Journal of Science and Applications:
BioMedicine
Research Paper

Correlation between single nucleotide polymorphisms in microRNAs and hepatitis B virus-related hepatocellular carcinoma risk

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Abstract: We aimed to investigate the correlation between the four common single nucleotide polymorphisms (SNPs) in microRNAs (miR-146a rs2910164, miR-196a2 rs11614913, miR-499 rs3746444 and miR-149 rs2292832) and hepatitis B virus-related hepatocellular carcinoma (HBV-related HCC) risk through a systematic review and meta-analysis. Odds ratios (ORs) along with 95% confidence intervals (95% CIs) were pooled to explore the strength of associations between SNPs in microRNAs and HBV-related HCC risk. All statistical analyses were performed using Stata 12.0 software. For miR-146a rs2910164, a significantly decreased risk of HBV-related HCC development was observed under recessive model (CC versus GC+GG: OR = 0.83, 95% CI 0.71-0.98, P = 0.03), and results remain robust in Asian populations. As for miR-196a2 rs11614913, significant association was found under four genetic models (T versus C: OR = 0.61, 95% CI 0.43-0.87, P = 0.01; TT versus CC: OR = 0.35, 95% CI 0.16-0.75, P = 0.01; TT+CT versus CC: OR = 0.59, 95% CI 0.36-0.96, P = 0.03; TT versus CT+CC: OR = 0.42, 95% CI 0.21-0.87, P = 0.02) among Caucasians, but no significant association on Asians. The current meta-analysis demonstrates that miR-146a rs2910164 polymorphism may play a potential role in genetic susceptibility to HBV-related HCC in Asian populations.

Published by www.inter-use.com. Available online April. 25 2015, Vol. 3 Issue 3 Page 45-55.

Keywords: MicroRNAs, Polymorphism, Hepatitis B virus, Hepatocellular carcinoma, Meta-analysis

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common types of cancer worldwide [1, 2], seriously threatening people's lives. It is well known that hepatitis B virus (HBV) infection is an important risk factor for HCC [3]. However, only a fraction of HBV-infected individuals develop HCC during their lifetime, which suggests that genetic factors may play an important role in tumor development [4, 5].

MicroRNAs (MiRNAs) are small non-coding RNA molecules (19-25 nucleotides in length) that regulate gene expression at transcriptional and post-transcriptional levels, usually resulting in gene silencing via translational repression or target degradation of gene mRNA [6]. MiRNAs have been demonstrated to play a role in susceptibility and prognosis of various types of human cancer [7]. Single nucleotide polymorphisms (SNPs) in miRNAs may affect transcription, processing, or target recognition and result in malignant diseases [8, 9]. Recently, many studies have explored the association between SNPs of miRNAs and HBV-related HCC susceptibility, but their results are not conclusive and consistent.

MiR-146a rs2910164 polymorphism is located in the stem region opposite to the mature miR-146a sequence, which results in a change from G: U pair to C: U mismatch in the stem structure of miR-146a precursor [10, 11]. Several case-control studies and meta-analyses

have investigated the associations between miR-146a rs2910164 polymorphism and risks of various cancers [12-14]. Moreover, polymorphism rs2910164 in miR-146a was thought to influence susceptibility to HBV-related HCC [7, 15], while some other studies' results indicated no significant association between miRNA-146a rs2910164 polymorphism and the risk of HBV-related HCC [16-19].

The C>T substitution polymorphism in miRNA196a2 rs11614913 is one of the most frequently studied SNPs and has been reported to be associated with several types of cancers [19]. Indeed, it was reported that miR-196a2 rs11614913 polymorphism was associated with reduced risk of HBV-related HCC [7, 20], but some other studies reported the opposite results [17, 21, 22].

MiR-499 was reported to play a role of mediator in a wide spectrum of biological processes, such as cellular senescence, apoptosis, immune response, tumor genesis and metastasis [23, 24]. The rs3746444 SNP in miR-499 is located in the stem region opposite to the mature miR-499 sequence [25, 26]. As a tumor suppressor gene, miR-149 might be involved in the proliferation and invasion of glioma cells via blockade of AKT1 signaling [27]. MiR-149 is also a tumor oncogene regulator which is involved in the increased expression levels of myeloid cell leukemia sequence 1 in melanoma cells [28]. Thus, SNPs in miR-499 and miR-149 genes maybe contribute to the HBV-related HCC risk. Similarly, the conclusions of investigations about miR-499 rs3746444 and miR-149 rs2292832 polymorphisms are controversial [4, 17].

Therefore, we performed a meta-analysis to evaluate the correlation between the four common SNPs in microRNAs (miR-146a rs2910164, miR-196a2 rs11614913, miR-499 rs3746444 and miR-149 rs2292832) and HBV-related HCC risk.

2. Materials and Methods

2.1 Search strategy

The major electronic databases including PubMed, Embase and Web of Science, were searched using key words ''microRNA'', ''HBV OR hepatitis B" and "polymorphism''. Our searches were not limited by publication date, country or language. The reference lists of retrieved studies and recent reviews were also manually searched to ensure comprehensive acquisition of literature. The last search was updated in December 7, 2014.

2.2 Inclusion and exclusion criteria

Studies in our meta-analysis met all of the following inclusion criteria: (1) design as case-control studies; (2) evaluation of the association between SNPs in microRNAs, namely miR-146a rs2910164 (G>C), miR-196a2 rs11614913 (C>T), miR-499 rs3746444 (T>C) or miR-149 rs2292832 (C>T), and HBV-related HCC risk; (3) providing complete genotypes distribution data; (4) fulfilling Hardy – Weinberg equilibrium (HWE) in the control group. Exclusion criteria were: (1) duplicate of previous publication; (2) other meta-analyses, letters, conference abstracts, comments, reviews and editorial articles; (3) study with no usable data; (4) no control population. If more than one article were published using the same case series, only the largest series were selected. Two reviewers independently identified eligible studies according to the selection criteria. Any dispute was solved by discussion.

2.3 Data extraction

Two reviewers independently extracted information from eligible studies using a predefined data extraction form. The following information were collected: name of first author, year of publication, country of origin, ethnicity, genotype frequency in cases and controls, source of cases, source of controls, case-control match, specimens used for determining genotypes, Hardy–Weinberg equilibrium in controls, total sample size, respectively. Different ethnic descents were categorized as Asian and Caucasian. Then another reviewer verified them. Discrepancies were resolved by consensus.

2.4 Methodological quality assessment

Three authors independently assessed the quality of the studies using a set of predetermined criteria that were extracted and modified from previous studies [29, 30] (Table 1). Six items, including the source of cases, source of controls, case-control match, specimens used for determining genotypes, Hardy – Weinberg equilibrium in controls, total sample size, were carefully checked in this scale. The quality scores ranged from 0 to 18. Those studies with scores $\geqslant$12 were classified as high quality, whereas those studies with scores < 12 were considered as low quality. The

scores of all included studies in this meta-analysis were shown in Table 2.

Table 1. Scale for Quality Assessment

Criterion	Score
Source of cases	
Selected from population or cancer registry	3
Selected from hospital	2
Selected from pathology archives, but without description	1
Not described	0
Source of controls	
Population-based	3
Blood donors or volunteers	2
Hospital-based (caner-free patients)	1
Not described	0
Case-control match	
Matched by age and gender	3
Not matched by age and gender	0
Specimens used for determining genotypes	
White blood cells or normal tissues	3
Tumor tissue or exfoliated cells of tissue	0
Hardy-Weinberg equilibrium in controls	
Hardy-Weinberg equilibrium	3
Hardy-Weinberg disequilibrium	0
Total sample size	
>1000	3
>500 and <1000	2
>200 and <500	1
<200	0

2.5 Statistical analysis

We conducted our meta-analysis according to the PRISMA checklists and followed the guideline [31]. HWE was evaluated by Pearson's goodness-of-fit χ^2 test for each study in control groups, and a P value of less than 0.05 was considered a significant Hardy-Weinberg disequilibrium. Based on the alleles and genotype frequencies in cases and controls, odds ratios (ORs) along with their 95 % confidence intervals (CIs) were calculated to assess the strength of association between the SNPs in miRNAs and HBV-related HCC risk. The statistical significant level was determined by Z-test with P value less than 0.05. The pooled ORs were performed for allelic, homozygous, heterozygous, dominant and recessive model, respectively. Heterogeneity of included studies was determined by using the Cochrane's Q test and I^2 statistics. If heterogeneity (P <0.05 or I^2 >50%) was statistically significant among studies, the random-effect model was used for the meta-analysis; otherwise, the fixed-effect model was chosen. We performed a subgroup analysis according to ethnicity (Asians or Caucasians). Sensitivity analyses were also carried out by systematically removing each study to ensure the stability of our results. Publication bias was assessed using Begg's funnel plot and Egger's linear regression method [32, 33]. All analyses were calculated by using the software Stata12.0 (Stata Corporation, College Station, Texas).

3. Results

3.1. Search results

A total of 226 records were found from initial searches of the electronic databases. After removing 65 duplicated records, 161 records were screened by title and abstract, and we applied the selection criteria to filter out 102 records. An additional 44 articles were further excluded after a full-text review. Besides, 5 studies were excluded due to Hardy–Weinberg disequilibrium in the control group. A total of 10 case-control studies that met inclusion criteria were included in the final analysis [2, 4, 15-19, 22, 34, 35]. The study selection process is summarized in Fig. 1.

3.2. Characteristics of the included studies

The main characteristics of the included studies are shown in Table 2. The publication years of included studies ranged from 2010 to 2014. In total, 4,720 cases and 6,310 controls were assessed in this meta-analysis. Two of the studies were conducted in Caucasian populations [18, 22], and other eight studies were conducted in Asian populations. The distribution of genotype in the control group was in agreement with the HWE in all studies. Quality scores ranged from 12 to 16, which suggested that all included studies had high quality in this meta-analysis.

3.3 For miR-146a rs2910164 polymorphism

No significant heterogeneity ($I^2 < 50\%$) was identified in all genetic models between miRNA-146a rs2910164 (G>C) polymorphism and HBV-related HCC risk. Therefore, fixed effects model was used to pool the results. Overall, a significantly decreased risk of HBV-related HCC development was observed under recessive model (CC versus GC+GG: OR = 0.83, 95% CI 0.71-0.98, P = 0.03, Fig. 2). However, no association was found to be significant under other genetic models (Table S1). In the five studies included, four studies

were conducted on Asian populations, and one on Caucasian populations. We conducted a subgroup analysis according to different ethnicities. In Asians, a significantly decreased risk of HBV-related HCC development was observed under the same recessive model (CC versus GC+GG: OR = 0.83, 95% CI 0.71-0.98, P = 0.03, Fig. 2). In addition, a trend of reduced risk could be seen in allelic comparison (C versus G: OR = 0.89, 95% CI 0.80-1.00, P = 0.05). In Caucasians, no significant association was observed in all genetic models.

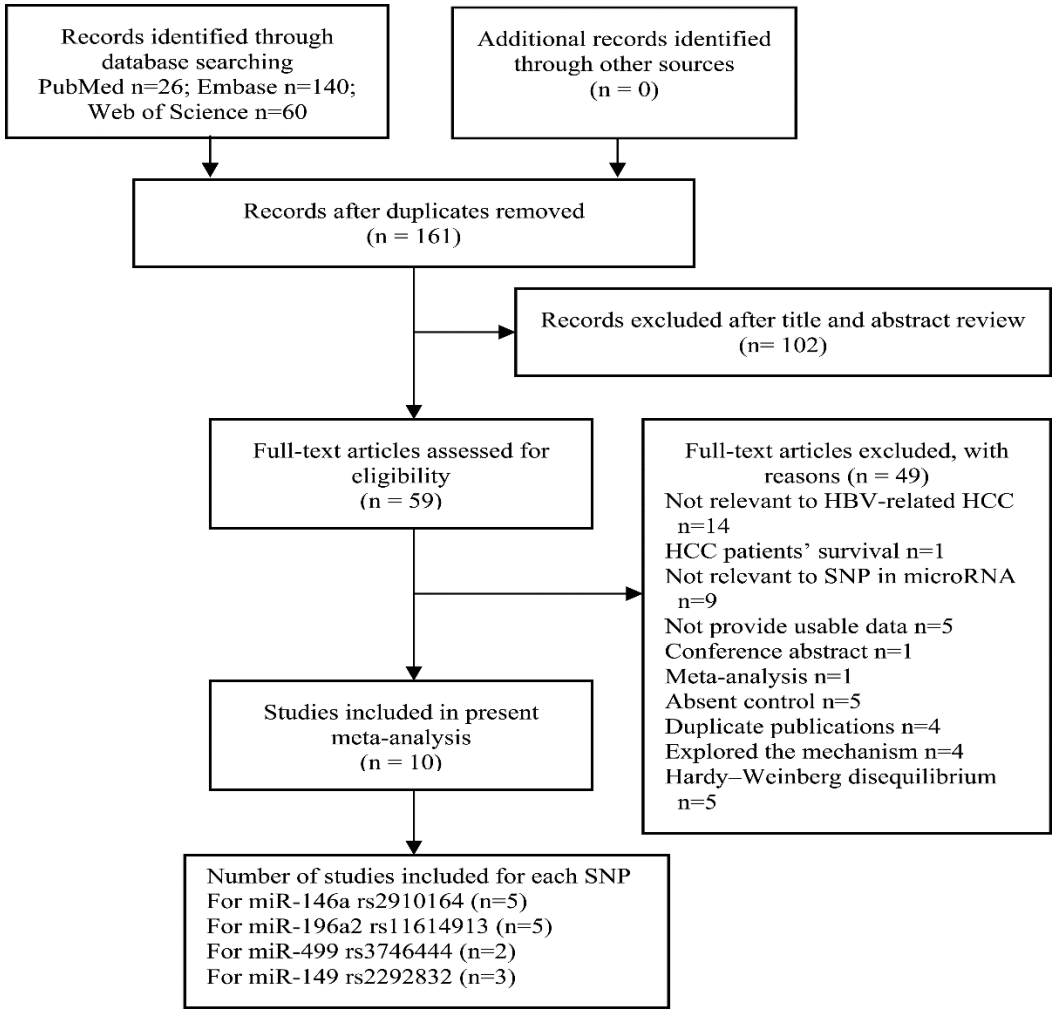

Fig. 1. Flow diagram of study identification.

3.4 For miR-196a2 rs11614913 polymorphism

There were five case-control studies referring to the association between the miR-196a2 rs11614913 (C > T) polymorphism and HBV-related HCC risk. Since significant statistical heterogeneity ($I^2 > 50\%$) was observed in most genetic models, the random effects model was used in these models except recessive model (TT versus CT+CC). Overall, no significant association between polymorphism rs11614913 in miR-196a2 and HBV-related HCC risk was identified in any genetic models (Table S1). Similarly, we performed a subgroup analysis according to different ethnicities. No significant association between the miR-196a2 rs11614913 polymorphism and HBV-related HCC risk was observed in the Asian populations (Table S1). As for the Caucasian populations, a significantly decreased risk of HBV-related HCC development was observed under four genetic models (T versus C: OR = 0.61, 95% CI 0.43-0.87, P = 0.01; TT versus CC: OR = 0.35, 95% CI 0.16-0.75, P = 0.01; TT+CT versus CC: OR = 0.59, 95% CI 0.36-0.96, P = 0.03; TT versus CT+CC: OR = 0.42, 95% CI 0.21-0.87, P = 0.02).

Table 2. Characteristics of the included studies in the meta-analysis

First author	Year	Country	Ethnicity	Cases (HBV-related HCC)			Controls			P for HWE	Quality score
miR-146a rs2910164: G>C				GG	GC	CC	GG	GC	CC		
Xiang Y	2012	China	Asian	18	34	21	21	46	33	0.51	12
Kim WH	2012	Korea	Asian	13	71	43	24	103	74	0.19	15
Akkiz H,-1	2011	Turkey	Caucasian	75	51	6	144	67	11	0.38	16
Cong N	2014	China	Asian	15	37	39	17	84	117	0.72	12
Zhang J	2013	China	Asian	124	390	257	156	475	367	0.91	14
miR-196a2 rs11614913: C>T				CC	CT	TT	CC	CT	TT		
Kim WH	2012	Korea	Asian	24	70	33	45	107	49	0.36	15
Akkiz H,-2	2011	Turkey	Caucasian	46	48	11	58	87	40	0.49	16
Qi P	2010	China	Asian	82	179	100	92	197	102	0.87	12
Zhang J	2013	China	Asian	171	376	224	165	502	328	0.24	14
Han Y	2013	China	Asian	207	505	305	220	485	304	0.31	13
miR-499 rs3746444: T>C				TT	TC	CC	TT	TC	CC		
Xiang Y	2012	China	Asian	27	30	16	54	36	10	0.28	12
Kim WH	2012	Korea	Asian	91	34	2	120	74	7	0.28	15
miR-149 rs2292832: C>T				CC	CT	TT	CC	CT	TT		
Kim WH	2012	Korea	Asian	10	49	68	21	97	83	0.34	15
Wang R	2014	China	Asian	81	307	341	92	414	478	0.86	15
Wang XH	2014	China	Asian	7	42	40	43	148	113	0.62	15

HWE: Hardy-Weinberg equilibrium in control groups

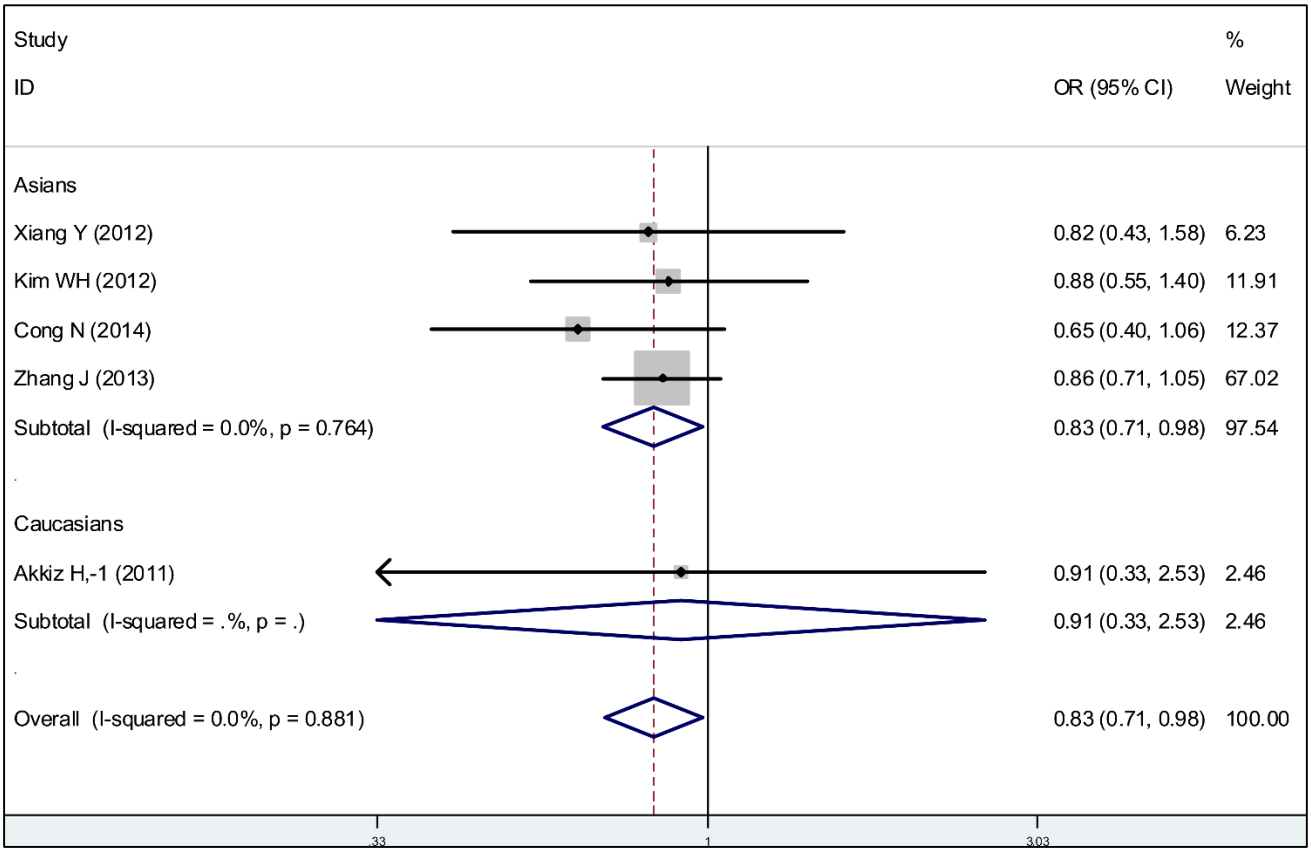

Fig. 2. Forest plot for the association of miRNA-146a rs2910164 polymorphism with HBV-related HCC risk is illustrated in subgroup analysis by ethnicity (CC versus GC+GG)

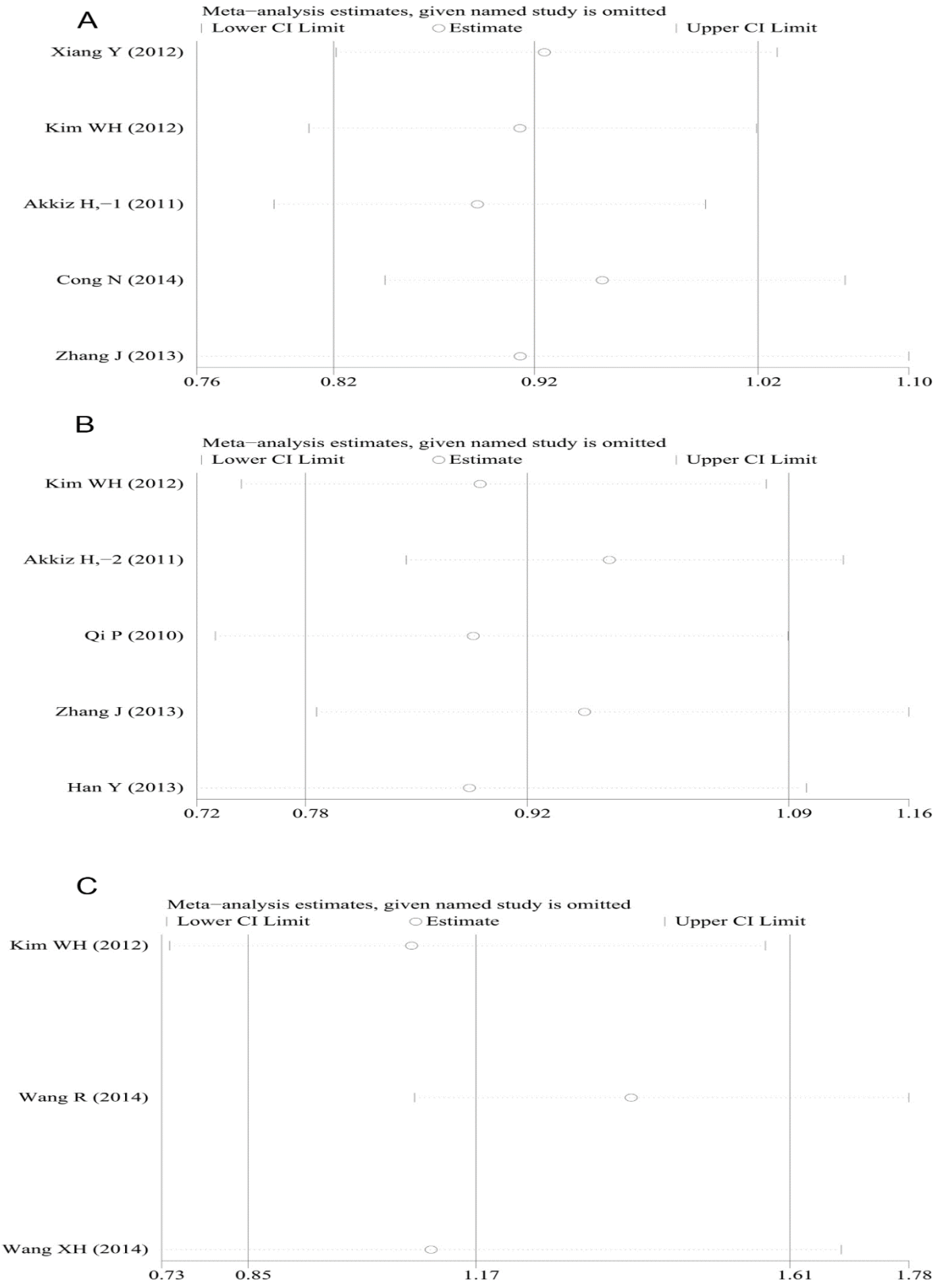

Fig. 3. Sensitivity analysis of the summary odds ratio coefficients of the three polymorphisms is illustrated under the allele model. Results were computed by omitting each study in turn. The two ends of the dotted lines represent the 95% CI (A: miR-146a; B: miR-196a2; C: miR-149)

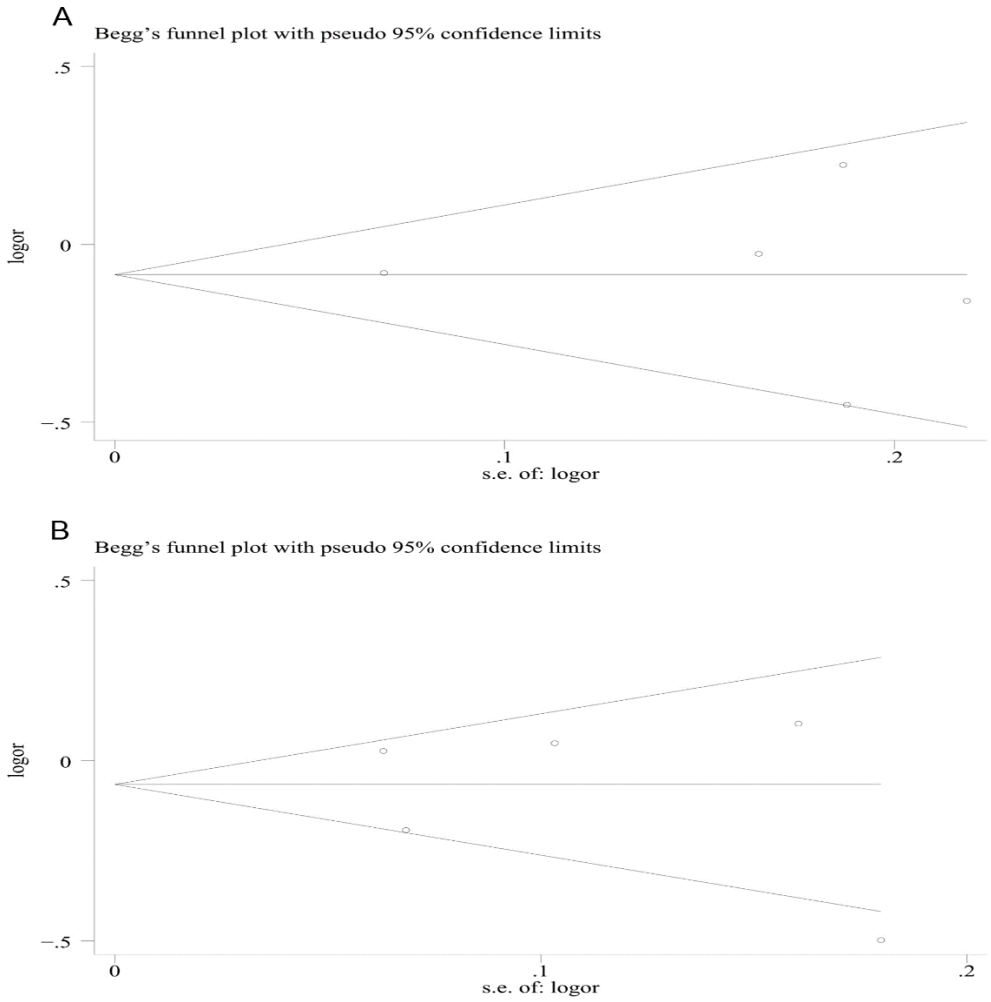

Fig. 4. Begg's funnel plot of publication bias is illustrated under the allele model (A: miR-146a; B: miR-196a2).

3.5 For miR-499 rs3746444 polymorphism

Only two studies involving 200 cases and 301 controls were evaluated for the association between miR-499 rs3746444 polymorphism and HBV-related HCC risk. Significant statistical heterogeneity ($I^2 >$ 50%) was observed in all genetic models. The pooled ORs therefore were calculated using random effects model. Our results showed no significant association between miRNA-499 rs3746444 polymorphism and HBV-related HCC risk, which was identified in any genetic models (Table S1). For the limited studies and relatively large heterogeneity, the results should be treated with caution.

3.6 For miR-149 rs2292832 polymorphism

The association between miR-149 rs2292832 polymorphism and HBV-related HCC risk was analyzed in three studies with 945 cases and 1,489 controls. No Significant statistical heterogeneity (I^2=18.1%) was identified in the heterozygous model (CT versus CC). Therefore, fixed effects model was used in this model, and random effects model was used in the other four models. No significant association between miR-149 rs2292832 polymorphism and HBV-related HCC risk was found in any genetic models. The results are summarized in Table S1.

3.7 Sensitivity analysis

Then sensitivity analyses were performed to check the influence of the removed data set to the pooled ORs. We deleted one single study from the overall pooled analysis in turn (Fig.3). Our results showed that pooled ORs were not materially altered, which suggested that no individual study significantly affected the pooled results. We did not perform a sensitivity analysis for miR-499 rs3746444 polymorphism.

3.8 Publication bias

No evidence of publication bias for the association between miRNA-146a rs2910164 polymorphism and HBV-related HCC risk was detected by either Begg or Egger's test (C versus G: Begg's test P = 0.81; Egger's test P = 0.90). Similarly, no evidence of publication bias for the association between miR-196a2 rs11614913 polymorphism and HBV-related HCC risk was detected by either Begg or Egger's test (T versus C: Begg's test P = 0.22; Egger's test P = 0.74). The shapes of the funnel plots for miR-146a rs2910164 and miR-196a2 rs11614913 polymorphisms under the allelic comparison seemed approximately symmetrical (Fig. 4). We did not detect publication bias for miR-499 rs3746444 and miR-149 rs2292832 polymorphisms due to limited studies.

4. Discussion

MicroRNAs have been demonstrated to have a role in several biochemical pathways in cell differentiation, proliferation, apoptosis and carcinogenesis [21]. SNPs reside at or near a miRNA binding site of a functional gene, influencing its expression by interfering with miRNA function [10]. Thus SNPs in miRNAs is considered as an important factor in oncogenesis. Recently, several meta-analyses have investigated the role of SNPs in miRNAs and their impacts on susceptibility to HCC [23, 25, 36], while none of the meta-analyses focuses on HBV-related HCC. Much effort has been made to identify the associations between SNPs in miRNAs and susceptibility to HBV-related HCC, whereas these studies have shown inconsistent conclusions. Therefore, we performed a meta-analysis to provide insight into the relationship of SNPs in miRNAs with HBV-related HCC risk and obtain more robust conclusions.

To study the relationship between the miR-146a rs2910164 polymorphism and HBV-related HCC risk, we performed a meta-analysis including five eligible case-control studies with 1,194 cases and 1,739 controls. Our results showed that the miR-146a*C variant was associated with a decrease in HBV-related HCC risk, while a previous meta-analysis did not find significant association between the miR-146a rs2910164 polymorphism and HBV-related HCC risk [25]. It is possible that larger sample sizes may lead to the identification of statistically significant correlation. In subgroup analysis by ethnicity, similar degree of reduction in HBV-related HCC risk was found in Asians but not in Caucasians, which suggested that genetic differences might underlie carcinogenesis in different populations.

We found that five studies reported the association between miR-499 rs3746444 polymorphism and HBV-related HCC risk, but we excluded three of the studies due to Hardy–Weinberg disequilibrium in the control group [4, 8, 37]. Finally, two eligible case-control studies were included in this meta-analysis. one study conducted by Xiang Y *et al* [16] found that HBV+ individuals with CC genotype of miR-499 gene were more susceptible for HCC compared with those carrying TT genotype, while another study conducted by Kim WH *et al* [17] suggested that the risk of HCC was significantly lower TC+CC genotype compared to the TT genotype in HBV-related HCC patients. The conflicting results from these two studies may be due to the different countries' populations as well as several environments. We calculated the pooled OR for these two studies. As a result, our study did not support a genetic association between miR-499 rs3746444 polymorphism and susceptibility to HBV-related HCC. Considering the important biological function of miR-499 in tumor genesis, the conclusion that miR-499 rs3746444 polymorphism has no role in HBV-related HCC development is arguable owing to limited number of studies in this meta-analysis.

We conducted a meta-analysis to comprehensively assess the relationship between the miR-149 rs2292832 polymorphism and HBV-related HCC risk. As a result, our study did not support a genetic association between miR-149 rs2292832 polymorphism and susceptibility to HBV-related HCC. A previous meta-analysis conducted by Xu L *et al* [38] suggested that miR-149 rs2292832 polymorphism was not associated with cancer risk in all genetic models, and their conclusion was supported by our result.

Our meta-analysis has several strengths. To the best of our knowledge, this is the first meta-analysis focusing on the potential association between four common SNPs (miR-146a rs2910164, miR-196a2 rs11614913, miR-499 rs3746444 and miR-149 rs2292832) and HBV-related HCC risk. A previous meta-analysis explored the association between SNPs in microRNAs and HBV-related HCC risk by means of subgroup analysis [25]. Compared with the former meta-analysis [25], more studies were included in our study and sensitivity analysis were performed.

Additionally, another polymorphism rs2292832 in miR-149 was also explored. Moreover, all the included studies had high qualities according to the methodological quality assessment and the distribution of genotypes was in agreement with HWE in the controls.

Similar to other meta-analyses, our study still has some limitations. Firstly, the number of cases and controls included in the study was limited. So, further well-designed studies are needed to confirm the relationships of miRNA-499 rs3746444 and miR-149 rs2292832 polymorphisms with HBV-related HCC susceptibility. Secondly, considering the limited study number for Caucasians in our meta-analysis, the results should be interpreted with caution in Caucasian populations. Thirdly, lack of available data prevented us from performing additional subgroup analyses by alcohol consumption, age, and gender, *etc*, which could be potential factors influencing the evaluation of the associations between SNPs in miRNAs and HBV-related HCC risk. Finally, although our searches were extensive and were not limited by language, language bias should not be completely avoided because of all included studies written in English.

5. Conclusions

Our study suggested that the miRNA-146a rs2910164CC genotype was significantly associated with a decreased risk of HBV-related HCC in Asian populations. We failed to find any significant correlation between miR-196a2 rs11614913 polymorphism and HBV-related HCC risk in Asians, but observed significant correlation in Caucasians. In addition, there was not any association of miRNA-499 rs3746444 and miR-149 rs2292832 polymorphisms with HBV-related HCC risk in our meta-analysis.

Acknowledgements

We thank all authors of included studies in current meta-analyses.

References

[1] Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA: a cancer journal for clinicians. 2011; 61(2):69-90.

[2] Wang R, Zhang J, Ma Y, et al. Association study of miR-149 rs2292832 and miR-608 rs4919510 and the risk of hepatocellular carcinoma in a large-scale population. Molecular Medicine Reports. 2014; 10(5):2736-44.

[3] Kao JH, Chen DS. Global control of hepatitis B virus infection. The Lancet infectious diseases. 2002; 2(7):395-403.

[4] Wang XH, Wang FR, Tang YF, et al. Association of miR-149C > T and miR-499A > G polymorphisms with the risk of hepatocellular carcinoma in the Chinese population. Genetics and Molecular Research. 2014; 13(3):5048-54.

[5] Wang R, Zhang J, Jiang W, et al. Association between a variant in microRNA-646 and the susceptibility to hepatocellular carcinoma in a large-scale population. The Scientific World Journal. 2014; 2014(312704):1-7.

[6] Tarocchi M, Polvani S, Marroncini G, et al. Molecular mechanism of hepatitis B virus-induced hepatocarcinogenesis. World Journal of Gastroenterology. 2014; 20(33):11630-40.

[7] Zhou B, Dong L-p, Jing X-y, et al. Association between miR-146aG > C and miR-196a2C > T polymorphisms and the risk of hepatocellular carcinoma in a Chinese population. Tumor Biology. 2014; 35(8):7775-80.

[8] Ma Y, Wang R, Zhang J, et al. Identification of miR-423 and miR-499 polymorphisms on affecting the risk of hepatocellular carcinoma in a large-scale population. Genetic Testing and Molecular Biomarkers. 2014; 18(7):516-24.

[9] Pastinen T, Ge B, Hudson TJ. Influence of human genome polymorphism on gene expression. Human molecular genetics. 2006; 15 Spec No 1:R9-16.

[10] Du W, Ma XL, Zhao C, et al. Associations of single nucleotide polymorphisms in miR-146a, miR-196a, miR-149 and miR-499 with colorectal cancer susceptibility. Asian Pacific journal of cancer prevention: APJCP. 2014; 15(2):1047-55.

[11] He B, Pan Y, Cho WC, et al. The association between four genetic variants in microRNAs (rs11614913, rs2910164, rs3746444, rs2292832) and cancer risk: evidence from published studies. PloS one. 2012; 7(11):e49032.

[12] Parlayan C, Ikeda S, Sato N, et al. 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 Pacific Journal of Cancer Prevention. 2014; 15(5):2101-7.

[13] Wu D, Wang F, Dai W-Q, et al. The miR-146a rs2910164 G > C Polymorphism and Susceptibility to Digestive Cancer in Chinese. Asian Pacific Journal of Cancer Prevention. 2013; 14(1):399-403.

[14] Li Y-J, Zhang Z-Y, Mao Y-Y, et al. A Genetic Variant in MiR-146a Modifies Digestive System Cancer Risk: a Meta-analysis. Asian Pacific Journal of Cancer Prevention. 2014; 15(1):145-50.

[15] Cong N, Chen H, Bu W-Z, et al. miR-146a G > C polymorphisms and risk of hepatocellular carcinoma in a Chinese population. Tumor Biology. 2014; 35(6):5669-73.

[16] Xiang Y, Fan S, Cao J, et al. Association of the microRNA-499 variants with susceptibility to hepatocellular carcinoma in a Chinese population. Molecular biology reports. 2012; 39(6):7019-23.

[17] Kim WH, Min KT, Jeon YJ, et al. Association study of microRNA polymorphisms with hepatocellular carcinoma in Korean population. Gene. 2012; 504(1):92-7.

[18] Akkiz H, Bayram S, Bekar A, et al. No association of pre-microRNA-146a rs2910164 polymorphism and risk of hepatocellular carcinoma development in Turkish population: a case-control study. Gene. 2011; 486(1-2):104-9.

[19] Zhang J, Wang R, Ma Y-Y, et al. Association Between Single Nucleotide Polymorphisms in miRNA196a-2 and miRNA146a and Susceptibility to Hepatocellular Carcinoma in a Chinese Population. Asian Pacific Journal of Cancer Prevention. 2013; 14(11):6427-31.
[20] Kou J-T, Fan H, Han D, et al. Association between four common microRNA polymorphisms and the risk of hepatocellular carcinoma and HBV infection. Oncology Letters. 2014; 8(3):1255-60.
[21] Hao Y-X, Wang J-P, Zhao L-F. Associations Between Three Common MicroRNA Polymorphisms and Hepatocellular Carcinoma Risk in Chinese. Asian Pacific Journal of Cancer Prevention. 2013; 14(11):6601-4.
[22] Akkiz H, Bayram S, Bekar A, et al. A functional polymorphism in pre-microRNA-196a-2 contributes to the susceptibility of hepatocellular carcinoma in a Turkish population: a case-control study. Journal of viral hepatitis. 2011; 18(7):e399-407.
[23] Hu M, Zhao L, Hu S, et al. The association between two common polymorphisms in MicroRNAs and hepatocellular carcinoma risk in Asian population. PloS one. 2013; 8(2):e57012.
[24] Wang JX, Jiao JQ, Li Q, et al. miR-499 regulates mitochondrial dynamics by targeting calcineurin and dynamin-related protein-1. Nature medicine. 2011; 17(1):71-8.
[25] Xu Y, Li L, Xiang X, et al. Three common functional polymorphisms in microRNA encoding genes in the susceptibility to hepatocellular carcinoma: a systematic review and meta-analysis. Gene. 2013; 527(2):584-93.
[26] Akkiz H, Bayram S, Bekar A, et al. Genetic variation in the microRNA-499 gene and hepatocellular carcinoma risk in a Turkish population: lack of any association in a case-control study. Asian Pacific journal of cancer prevention: APJCP. 2011; 12(11):3107-12.
[27] Pan SJ, Zhan SK, Pei BG, et al. MicroRNA-149 inhibits proliferation and invasion of glioma cells via blockade of AKT1 signaling. International journal of immunopathology and pharmacology. 2012; 25(4):871-81.
[28] Jin L, Hu WL, Jiang CC, et al. MicroRNA-149*, a p53-responsive microRNA, functions as an oncogenic regulator in human melanoma. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108(38):15840-5.
[29] Yu H, Sun S, Liu F, et al. Meta-analysis of Associations between Interleukin-17 Gene Polymorphisms and Risk of Gastric Cancer. Asian Pacific journal of cancer prevention: APJCP. 2014; 15(20):8709-13.
[30] Li L, Sheng Y, Lv L, et al. The association between two microRNA variants (miR-499, miR-149) and gastrointestinal cancer risk: a meta-analysis. PloS one. 2013; 8(11):e81967.
[31] Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. International journal of surgery (London, England). 2010; 8(5):336-41.
[32] Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics. 1994; 50(4):1088-101.
[33] Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. BMJ (Clinical research ed). 1997; 315(7109):629-34.
[34] Han Y, Pu R, Han X, et al. Associations of pri-miR-34b/c and pre-miR-196a2 Polymorphisms and Their Multiplicative Interactions with Hepatitis B Virus Mutations with Hepatocellular Carcinoma Risk. PloS one. 2013; 8(3):e58564.
[35] Qi P, Dou TH, Geng L, et al. Association of a variant in MIR 196A2 with susceptibility to hepatocellular carcinoma in male Chinese patients with chronic hepatitis B virus infection. Human immunology. 2010; 71(6):621-6.
[36] Wang Z, Cao Y, Jiang C, et al. Lack of association of two common polymorphisms rs2910164 and rs11614913 with susceptibility to hepatocellular carcinoma: a meta-analysis. PloS one. 2012; 7(6):e40039.
[37] Zou HZ, Zhao YQ. Positive association between miR-499a>G and hepatocellular carcinoma risk in a chinese population. Asian Pacific Journal of Cancer Prevention. 2013; 14(3):1769-72.
[38] Xu L, Zhou X, Qiu MT, et al. Lack of association between hsa-miR-149 rs2292832 polymorphism and cancer risk: a meta-analysis of 12 studies. PloS one. 2013; 8(9):e73762.

Appendix

Table S1. Summary of polled ORs in the meta-analysis.

SNPs	Allelic model				Heterozygous model				Homozygous model				Dominant model				Recessive model			
	OR	95%CI	P	I^2(%)	OR	95%CI	P	I^2(%)	OR	95%CI	P	I^2(%)	OR	95%CI	P	I^2(%)	OR	95%CI	P	I^2(%)
miR-146a rs2910164	C versus G				GC versus GG				CC versus GG				CC+GC versus GG				CC versus GC+GG			
Overall	0.92	0.82-1.02	0.13	40.9	1.06	0.86-1.30	0.58	31.9	0.83	0.66-1.05	0.12	15.5	0.99	0.81-1.20	0.91	49.1	0.83	0.71-0.98	**0.03**	0
Asians	0.89	0.80-1.00	0.05	20.6	0.98	0.78-1.23	0.85	14.2	0.82	0.64-1.04	0.11	33.7	0.91	0.73-1.13	0.39	37.4	0.83	0.71-0.98	**0.03**	0
Caucasians	1.25	0.87-1.80	0.23	—	1.46	0.92-2.31	0.11	—	1.05	0.37-2.94	0.93	—	1.4	0.90-2.18	0.13	—	0.91	0.33-2.53	0.86	—
miR-196a2 rs11614913	T versus C				CT versus CC				TT versus CC				TT+CT versus CC				TT versus CT+CC			
Overall	0.92	0.78-1.09	0.33	70.8	0.92	0.74-1.16	0.5	52.4	0.85	0.60-1.20	0.35	72.5	0.9	0.69-1.16	0.41	67.8	0.92	0.82-1.04	0.2	46.4
Asians	0.97	0.85-1.12	0.71	60.7	0.97	0.75-1.24	0.78	58	0.95	0.70-1.29	0.74	65.6	0.96	0.74-1.26	0.79	67.5	0.95	0.84-1.07	0.4	0
Caucasians	0.61	0.43-0.87	**0.01**	—	0.7	0.41-1.17	0.17	—	0.35	0.16-0.75	**0.01**	—	0.59	0.36-0.96	**0.03**	—	0.42	0.21-0.87	**0.02**	—
miR-499 rs3746444	C versus T				TC versus TT				CC versus TT				CC+TC versus TT				CC versus TC+TT			
Asians	1.09	0.37-3.22	0.88	92	0.98	0.36-2.63	0.97	82.5	1.22	0.15-9.94	0.86	81	1.07	0.32-3.55	0.92	89.5	1.21	0.22-6.57	0.83	72.3
miR-149 rs2292832	T versus C				CT versus CC				TT versus CC				TT+CT versus CC				TT versus CT+CC			
Asians	1.17	0.85-1.61	0.33	74.8	0.95	0.71-1.27	0.73	18.1	1.31	0.67-2.59	0.43	68.2	1.16	0.68-1.97	0.6	54	1.22	0.83-1.79	0.3	69.6

SNPs: single nucleotide polymorphisms; N: number of studies; OR: odds ratios; 95%CI: 95% confidence interval; I^2＞50%: Estimates for random effects model; —not available