000	**1.164 (1.013 - 1.337)**	**0.032**	0.000	**1.139 (1.047 - 1.240)**	**0.003**	0.000
Cancer Type																
CRC	6	**1.325 (1.102 - 1.594)**	**0.003**	0.108	1.124 (0.959 - 1.318)	0.147	0.227	**1.193 (1.027 - 1.386)**	**0.021**	0.165	1.143 (0.929 - 1.405)	0.206	0.082	1.098 (0.959 - 1.259)	0.177	0.061
ESCC	4	1.213 (0.777 - 1.894)	0.395	0.000	1.279 (0.916 - 1.785)	0.148	0.000	1.256 (0.897 - 1.760)	0.185	0.000	1.017 (0.776 - 1.332)	0.903	0.005	1.080 (0.901 - 1.296)	0.405	0.001
GBC	1	1.039 (0.511 - 2.112)	0.916	-	1.504 (0.724 - 3.123)	0.274	-	1.204 (0.603 - 2.405)	0.598	-	0.741 (0.512 - 1.071)	0.111	-	0.854 (0.636 - 1.147)	0.293	-
GC	6	1.893 (0.978 - 3.663)	0.058	0.000	**1.189 (1.017 - 1.389)**	**0.030**	0.171	**1.475 (1.007 - 2.162)**	**0.046**	0.000	1.595 (0.851 - 2.987)	0.145	0.000	1.401 (0.942 - 2.083)	0.096	0.000
HCC	11	**1.302 (1.019 - 1.663)**	**0.035**	0.000	1.129 (0.940 - 1.355)	0.194	0.002	1.182 (0.977 - 1.431)	0.085	0.000	1.191 (0.997 - 1.422)	0.054	0.002	**1.130 (1.004 - 1.272)**	**0.043**	0.000
OSCC	4	1.178 (0.658 - 2.109)	0.582	0.001	1.057 (0.720 - 1.552)	0.778	0.021	1.154 (0.825 - 1.615)	0.403	0.029	1.090 (0.660 - 1.800)	0.735	0.000	1.116 (0.861 - 1.447)	0.406	0.002
PSCC	2	1.331 (0.493 - 3.598)	0.572	0.011	1.352 (0.789 - 2.316)	0.273	0.134	1.364 (0.658 - 2.826)	0.404	0.044	0.995 (0.690 - 1.679)	0.986	0.021	1.086 (0.723 - 1.631)	0.693	0.012
Ethnicity																
Asian	25	**1.253 (1.081 - 1.451)**	**0.003**	0.000	**1.140 (1.032 - 1.260)**	**0.010**	0.001	**1.179 (1.067 - 1.304)**	**0.001**	0.000	1.127 (0.999 - 1.271)	0.052	0.000	**1.109 (1.035 - 1.188)**	**0.003**	0.000
Caucasian	9	1.544 (0.885 - 2.693)	0.126	0.000	1.275 (0.969 - 1.679)	0.083	0.006	1.400 (0.957 - 2.049)	0.083	0.000	1.269 (0.827 - 1.946)	0.276	0.000	1.236 (0.912 - 1.674)	0.172	0.000
Source of Control																
HB	25	**1.232 (1.026 - 1.478)**	**0.025**	0.000	**1.028 (1.017 - 1.201)**	**0.018**	0.056	**1.150 (1.033 - 1.280)**	**0.011**	0.000	1.143 (0.966 - 1.351)	0.119	0.000	**1.112 (1.007 - 1.228)**	**0.036**	0.000
PB	9	**1.630 (1.148 - 2.314)**	**0.006**	0.000	1.361 (0.992 - 1.868)	0.056	0.000	**1.480 (1.104 - 1.983)**	**0.009**	0.000	1.219 (0.966 - 1.538)	0.095	0.001	**1.225 (1.049 - 1.431)**	**0.010**	0.001

Continued

HWE in Control																
Yes	29	**1.252 (1.041 - 1.505)**	**0.017**	0.000	**1.171 (1.049 - 1.307)**	**0.005**	0.000	**1.203 (1.063 - 1.361)**	**0.003**	0.000	1.109 (0.949 - 1.296)	0.195	0.000	**1.111 (1.009 - 1.224)**	**0.032**	0.000
No	5	**1.317 (1.119 - 1.549)**	**0.002**	0.021	1.076 (0.943 - 1.229)	0.278	0.506	**1.179 (1.114 - 1.243)**	**0.010**	0.153	**1.501 (1.169 - 1.927)**	**0.001**	0.047	**1.304 (1.089 - 1.562)**	**0.004**	0.009

N.: number of involved studies; OR: odds ratio; 95%CI: 95% confidence interval; P: values of significance; P_Q: values of Q test for heterogeneity; Random model was used for data pooling when $P_Q < 0.10$ or $I^2 > 50\%$; otherwise fixed model was used; The results marked in boldface indicates statistical significance.

After stratification, the heterogeneities decreased merely in the subgroups of CRC ($P_Q \geq 0.10$ and $I^2 < 50\%$). But we found that the variable ethnicity, source of controls and HWE status could not explain heterogeneity (data not shown).

3.4. Sensitivity Analysis

In the sensitivity analysis, the influence of each study on the pooled OR was checked by repeating the meta-analysis while deleting each study, one at a time. The significance of pooled ORs was not influenced materially by omitting any single study (**Figure 3**). Moreover, sensitivity analysis was performed by omitting the studies conducted by Zhang *et al.*, Shen *et al.*, and Sushma *et al.* [39] [42] [43] in which the control groups were not in accordance with HWE. The significance of all ORs remained unaltered after excluding these 3 studies.

3.5. Publication Bias

Begg's funnel plot, Begg's test and Egger's tests were performed to evaluate publication bias of the literature on digestive system cancers. **Figure 4** displays funnel plots that examine the publication bias of studies included in the meta-analysis in dominant model. The shape of funnel plots did not reveal any evidence of asymmetry. The statistical results still did not show publication bias (CC vs. TT: Begg's Test P = 0.236, Egger's test P = 0.268; CT vs. TT: Begg's Test P = 0.614, Egger's test P = 0.205; CC vs. CT + TT: Begg's Test P = 0.477, Egger's test P = 0.446; CC + CT vs.TT: Begg's Test P = 0.173, Egger's test P = 0.112; C vs. T: Begg's Test P = 0.299, Egger's test P = 0.263).

4. Discussion

MiRNAs get involved in various biological and pathological processes and are regarded as a key component in carcinogenesis. SNPs are the most common sequence variation in the human genome [44]. SNPs could alter sequences' coding and binding ability, thus modifying the cancer susceptibility in population. Recently, many studies demonstrated that SNPs in miR196a2 rs11614913 was significantly associated with the susceptibility of diverse cancers. Tong *et al.* [45] observed that CT and CC/CT genotypes were associated with a significantly increased childhood ALL (Acute lymphoblastic leukemia) risk compared with the wild TT genotype (OR = 1.50, 95%CI = 1.15 - 1.95; OR =

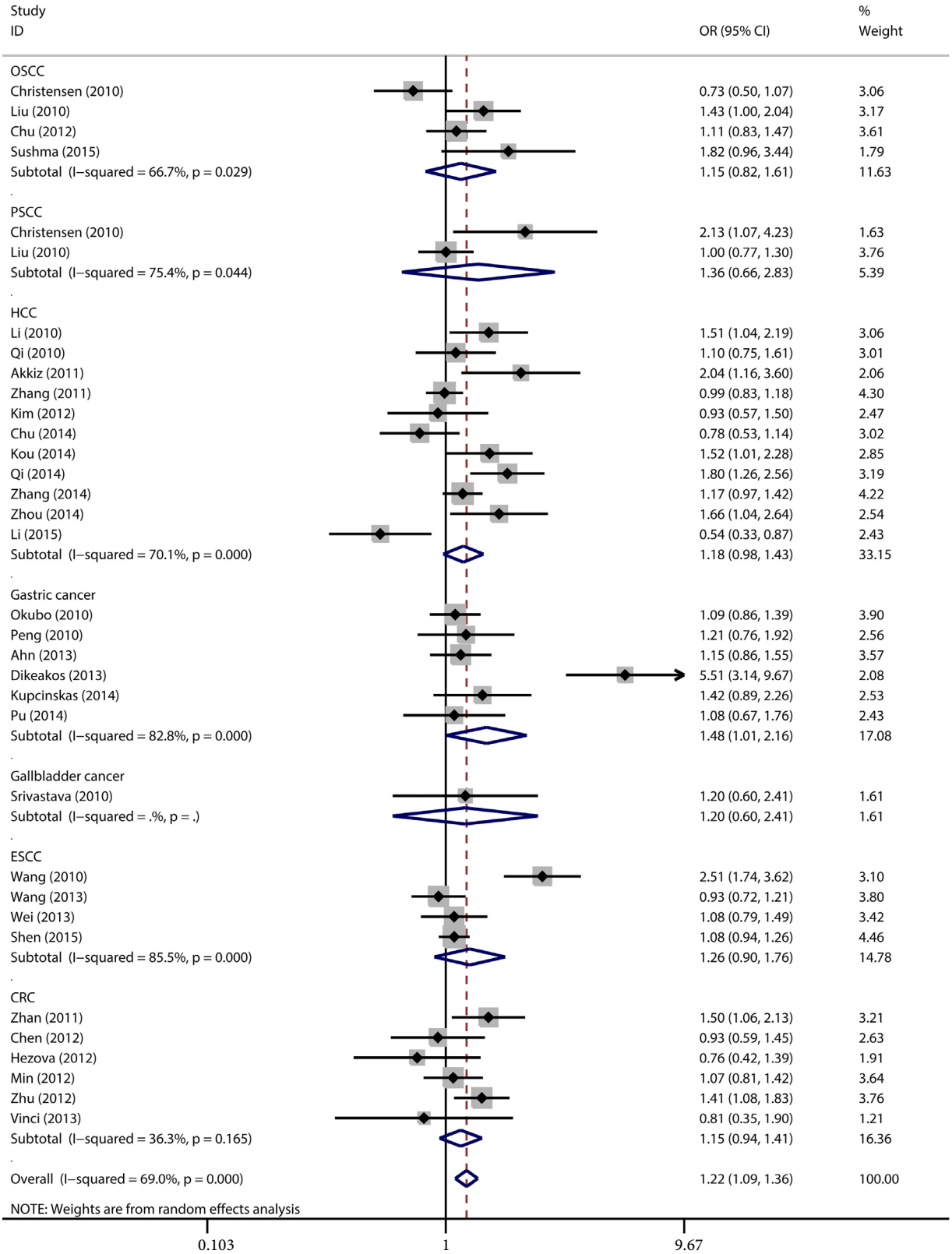

Figure 2. Forest plot of digestive system cancer risk in different cancer types associated with miR-196a2 rs11614913 polymorphism for dominant model (CC + CT vs. TT).

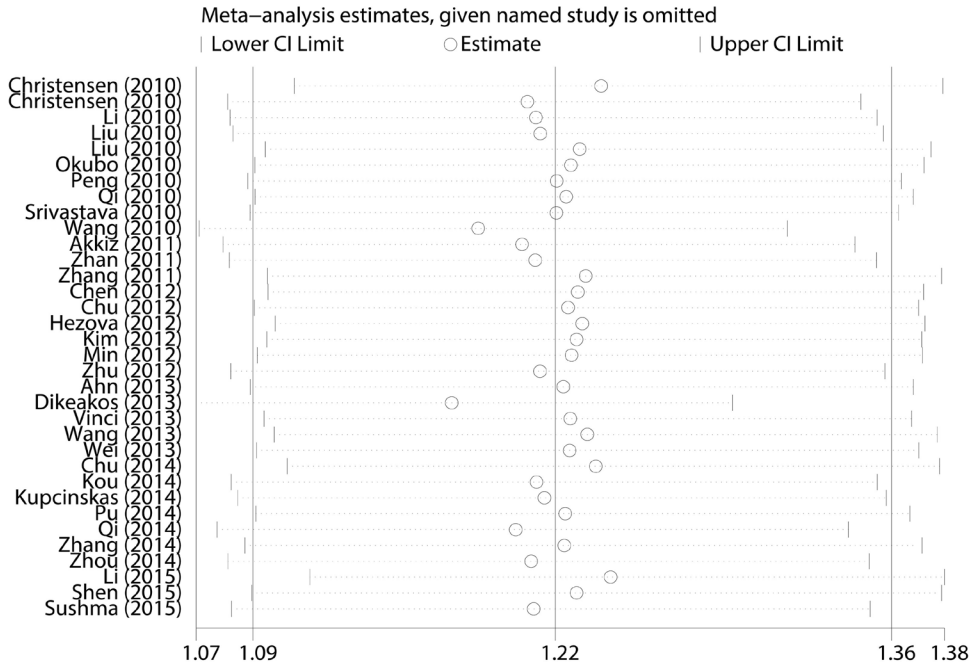

Figure 3. The influence of individual study on the pooled OR for dominant model (CC + CT vs. TT). The middle vertical axis indicates the overall OR and the two vertical axesindicate its 95%CI. Every circle indicates the pooled OR when the left study is omitted in this meta-analysis. The two ends of every broken line represent the 95%CI.

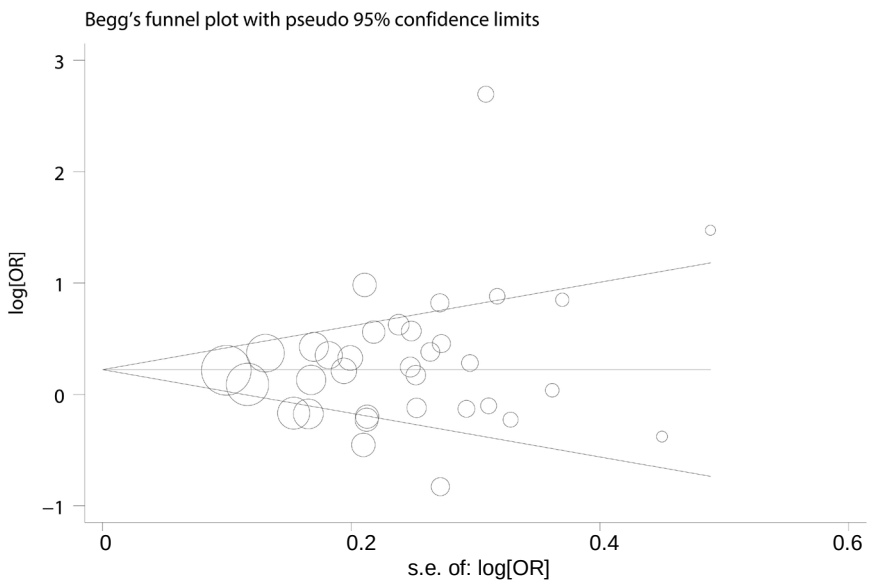

Figure 4. Begg's funnel plot for publication bias test in dominant model (CC + CT vs.TT). Each point represents a separate study for the indicated association. Log (OR): nature logarithm of OR. Horizontal line represents size of effect. OR: odds ratio.

1.40, 95%CI = 1.09 - 1.79; respectively). On the contrary, Du *et al.* [46] found that carriers of CC genotype had a significantly decreased risk for renal cell cancer, compared with the carriers of CT/TT genotype.

Moreover, several meta-analyses have been conducted on the association between SNPs in miR196a2 and cancer risk, but a unanimous conclusion still has not been achieved. Given the controversial result, we performed this meta-analysis to clarify the association of this variant with digestive system cancer risk. Compared to Guo *et al*.'s meta-analysis published in 2011 [47], we included another 19 studies, which kept our results more stable and authentic.

In the present meta-analysis, we observed a significant association between rs11614913 and increased risk in digestive system cancers. However, when stratified by cancer type, rs11614913 merely displayed relevance with CRC, HCC and gastric cancer. This may be explained by the effect of gene polymorphism on cancer susceptibility varies by specific cancer type. Otherwise, the relatively small amount of eligible studies in stratified analysis might induce statistically significant or insignificant association by chance due to insufficient statistical power [48]. In addition, we observed that rs11614913 polymorphism presented a risk factor in Asians, but not in Caucasians. The inconsistent results among different ethnicities may be due to diverse heredity backgrounds. And relatively small number of eligible studies with only 8 Caucasian studies included may be insufficient to detect statistical significance. Therefore, the results from the Caucasian subgroup should be treated with caution. Additional studies, especially Caucasians studies are urgently needed to further validate the ethnic differences in the effect of rs11614913 on digestive system cancer risk. Significant association was observed in both subgroups when stratified by source of control and HWE, which exactly supports the conclusion that rs11614913 polymorphism contribute to the susceptibility of digestive system cancers.

One of the major concerns in a sound meta-analysis is the stability of results, which is examined by sensitivity analysis. The pooled ORs kept unaltered during the sensitivity analysis. When excluding the studies that were inconsistent with HWE, the estimated pooled ORs still did not change at all, indicating that our results are reliable and robust.

Heterogeneity is a major problem that can distort the findings in meta-analysis. Obvious heterogeneity between studies was observed in overall comparisons and most subgroup analyses. In an attempt to find the sources of heterogeneity, a Galbraith plot was drawn (data not shown), and Dikeakos *et al*.'s study [30] was thought to serve as the main contributor for the heterogeneity. But the heterogeneity still kept considerable after excluding it, indicating that there may have other reasons for the heterogeneity. Then we conducted stratified analysis to reduce heterogeneity, but we found that the variable ethnicity, source of controls, and HWE status could not explain heterogeneity. Notably, in the current study, only studies published in English or Chinese were included, which may partially explain the intractable heterogeneity. Another important problem for any meta-analysis is publication bias due to selective publication of reports. In this meta-analysis, both the shape of funnel plots and statistical results did not show publication bias, suggesting reliability of our study.

Nevertheless, some limitations of this meta-analysis should be addressed. Firstly, oncogenesis is influenced by various factors such as genetic factors, tumor biological

characteristics environmental damage, while we only focused on the variant in miRNAs. Secondly, our analysis was limited to Asian and Caucasian ethnicities, which limited the general application of the findings from the meta-analysis. Thirdly, only studies published in English and Chinese were included in our meta-analysis. The exclusive reliance on English and Chinese studies may not represent all of the evidence. Excluding languages other than English or Chinese may introduce a language bias and lead to erroneous conclusions. In addition, many studies with negative data, such as absence of links to cancer risks and progression, are most times not considered for submission by researchers, resulting in unavoidable bias. But fortunately, our results of publication bias test show this bias is limitable and acceptable.

5. Conclusion

In summary, our meta-analysis reveals that miR196a2 rs11614913 polymorphism contributes to increased digestive system cancer susceptibility, especially in Asian populations. Further, well-designed studies with diverse ethnic groups and larger sample size are required to validate this association.

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