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Journal of the Chinese Medical Association 80 (2017) 191−203

www.jcma-online.com

Original Article

Study of the association between five polymorphisms and risk of hepatocellular carcinoma: A meta-analysis

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Received April 11, 2016; accepted September 21, 2016

Abstract

Background: Recently, several studies have investigated the association between polymorphisms in miR-146a rs2910164, miR-196a2 rs11614913, miR-499 rs3746444, miR-149 rs229283, miR-34b/c rs4938723, and hepatocellular carcinoma (HCC), which showed inconclusive results.

Methods: A publication search was performed in PubMed, ExcerptaMedica Database, Chinese Biomedical Literature Database, and Chinese National Knowledge Infrastructure to collect relevant medical data published through February 2016. The aim of this study was to ascertain the association between HCC and micro-RNAs. A total of 21 studies were included in our study, which showed that miR-146a rs2910164 polymorphism has a significant association with HCC in the allele, recessive, and homozygous models overall [allele model: odds ratio (OR) = 0.927, 95% confidence interval (CI): 0.869−0.988, $p = 0.02$; recessive model: OR = 0.893, 95% CI: 0.814−0.981, $p = 0.018$; homozygous model: OR = 0.853, 95% CI: 0.744−0.978, $p = 0.023$] and in Asian populations (allele model: OR = 0.921, 95% CI: 0.863−0.983, $p = 0.014$; recessive model: OR = 0.893, 95% CI: 0.814−0.981, $p = 0.019$; homozygous model: OR = 0.851, 95% CI: 0.741−0.977, $p = 0.022$). For miR-196a2 rs11614913, significant statistical heterogeneity overall and in Asian populations was identified in the comparison of the allele, recessive, homozygous, and heterozygous models (overall: allele model: OR = 0.889, 95% CI: 0.842−0.94, $p < 0.001$; recessive model: OR = 0.837, 95% CI: 0.723−0.970, $p = 0.018$; homozygous model: OR = 0.722, 95%CI: 0.575−0.906, $p = 0.005$; heterozygous model: OR = 0.532, 95% CI: 0.37−0.765, $p = 0.001$), and also has a decreased risk of HCC in Caucasians in all genetic models except for the heterozygous model (allele model: OR = 0.658, 95% CI: 0.49−0.885, $p = 0.006$; dominant model: OR = 0.641, 95% CI: 0.418−0.981, $p = 0.041$; recessive model: OR = 0.489, 95% CI: 0.278−0.862, $p = 0.013$; homozygous model: OR = 0.414, 95% CI: 0.222−0.772, $p = 0.005$). Only the recessive models produced a significant association between miR-499 rs3746444 polymorphism and HCC risk (recessive model: OR = 1.283, 95% CI: 1.008−1.632, $p = 0.043$).

Results: The analysis for miR-146a rs2910164 polymorphisms by racial decent found the same association between miR-146a rs2910164 polymorphism and susceptibility to HCC in Asians, but no significance risk association was observed in Caucasians. The meta-analysis results showed that miR-196a2 rs11614913 was associated with a decreased risk of HCC in Caucasians in all genetic models except for the heterozygous model. In the Asian population, miR-499 rs3746444 polymorphism was associated with a decreased risk of HCC in recessive models. This meta-analysis showed that no significant statistical heterogeneity was identified in miR-149 rs2292832 and miR-34b/c rs4938723.

Conclusion: Our findings supported the proposition that the polymorphisms of miR-146a rs2910164, miR-196a2 rs11614913, and miR-196a2 rs11614913 may contribute to the susceptibility of HCC.

Keywords: hepatocellular carcinoma; meta-analysis; micro-RNA; polymorphisms

Conflicts of interest: The authors declare that they have conflicts of interest related to the subject matter or materials discussed in this article.

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http://dx.doi.org/10.1016/j.jcma.2016.09.009

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers and is the second leading cause of cancer death worldwide.[1] In 2012, 782,500 new HCC cases and 745,500 deaths were caused by this persistent disease worldwide, with about 50% of the total number of cases and deaths occurring in China. Currently, HCC is a predominant histological subtype of human liver malignancy, which accounts for 70−85% of primary malignancies in the liver.[2] Hepatitis B virus, hepatitis C virus, obesity, and alcohol abuse lead to HCC, which has been recognized as an insidious malignancy with a very poor prognosis. Unfortunately, most HCC patients enter the late stage when diagnosed and have already missed the window of opportunity to have radical treatments. However, HCC is usually highly malignant and quick to metastasize. Therefore, it is of great importance and benefit for patients with HCC to develop early and noninvasive diagnostic biomarkers.

Micro-RNAs (miRNAs) are small, noncoding, single-stranded RNA molecules with a typical length of 22 nucleotides. They also play an important role in physiologic and pathologic processes including cell differentiation, proliferation, apoptosis, and carcinogenesis,[3] and have been implicated in the initiation and progression of various cancers.[4] A miRNA, which has even a slight variation in the function or expression, may affect a wide spectrum of mRNA targets, including many oncogenes and tumor suppressor genes.[5] Single-nucleotide polymorphisms (SNPs) can reportedly alter expressions or functions of miRNAs; related to cancer risk, they are the most common type of genetic variation, which are associated with population diversity, disease susceptibility, and individual response to medicine.[6] Research focusing on both SNPs in miRNAs and human cancer has provided another insight into the molecular mechanisms of cancer development. Recent studies have demonstrated that genetic factors could also contribute to the etiology of HCC.[7] In recent years, we have paid significant attention to genetic polymorphisms due to their etiological roles in defining the risk of HCC development. According to recent research, miR-34b/c, miR-218, miR-146a, miR-149, miR-196a-2, miR-499, and miR106b-25 are related to HCC.[8−22] A small sample size may not be adequate to detect the effects of SNPs on HCC, so we collected 21 studies for this meta-analysis and explored the associations between polymorphisms of miRNAs and HCC.

2. Methods

A publication search was performed in PubMed, ExcerptaMedica Database, Chinese Biomedical Literature Database, and Chinese National Knowledge Infrastructure, to collect relevant medical literature published up to February 2016, using the combined words “microRNA or mir or miRNA,” “gene or allele or polymorphism or variation,” or “HCC or liver cancer.” Publication language was not restricted in our search. Examining the reference lists manually to further identify potentially relevant studies, we also made use of e-mail addresses to contact the corresponding authors of conference abstracts that lacked sufficient data to get additional information.

2.1. Selection

The studies were incorporated into our meta-analysis only if they met the following criteria: (1) HCC and miRNA polymorphism data; (2) independent case−control studies for humans; (3) sources of cases and sufficient available data to estimate an odds ratio (OR) with 95% confidence interval (CI); (4) available genotype frequency; and (5) only full-text manuscripts. We included the latest study if serial studies of the same population were from the same group. The exclusion criteria were as follows: (1) non-HCC studies; (2) the paper being an abstract, comment, editorial, and review; or (3) insufficient data.

2.2. Data abstraction

The included studies were carefully extracted by two well-qualified investigators. The information derived from each study included author, publication date, country of origin, ethnicity, genotyping method, total number of cases and controls, and genotype frequencies of cases and controls. The two investigators reviewed the data extraction for the purpose of arriving at a consensus.

2.3. Quantitative data synthesis

Strength of the association between the SNPs and HCC, including five genetic models (the allele, dominant, recessive, homozygous, and heterozygous models), was measured by ORs with 95% CIs. The *Z*-test was used to determine the significance of the pooled ORs, and $p < 0.05$ was considered statistically significant. Publication bias of literature was assessed by the use of Begg's funnel plots and Egger's test. If a p value of Egger's test was <0.05, we regarded it as representative of statistically significant publication bias.[23] When we used Begg's funnel plot, the standard error of logarithm (Log) for OR was plotted against its OR, and Log OR was plotted versus standard error of Log OR for each enrolled study.[24] Heterogeneity of the studies was checked by the chi-square test-based Q-test,[25,26] and the random-effect model was chosen (DerSimonian and Laird method)[27] when the existence of heterogeneity was detected ($p < 0.10$ for the Q-test, $I^2 > 50\%$). If not, the fixed-effect model (the Mantel−Haenszel method)[28] was selected. The Hardy−Weinberg equilibrium was calculated using the goodness-of-fit chi-square test. A p value < 0.05 was considered significant disequilibrium. All statistical analyses were carried out using STATA 12.0 StataCorp LLC (4905 Lakeway Drive College Station, Texas 77845, USA) and all p values were two sided.

3. Results

3.1. Study characteristics

A total of 52 articles were retrieved after the first search in PubMed, ExcerptaMedica Database, Chinese Biomedical

Literature Database, and Chinese National Knowledge Infrastructure. After our selection (Fig. 1), 21 case–control studies fulfilled the inclusion criteria, including those from China[8–17,29–34] (16 studies), Korea[18,19] (2 studies), and Turkey[20–22] (3 studies). In total, those studies involved 10,145 HCC cases and 9907 healthy controls, evaluating the relationship between the polymorphisms in miRNA and HCC (Figs. 2 and 3). Their main genotyping method was polymerase chain reaction restriction fragment length polymorphism, and the controls of most studies were population based (Table 1). Genotype distribution of the controls was in agreement with the Hardy–Weinberg equilibrium in the included studies, except for one study.[22] We used the modified strengthening the Reporting of Observational Studies in Epidemiology (STROBE) quality score system to evaluate the quality of the included studies, resulting in scores that were moderately high (higher than 20 points). The characteristics and methodological qualities of the included studies are summarized in Table 1.

3.2. Association between four novel polymorphisms in miRNA genes and risk of HCC

3.2.1. MiR-146a rs2910164

Eleven studies were evaluated for the association between miR-146a rs2910164 polymorphism and HCC risk, consisting of 10 Asian- and one Caucasian-population studies. After excluding the studies of Hao et al[32] and Zhou et al,[31], wherein the data were significantly departed from the Hardy–Weinberg equilibrium ($p = 0.0005$ and $p = 0.006$), for the remaining pool of studies included in this meta-analysis, the study heterogeneities were reduced and the genotypic results were more credible. We observed a significant association between miR-146a rs2910164 polymorphism and susceptibility to HCC in the allele, recessive, and homozygous models

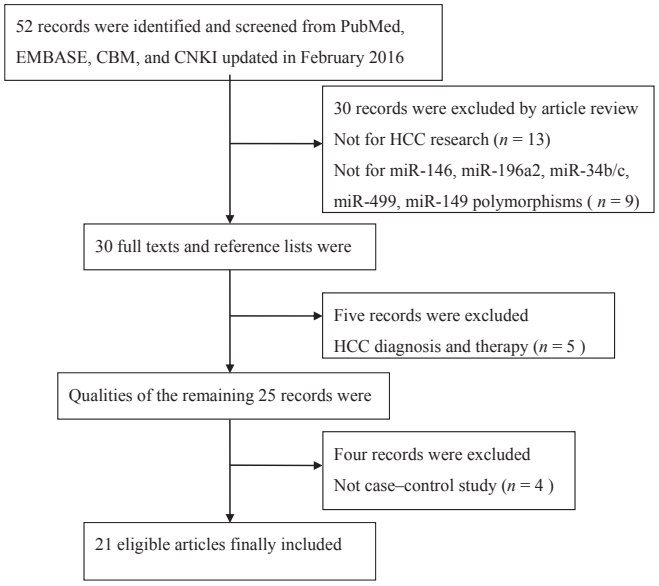


Fig. 1. Flow diagram of study identification with criteria for inclusion and exclusion in the meta-analysis. CBM = Chinese Biomedical Literature Database; CNKI = Chinese National Knowledge Infrastructure; EMBASE = ExcerptaMedica Database.

(Table 2). Thereafter, we performed an analysis for miR-146a rs2910164 polymorphisms by racial decent and found the same association between miR-146a rs2910164 polymorphism and susceptibility to HCC in Asians, but no significance risk association was observed in Caucasians.

3.2.2. MiR-196a2 rs11614913

Ten studies referred to the association between the miR-196a2 rs11614913 polymorphism and risk of HCC development, which included 5505 cases and 4771 controls. Significant statistical heterogeneity in the overall pool of study participants and Asians was identified, in comparing the allele, recessive, homozygous, and heterozygous models (Table 2). The meta-analysis results showed that miR-196a2 rs11614913 was associated with a decreased risk of HCC in Caucasians in all genetic models except for the heterozygous model, which is shown in Table 2 (allele model: OR = 0.658, 95% CI: 0.490–0.885, $p = 0.006$; dominant model: OR =0.641, 95% CI: 0.418–0.981, $p = 0.041$; recessive model: OR = 0.489, 95% CI: 0.278–0.862, $p = 0.013$; homozygous model: OR = 0.414, 95% CI: 0.222–0.772, $p = 0.005$).

3.2.3. MiR-499 rs3746444

Our analysis included only seven studies involving 1439 cases and 2100 controls, which referred to the association between miR-499 rs3746444 polymorphism and risk of HCC development. However, only the recessive models produced a significant association between miR-499 rs3746444 polymorphism and HCC risk. When we analyzed them by racial descent, no significant risks were found in the Caucasian population, but in the Asian population, miR-499 rs3746444 polymorphism was associated with a decreased risk of HCC in recessive models. The results are summarized in Table 2.

3.2.4. MiR-149rs2292832

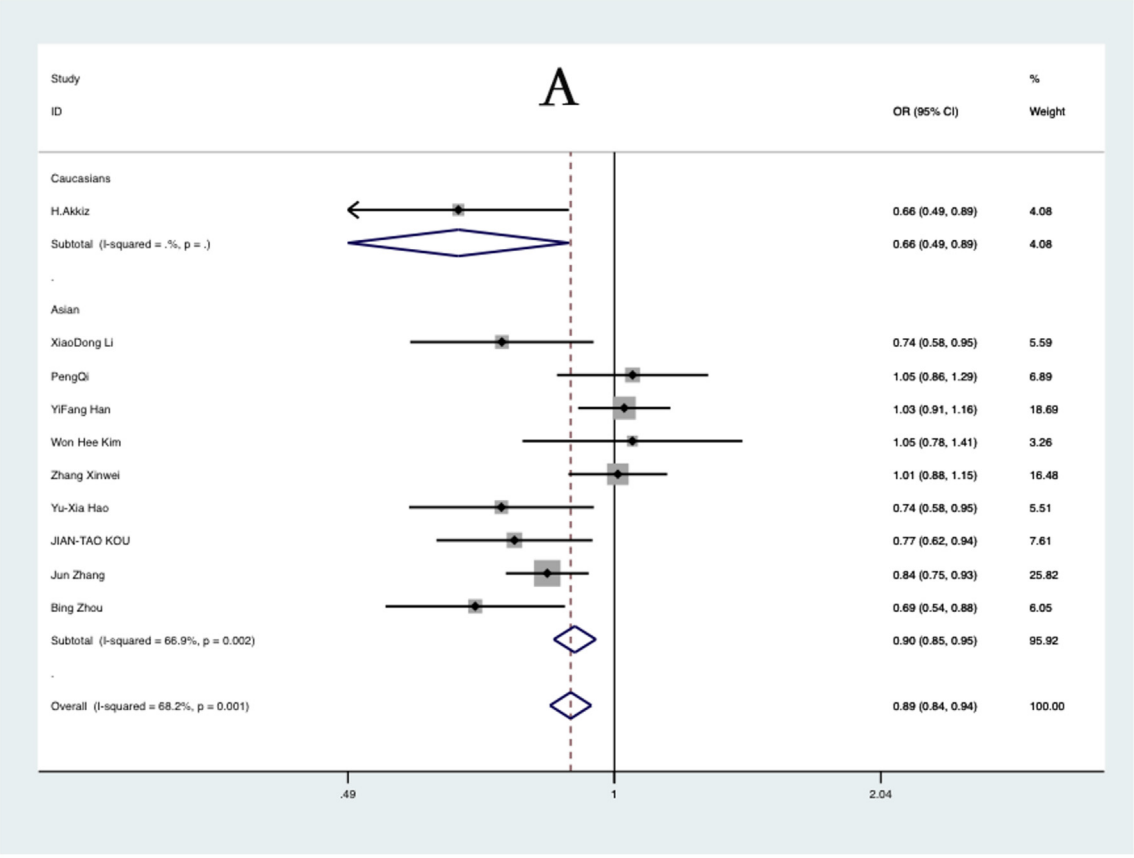
The association between miR-149 rs2292832 polymorphism and susceptibility to HCC was analyzed in three independent studies with 757 cases and 1060 controls. This meta-analysis showed that no significant statistical heterogeneity was identified in any of the genetic models (allele model: OR =1.055, 95% CI: 0.731–1.524, $p = 0.29$; dominant model: OR = 0.897, 95% CI: 0.682–1.181, $p = 0.77$; recessive model: OR = 1.142, 95% CI: 0.688–1.897, $p = 0.607$; homozygous model: OR = 0.958, 95% CI: 0.65–1.411, $p = 0.827$; heterozygous model: OR =1.189, 95% CI: 0.869–1.626, $p = 0.279$).

3.2.5. MiR-34b/c rs4938723

Only three studies referred to the association between the miR-34b/c rs4938723 polymorphism and the risk of HCC development. None of the genetic models produced a significant association between miR-34b/c rs4938723 polymorphism and HCC susceptibility.

3.3. Sensitivity analysis

Sensitivity analysis assessed the influence of each individual study on the overall pooled ORs by deleting individual



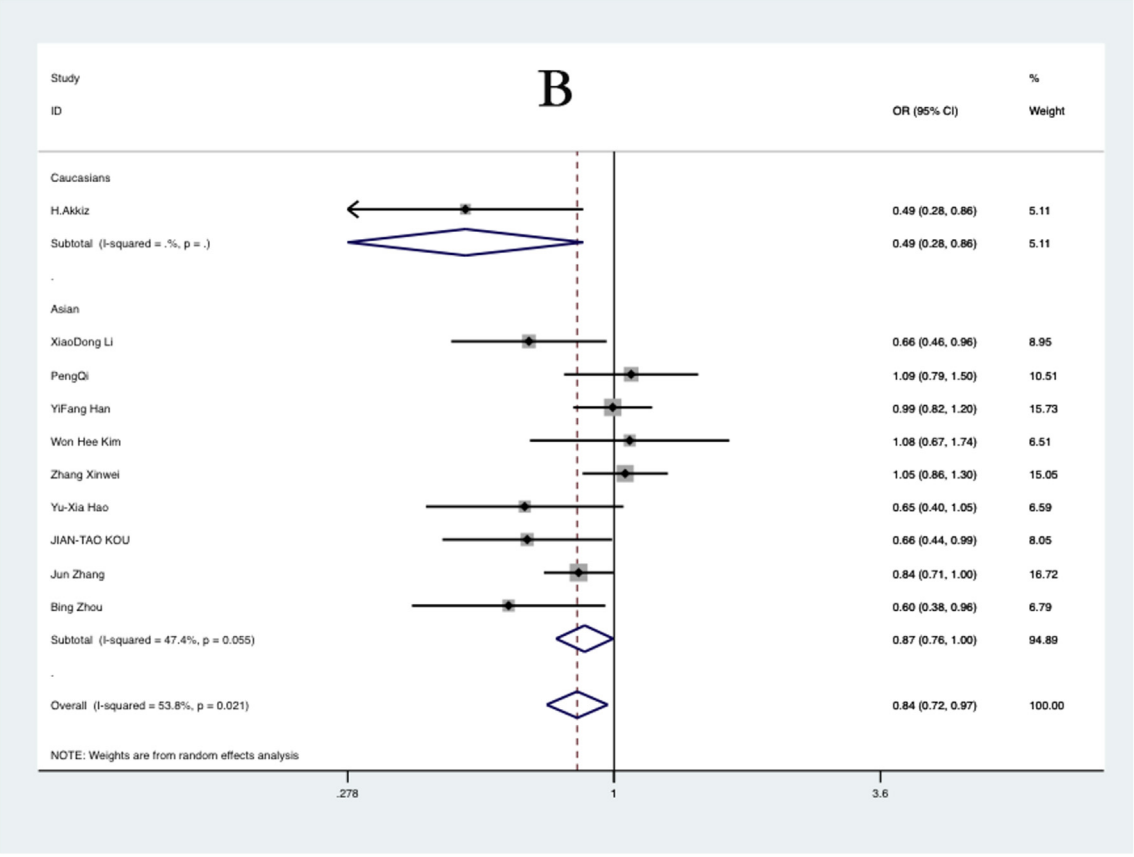
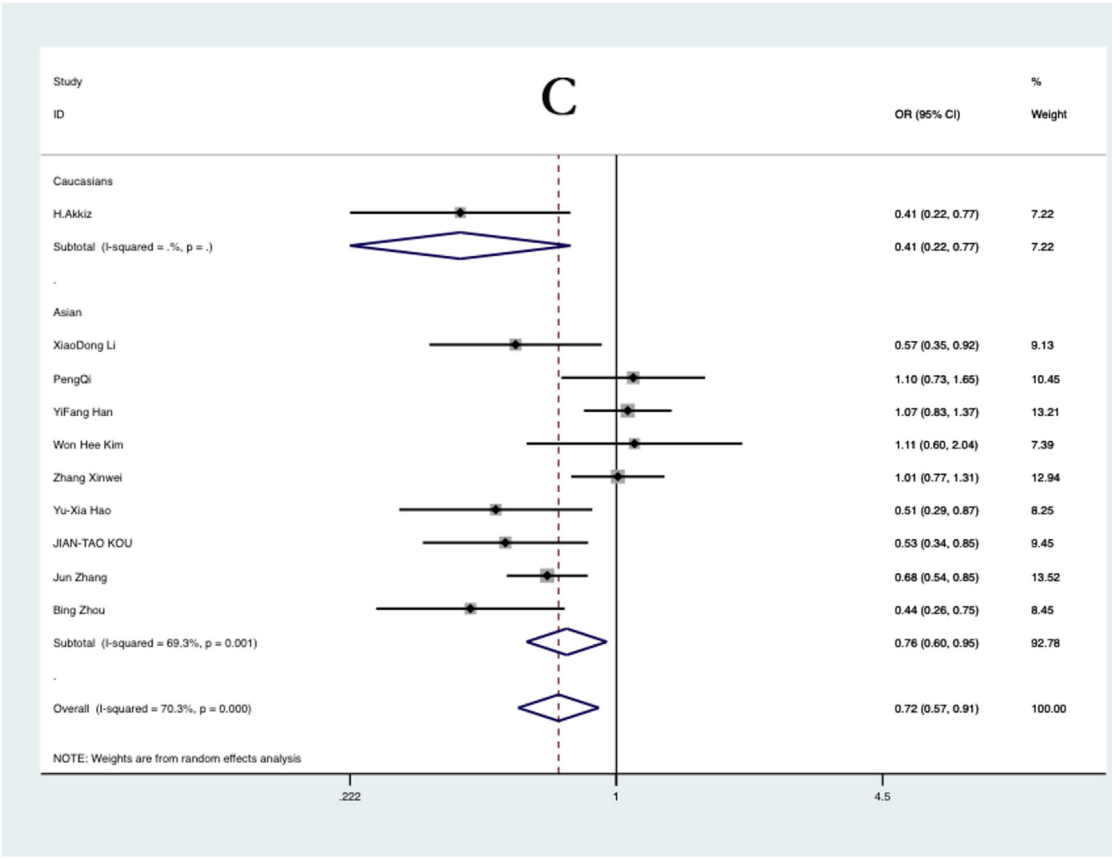


Fig. 2. Forest plot of ORs for the association of miR-196A2 Rs11614913 polymorphism with HCC risk is illustrated in subgroup analysis by ethnicity. (A) allele model; (B) recessive model; (C) homozygous model; and (D) heterozygous model. CI = confidence interval; HCC = hepatocellular carcinoma; OR = odds ratio.



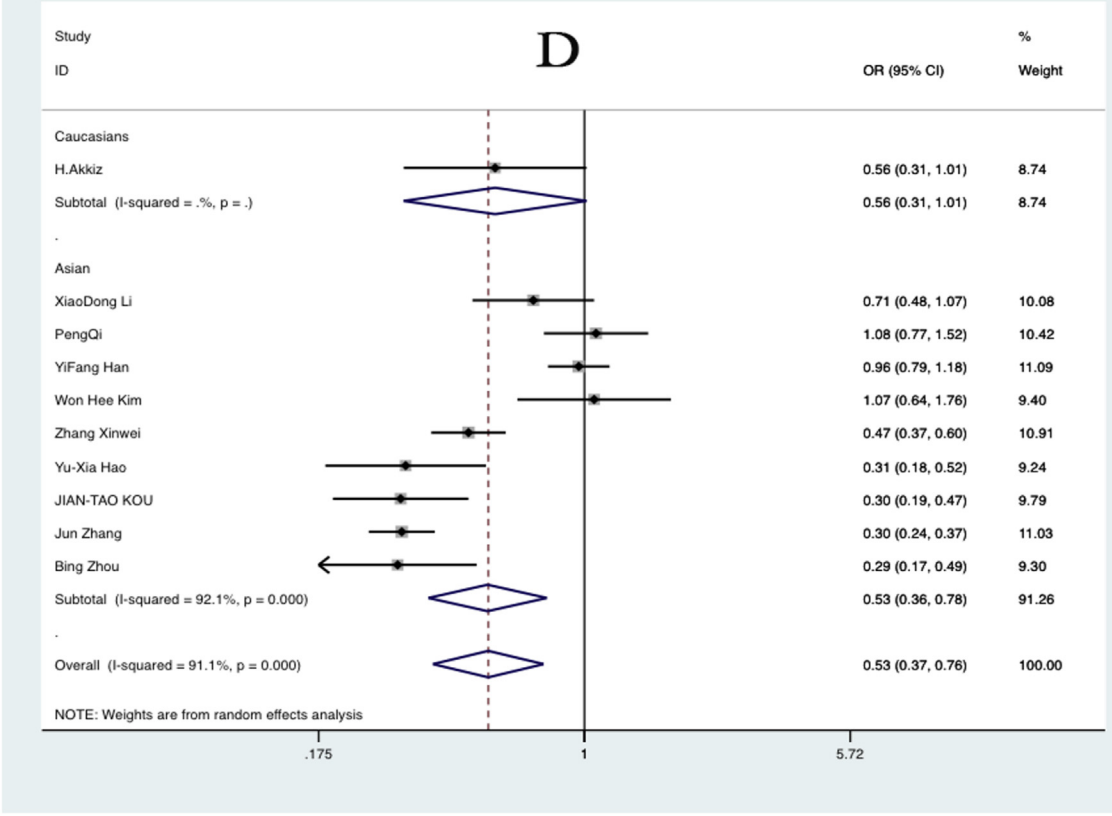
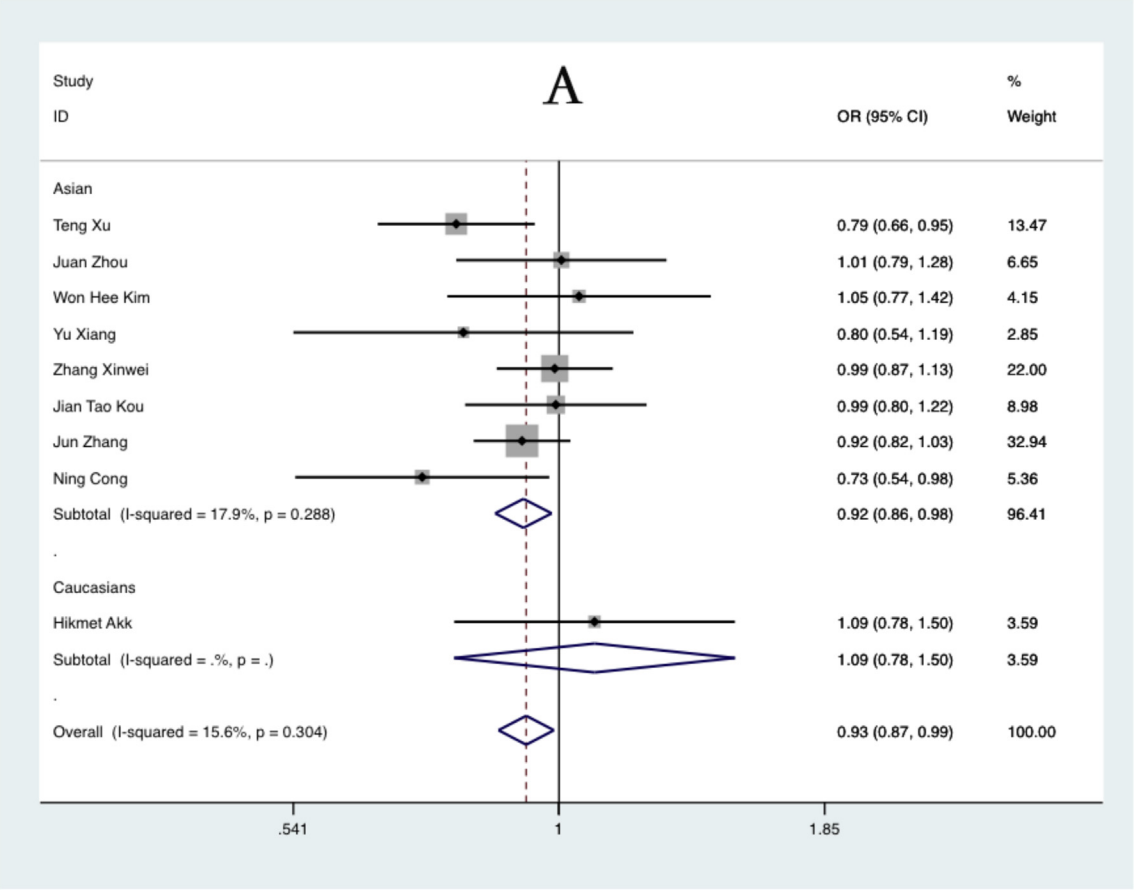


Fig. 2. (*continued*).



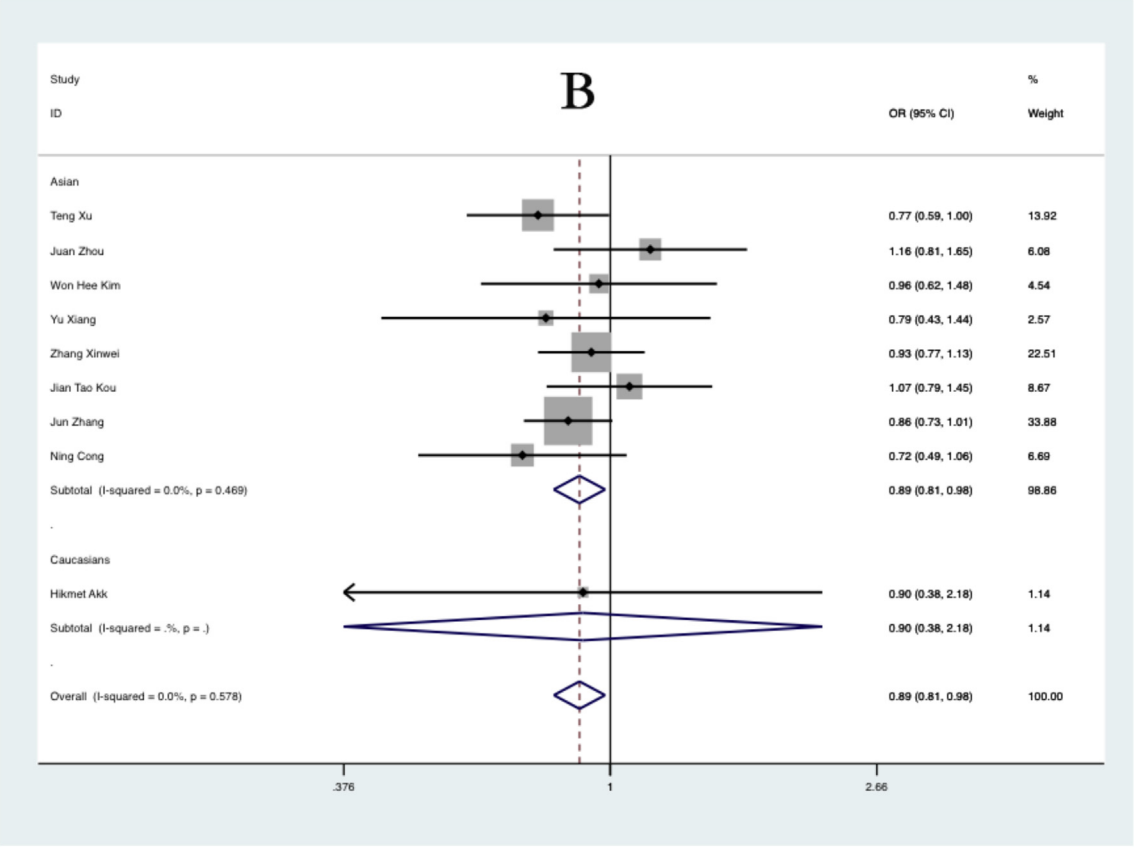


Fig. 3. Forest plot of ORs for the association of miR-146 rs2910164 polymorphism with HCC risk illustrated in subgroup analysis by ethnicity. (A) allele model, (B) recessive model, and (C) homozygous model. CI = confidence interval; HCC = hepatocellular carcinoma; OR = odds ratio.

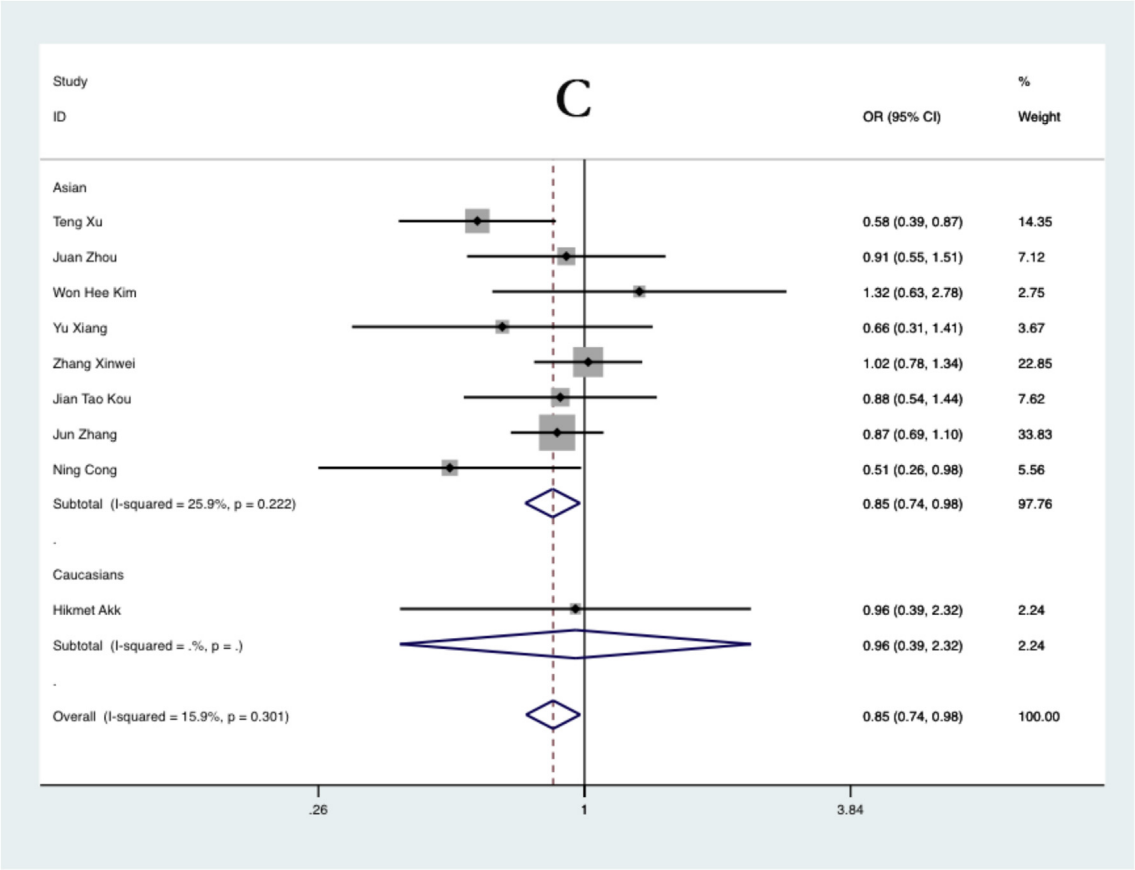


Fig. 3. (*continued*).

Table 1
Characteristics of the studies included in this meta-analysis.

First author	Year	Country	Ethnicity	Genotyping method	No.		Micro-RNA gene	SNPs	Quality scores
					Case	Control			
Yu Xiang	2012	China	Asian	PCR-RFLP	100	100	miR-146a, miR-499	rs2910164 (G>C), rs3746444(A>G)	25
Won Hee Kim	2012	Korea	Asian	PCR-RFLP	159	201	miR-146a, miR-149, miR-196a2, miR-499	rs2910164(G>C), rs2292832(C>T), rs11614913(C>T), rs3746444(A>G)	27
Yifang Han	2013	China	Asian	PCR	1013	999	miR-34b/c	rs4938723(T >C)	24
Yifang Han	2013	China	Asian	PCR	1017	1009	miR-196a2	rs11614913(C>T)	28
Myung Su Son	2013	Korea	Asian	PCR-RFLP	157	201	miR-34b/c	rs4938723 (T>C)	25
Yan Xu	2010	China	Asian	PCR-RFLP	501	548	miR-34b/c	rs4938723 (T > C)	26
Zhang Xinwei	2011	China	Asian	PCR-RFLP	925	840	miR-146a	rs2910164 (G>C)	27
Zhang Xinwei	2011	China	Asian	PCR-RFLP	934	837	miR-196a2	rs11614913(C>T)	25
Teng Xu	2008	China	Asian	PCR	479	504	miR-146a	rs2910164(G>C)	27
Xiao Dong Li	2010	China	Asian	PCR-RFLP	310	222	miR-196a2	rs11614913(C>T)	24
H. Akkiz	2010	Turkey	Caucasian	PCR-RFLP	185	22	miR-196a2	rs11614913(C>T)	26
Peng Qi	2010	China	Asian	PCR-LDR	361	391	miR-196a2	rs11614913(C>T)	27
H. Akkiz	2011	Turkey	Caucasian	PCR-RFLP	222	222	miR-499	rs3746444(A>G)	26
Juan Zhou	2011	China	Asian	PCR	186	483	miR-499, miR-146a	rs3746444(A>G), rs2910164(G>C)	27
Hikmet Akkız	2011	Turkey	Caucasian	PCR-RFLP	222	222	miR-146a	rs2910164(G>C)	28
Yu Xia Hao	2013	China	Asian	PCR-RFLP	235	281	miR-196a2, miR-499, miR-146a	rs11614913(C>T), rs3746444(A>G), rs2910164 (G>C)	26
Jun Zhang	2013	China	Asian	PCR-RFLP	1767	955	miR-196a2, miR-146a	rs11614913(C>T), rs2910164 (G>C)	25
Bing Zhou	2014	China	Asian	PCR-RFLP	266	281	miR-196a2, miR-499, miR-146a	rs11614913(C>T), rs3746444(A>G), rs2910164(G>C)	28
M.F. Liu	2014	China	Asian	PCR	327	327	miR-149	rs2292832(C>T)	27
Ning Cong	2013	China	Asian	PCR-RFLP	206	217	miR-146a	rs2910164(G>C)	24
Jian Tao Kou	2013	China	Asian	PCR	271	532	miR-146a, miR-149, miR-196a2, miR-499	rs2910164(G>C), rs2292832(C>T), rs11614913(C>T), rs3746444(A>G)	27

LDR = ligation detection reaction; PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism; SNP = single-nucleotide polymorphism.

Table 2
Meta-analysis results for the four polymorphisms and HCC risk.

SNPs	2 allele versus 1 allele (allele model)				1/2 + 2/2 versus 1/1 (dominant model)				2/2 versus 1/1 + 1/2 (recessive model)				2/2 versus 1/1 (homozygous model)				2/2 versus 1/2 (heterozygous model)			
	OR	95% CI	Z	p	OR	95% CI	Z	p	OR	95% CI	Z	p	OR	95% CI	Z	p	OR	95% CI	Z	p
miR-146a rs2910164																				
Overall	0.927	0.869−0.988	2.32	0.02[a]	0.923	0.819−1.040	1.32	0.187	0.893	0.813−0.981	2.36	0.018	0.853	0.744−0.978	2.27	0.023	0.906	0.821−1.000	1.96	0.05
Asians	0.921	0.863−0.983	2.47	0.014[a]	0.902	0.795−1.023	1.61	0.107	0.893	0.813−0.981	2.35	0.019	0.851	0.741−0.977	2.28	0.022	0.907	0.821−1.002	1.92	0.055
Caucasians	1.086	0.785−1.502	0.5	0.619[a]	1.145	0.778−1.685	0.069	0.491	0.905	0.376−2.176	0.22	0.823	0.956	0.393−2.322	0.1	0.92	0.812	0.324−2.033	0.44	0.657
miR-196a2 rs11614913																				
Overall	0.889	0.842−0.94	4.14	<0.001	0.879	0.610−1.266	0.69	0.489	0.837	0.723−0.970	2.37	0.018	0.722	0.575−0.906	2.81	0.005	0.532	0.37−0.765	3.41	0.001
Asians	0.899	0.85−0.952	3.68	<0.001	0.909	0.614−1.344	0.48	0.631	0.868	0.756−0.996	2.02	0.044	0.756	0.602−0.948	2.42	0.015	0.53	0.359−0.781	3.21	0.001
Caucasians	0.658	0.49−0.885	2.77	0.006	0.641	0.418−0.981	2.05	0.041	0.489	0.278−0.862	2.47	0.013	0.414	0.222−0.772	2.78	0.005	0.556	0.305−1.013	1.92	0.055
miR-499 rs3746444																				
Overall	1.109	0.886−1.389	0.9	0.367[a]	1.042	0.894−1.214	0.53	0.597	1.283	1.008−1.632	1.03	0.043	0.715	0.363−1.411	0.97	0.334	1.258	0.965−1.641	1.7	0.09
Asians	1.113	0.845−1.467	0.76	0.446[a]	1.04	0.885−1.223	0.48	0.632	11.369	1.004−1.866	1.99	0.047	0.653	0289−1.474	1.03	0.305	1.32	0.936−1.860	1.58	0.113
Caucasians	1.098	0.84−1.434	0.68	0.495[a]	1.056	0.667−1.672	0.23	0.815	1.164	0.794−1.706	0.78	0.436	1.146	0.691−1.902	0.53	0.597	1.173	0.772-1.783	0.75	0.454
miR-34b/c rs4938723	1.151	0.974−1.36	1.65	0.1	1.248	0.987−1.577	1.85	0.065	1.062	0.859−1.314	0.55	0.579	1.15	0.919−1.439	1.22	0.221	0.977	0.780−1.223	0.2	0.838
miR-149 rs2292832	1.055	0.731−1.524	0.29	0.774	0.897	0.682−1.181	0.77	0.44	1.142	0.688−1.897	0.51	0.607	0.958	0.65−1.411	0.22	0.827	1.189	0.869−1.626	1.08	0.279

[a] Estimates for the random effect model. 1 = wild allele; 2 = mutant allele; 1/1 = wild homozygote; 1/2 = heterozygote; 2/2 = mutant homozygote; CI = confidence interval; HCC = hepatocellular carcinoma; OR = odds ratio; SNP = single-nucleotide polymorphisms.

studies in turn. The results demonstrated that the studies were statistically robust, and therefore no individual study significantly altered the pooled ORs for the five novel functional polymorphisms in miRNA genes under the allele model (Fig. 4).

3.4. Publication bias

Begg's funnel plot and Egger's test were performed to assess the publication bias of the literature. Symmetrical funnel plots were obtained in those five miRNAs tested in all the models. No statistically significant evidence of publication bias was found in any comparison model (Fig. 5).

4. Discussion

A growing volume of research has shown that miRNAs play an important role in normal development and cellular homeostasis. Therefore, dysfunctions in these molecules have been associated with several human carcinomas, including HCC.[35] Recently, numerous reports have shown that SNPs in miRNAs may not only be involved in the process of HCC, but also contribute to the development of HCC. Therefore, we attempted to search all the eligible studies to show the association between the five SNPs in miRNA genes [miR-146a G > C (rs2910164), miR-196a-2 C > T (rs11614913), miR-34b/c T>C (rs4938723), miR-499 A > G (rs3746444), and miR-149 (rs2292832)] and susceptibility to HCC.

MiR-146a rs2910164 is located in the stem region opposite the mature miR-146a sequence. Owing to the mispairing in the hairpin of the precursor, the mature sequence has altered the process and has lower expression.[36] In other words, the G:U pair to C:U mismatch in the stem structure of miR-146a precursor results in G > C polymorphism. Compared with the G allele, the C allele gene has less efficient inhibition of target genes such as papillary thyroid carcinoma 1 gene and cytokine signaling pathway (tumor necrosis factor receptor-associated factor -6 (TRAF6), interleukin 1 receptor - asspciated kinase 1 (IRAK1)).[37] According to a series of studies, the miR-146a GG genotype could promote colon formation and cell proliferation in HCC cells, in part by having a higher expression level of mature miR-146a.[13] Moreover, the association between miR-146a rs2910164 polymorphism and risks of various cancers has been investigated by several case−control studies, and some believed that miR-146a rs2910164 contributes to modified HCC risks.[11,13,17,19,22] Our meta-analysis had positive results, as the biological consequence of miR-146a rs2910164 supports a significant association between miR-146a rs2910164 polymorphism and susceptibility to HCC found in the allele, recessive, and homozygous models. In subgroup analysis of the Asian and Caucasian populations, no significant result was shown in Caucasian populations. However, in Asian populations, we found the same association between miR-146a rs2910164 polymorphism and susceptibility to HCC. In several case−control studies, researchers investigated the association between miR-146a rs2910164 polymorphism and the risk of some cancers. Some previous

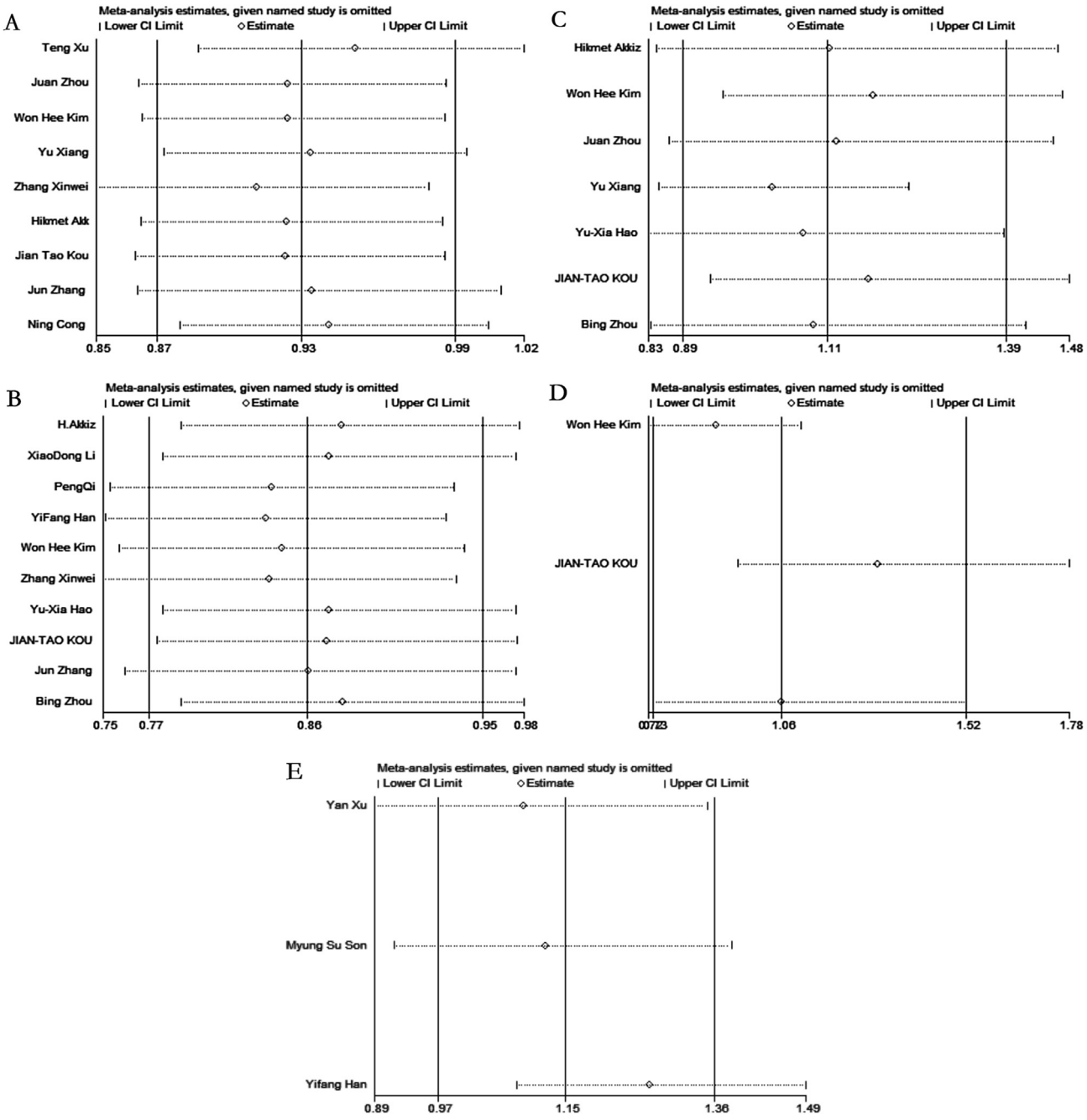


Fig. 4. Sensitivity analysis of the summary odds ratio coefficients of the five functional polymorphisms illustrated under the allele model. (A) miR-146a, (B) miR-196a-2, (C) miR-499, (D) miR-149, and (E) miR-34b/c. Results were computed by omitting each study in turn. The two ends of the dotted lines represent the 95% CI. CI = confidence interval.

findings showed that there was no association between miR-146a rs2910164 polymorphism and breast cancer, bladder cancer, and nonsmall cell lung cancer. Furthermore, some studies suggested that G allele or GG genotype of miR-146a polymorphism is related to an increased risk of gastric cancer, esophageal cell carcinoma, and prostate cancer.[38] Race differences might be the reason that miR-146a rs2910164 polymorphism has distinct effects, including living habits, genetic background, and the environment, which could be responsible for causing the noted differences. Another rational explanation for the result may be that the allele of miR-146a rs2910164 polymorphism might have a different effect on carcinogenesis in different organs. This phenomenon reflects the diversities of the etiological factors for different cell types.[22] The number of studies used to evaluate the association between miR-146a rs2910164 polymorphism and HCC risk may also lead to the difference that there were 10 studies in Asian populations and only one in Caucasian populations. To further study, deeper insights of biological mechanism, as well as well-designed studies and unbiased larger sample sizes, are needed to elucidate the exact role of miR-146a rs2910164 in HCC susceptibility.

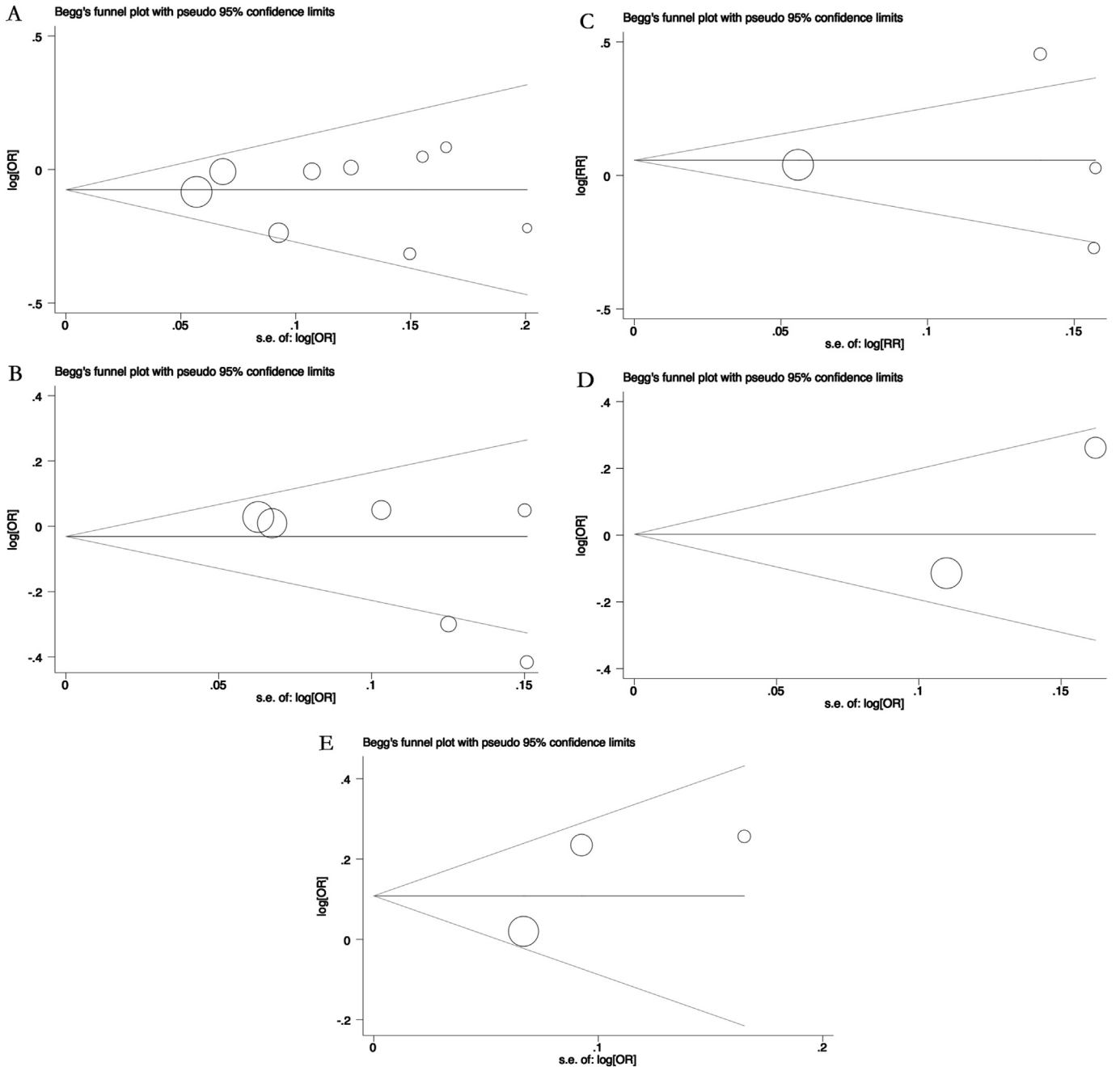


Fig. 5. Begg's funnel plot of publication bias for the five polymorphisms illustrated under the allele model. (A) miR-146a, (B) miR-196a-2, (C) miR-499, (D) miR-149, and (E) miR-34b/c. Log[OR] represents natural logarithm of OR. The horizontal line indicates the mean magnitude of the effect. Each point represents a separate study by the indicated association. OR = odds ratio; s.e. = standard error.

MiR-196a2 rs11614913 is situated in the 3′ passenger strand mature sequence of miR-196a2. The C to T mutation of the rs11614913 SNP, which was located in the stem region of an miR-196a-2 precursor, leads to a change from a G:C to a G:U mismatch.[39] A previous study has shown that miR-196a2 rs11614913 could influence the mature miRNA and the expression of the target genes including homeobox (*HOX*) and annexin A1 (*ANXA1*).[40] In various physiological and pathological processes, *HOX* genes encode important transcription factors, while *ANXA1* genes act as a mediator of apoptosis and inhibitor of cell proliferation.[41,42] Indeed, the association of miR-196a2 rs11614913 polymorphism with HCC risk has been reported in recent studies, and some studies supported the proposition that the polymorphism of miR-196a2 rs11614913 may contribute to the susceptibility of colorectal cancer, breast cancer, head and neck cancer, lung cancer, and gastric cancer.[43] However, no association was found in gallbladder cancer.[44] Interestingly, the CC genotype of the miR-196a-2 rs11614913 polymorphism was associated with a decreased risk of glioma.[45] It is reported that in HCC tissues, the C allele of rs11614913 increased the expression of mature miR-196a2.[14] However, in another study, no association between

rs11614913 and HCC was observed.[12] In our meta-analysis, overall and in Asian populations, significant statistical heterogeneity was identified in the comparison of the allele, recessive, homozygous, and heterozygous models. A decreased risk of HCC in Caucasians could be observed in the allele, dominant, recessive, and homozygous models. The reason why miR-146a rs2910164 polymorphism showed no significant result in Caucasians may be attributed to differences in the pathways of carcinogenesis. In addition, this meta-analysis enrolled only 10 studies for MiR-196a2 rs11614913 polymorphism, and an inadequate number of studies would be another influence factor.

Polymorphism miR-499 rs3746444 is located in the stem region opposite the mature miR-499 sequence, resulting in a change from A:U to G:U in its stem region.[21] An earlier study revealed that miR-499 was able to regulate C-reactive protein, a protein closely related to cerebral ischemia. Moreover, mi-499 is also associated with cell apoptosis and cell death in the condition of anoxia and ischemia by dynamin-related protein-1 and targeting calcineurin.[46] As rs3746444 A/G mutation might affect the binding of target mRNAs and maturation of pre-miRNA, this SNP might possibly affect downstream biological functions.[47] A previous meta-analysis found that miR-499 rs3746444 polymorphism is a low-penetrant risk factor for cancer development among Asians and may contribute to breast cancer susceptibility.[48] There was a study suggesting that miR-499 rs3746444 polymorphisms may not be associated with the risk of HCC.[49] However, more studies were pooled into our study, and we identified that in Chinese populations, miR-499 rs3746444 G allele was a risk factor, with the association varying between different cancer types.[50] However, in this meta-analysis, only the recessive models produced a significant association between miR-499 rs3746444 polymorphism and HCC risk. As Hikmet Akkiz's study[21] showed that miR-499 rs3746444 polymorphism has not played any major role in genetic susceptibility to hepatocellular carcinogenesis, none of the genetic models produced any significant association between miR-499 rs3746444 polymorphism and HCC risk in Caucasian.

In the development of a solid tumor, miR-149 rs2292832 can function as a tumor suppressor[51] and an oncogene.[52] The miR-149 rs2292832 SNP might affect the expression of mature miRNAs or their binding activities to target mRNA, with the influence of cancer risk through variable mechanisms. As for miR-149, it is a proapoptotic miRNA that represses the expression of Akt1 and E2F1. Silencing of Akt1 and E2F1 results in apoptosis in human cancer cell lines.[51,53] Moreover, miR-149 is considered as a tumor suppressor, which could inhibit cell growth and invasion by binding to the target gene specificity protein 1.[54] A previous article showed that, based on the pooled studies especially for Asians, miR-149 polymorphism can marginally contribute to gastrointestinal cancer and breast cancer susceptibility.[55] However, there were several other studies that failed to find an association between miR-149 polymorphism and colorectal and gastric cancer,[55,56] which have conclusions consistent with our own. This suggests that no significant statistical heterogeneity was identified in any of the genetic models.

The studies from several laboratories suggest that miR-34 family members are the direct targets of TP53, and their upregulation can induce cancer cell apoptosis and cell-cycle arrest.[57] It has been found that miR-34 is associated with prognosis, carcinogenesis, and survival of various cancers, including ovarian cancer, gastric cancer, and many other cancers.[58] Only three studies reported a potential association between miR-34b/c rs4938723 and HCC risk. However, all these studies were conducted in Asian populations. A common primary transcript (pri-miR-34b/c), which was induced by p53 in response to genotoxic stress, was shared by mMiR-34b and miR-34c; miR-34b/c is considered to be a tumor-suppressor miRNA.[59] In another meta-analysis, the authors revealed that there is no association between miR-34b/c rs4938723 polymorphism, and colorectal cancer and breast cancer.[60] In our meta-analysis, none of the genetic models produced a significant association between miR-34b/c rs4938723 polymorphism and HCC susceptibility.

Compared with other meta-analyses, this study focused on five miRNAs, and found three miRNAs that are associated with HCC. However, well-designed studies with larger sample size of the same ethnic background and biological characterization should be considered to further clarify the association.

4.1. Limitations

Considering the size of the study populations and the limited number of studies, our results should be interpreted with caution. Additionally, there were several other limitations of our investigation. First, there were no more precise analyses involving other covariates such as sex, age, family history, hepatitis B virus/hepatitis C virus infection status, life style, and environmental factors. Second, the included studies incorporated only Asian and Caucasian populations. Third, we did not consider gene−gene and gene−environment interactions, which could alter the associations between cancer and miRNA polymorphisms.

In conclusion, our meta-analysis suggests that miR-146a rs2910164 polymorphism has a significant association with HCC in the allele, recessive, and homozygous models overall and in Asian populations. For miR-196a2 rs11614913, significant statistical heterogeneity overall and in Asian populations was identified in the comparison of the allele, recessive, homozygous, and heterozygous models, and also has a decreased risk of HCC in Caucasians in all genetic models excepted for the heterozygous model. Only the recessive models produced a significant association between miR-499 rs3746444 polymorphism and HCC risk.

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