RESEARCH ARTICLE

Lack of Association between Hsa-Mir-499 rs3746444 Polymorphism and Cancer Risk: Meta-analysis Findings

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Abstract

Epidemiologic findings concerning the association between the hsa-mir-499 rs3746444 A>G polymorphism and cancer risk have yielded mixed results. We aimed to investigate the association by performing a meta-analysis of all available studies. We searched PubMed and EMBASE for studies published up to November 2014, using odds ratios (ORs) with 95% confidence intervals (CIs) to assess the strength of any association. The Benjamini-Hochberg (BH) method was used to correct the *p* values for multiple comparisons. We included 39 studies, including 14,136 cases and 16,937 controls. The results of overall meta-analysis suggested a borderline association between hsa-mir-499 rs3746444 polymorphism and cancer susceptibility (AG+GG vs. AA: OR=1.15, 95% CI= 1.04-1.26, corrected *p* value=0.04). After removing studies not conforming to Hardy–Weinberg equilibrium (HWE), however, this association disappeared (AG+GG *vs* AA: OR=1.18, 95% CI=1.03-1.34, corrected *p* value=0.21). When stratified analysis by ethnicity, cancer type or HWE in controls, although some associations between hsa-mir-499 rs3746444 polymorphism and cancer susceptibility were detected, these associations no longer existed after adjustment using BH method. In conclusion, our meta-analysis suggests that hsa-mir-499 rs3746444 A>G polymorphism is not associated with risk of cancer based on current evidence.

Keywords: Cancer - meta-analysis - hsa-mir-499 - polymorphism - susceptibility

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Introduction

Cancer is a major public health problem all over the world. In the United States, one in four deaths is due to cancer (Siegel et al., 2014). The etiology of cancer is still not fully understood. It has been suggested that genetic factors play a significant role in cancer development. MicroRNAs (miRNAs, miRs) are a class of small non-coding RNAs with 18-25 nucleotides (Kutanzi et al., 2011) that negatively control gene expression at the mRNA and protein level (He and Hannon, 2004). It has been demonstrated that miRNAs are master regulators of key genes implicated in important biological processes, such as embryonic development, cell proliferation, differentiation, migration, apoptosis and signal transduction, etc (Anglicheau et al., 2010; Kutanzi et al., 2011). It has also been reported that miRNAs play key roles in tumor formation. Recently, single nucleotide polymorphisms (SNPs) in miRNAs have been paid much more attention. Studies have reported that miRNA SNPs could alter expressions or functions of miRNAs thus affecting cancer risk. An important SNP in the hsa-mir-499 with an A to G change (rs3746444) was identified. A series of studies have explored the role of hsa-mir-499 rs3746444 polymorphism in cancer risk, but their results are conflicting rather than conclusive. Many meta-analyses have examined the association of the polymorphism with cancer risk and the lasted one (Ma et al., 2013) suggested that hsa-mir-499 rs3746444 polymorphism was a risk factor for cancer development. Since the latest meta-analysis had been performed, nineteen case-control studies (Ling et al., 2011; Ahn et al., 2013; Lv et al., 2013; Shan et al., 2013; Umar et al., 2013; Wu et al., 2013; Zou and Zhao, 2013; Bansal et al., 2014; Chu et al., 2014; Du et al., 2014; Gutierrez-Camino et al., 2014; Hasani et al., 2014; Hou et al., 2014; Hu et al., 2014; Ma et al., 2014; Omrani et al., 2014; Pu et al., 2014; Qi et al., 2014; Wang et al., 2014) have been published. Therefore, we performed a meta-analysis of all studies available now to derive a more precise estimation of the association between hsa-mir-499 rs3746444 polymorphism and cancer risk.

Materials and Methods

Publication search

We searched PubMed (from 2009 to present) and Embase (from 2009 to present) for studies in humans of the association between hsa-mir-499 rs3746444 polymorphism and risk of cancer. The search strategy used the terms "rs3746444 or miR-499" and "cancer OR

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carcinoma". The latest date of this search was November 2014. Reference lists were examined manually to further identify potentially relevant studies. All studies matching the eligible criteria listed below were included in our meta-analysis, without language restriction.

Inclusion criteria

The following inclusion criteria were used in selecting literature for further meta-analysis: (a) evaluation of hsa-mir-499 rs3746444 polymorphism and cancer risk; (b) a case-control design; and (c) sufficient published data for calculating odds ratios (ORs) with their 95% confidence intervals (95% CIs).

Data extraction

Two investigators independently extracted the data. Discrepancies were solved by discussion until consensus was achieved on every item. From each of included articles the following information was abstracted: the name of first author, year of publication, country origin, ethnicity, cancer type, source of controls, total number of cases and controls, the number of cases and controls with hsa-mir-499 rs3746444 polymorphism genotypes and *p* value for Hardy-Weinberg equilibrium (HWE), respectively. Only controls randomly selected from general population were defined as population-based (PB) otherwise hospital-based (HB).

Statistical methods

For the controls of each study, HWE was assessed using the chi-square goodness-of-fit test and a P<0.05 was considered representative of a departure from HWE. The OR and its 95% CI were used to assess the strength of association between hsa-mir-499 rs3746444 polymorphism and cancer risk. The pooled ORs were performed for allelic comparison (G *vs* A), homozygote comparison (GG *vs* AA), heterozygote comparison (AG *vs* AA), recessive model (GG *vs* AA+AG) and dominant model (AG+GG *vs* AA), respectively. Summary estimates of OR and 95% CIs were obtained using a random effects model where the restricted maximum likelihood estimator was used to evaluate the inter-study heterogeneity (Viechtbauer, 2005; Raudenbush, 2009). Subgroup analyses were performed based on ethnicity (Asian and Caucasian), HWE in controls (yes/no) and cancer type (acute lymphoblastic leukemia, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, hepatocellular carcinoma, lung cancer, squamous cell carcinoma of the head and neck and others) to explore the source of heterogeneity. To correct the *p* values for multiple comparisons, we applied the benjamini-hochberg (BH) method (Benjamini and Hochberg, 1995) which control for false discovery rate (FDR). Meta-regression analyses (Sharp, 1998) were run including sample size (<700 and ≥700 subjects), ethnicity, HWE in controls, cancer type, publication year, source of controls and genotyping method as factors to further evaluate the source of heterogeneity in the effect-sizes and to check the influence of potential confounding variables (the analysis was based on allelic comparison). In addition, sensitivity analyses were performed to reflect the influence of individual data on summary ORs. Finally, the potential for publication bias was examined using a funnel plot and Egger regression test (Egger et al., 1997). All of the statistical analyses were done with R software, version 3.1.1.

Results

Characteristics of the studies

Figure 1 outlines the search strategy used to obtain relevant literature. One hundred and three titles and abstracts were identified and screened and forty-two studies were reviewed in detail. One study was excluded as it was not associated with hsa-mir-499 rs3746444 A>G (Kupcinskas et al., 2012). After further excluding two abstracts (Hu et al., 2009; Hwang et al., 2010), thirty-nine case-control studies involving 14,136 cases and 16,937 controls were selected for meta-analysis (Hu et al., 2009; Tian et al., 2009; Catucci et al., 2010; Liu et al., 2010; Okubo et al., 2010; Srivastava et al., 2010; Akkiz et al., 2011; George et al., 2011; Ling et al., 2011; Mittal et al., 2011; Vinci et al., 2011; Zhou et al., 2011; Alshatwi et al., 2012; Chu et al., 2012; Kim et al., 2012; Min et al., 2012; Xiang et al., 2012; Zhou et al., 2012; Ahn et al., 2013; Lv et al., 2013; Shan et al., 2013; Song et al., 2013; Umar et al., 2013; Vinci et al., 2013; Wei et al., 2013; Wu et al., 2013; Zou and Zhao, 2013; Bansal et al., 2014; Chu et al., 2014; Du et al., 2014; Gutierrez-Camino et al., 2014; Hasani et al., 2014; Hou et al., 2014; Hu et al., 2014; Ma et al., 2014; Omrani et al., 2014; Pu et al., 2014; Qi et al., 2014; Wang et al., 2014). Table 1 presents the main characteristics of each study included in the meta-analysis. The cancer type includes acute lymphoblastic leukemia, breast cancer, lung cancer, hepatocellular carcinoma, colorectal cancer, squamous cell carcinoma of the head and neck, gastric cancer, esophageal cancer, renal cell carcinoma, prostate cancer, gallbladder cancer, bladder cancer and cervical squamous cell carcinoma. There were fifteen studies of caucasian descent and twenty-four studies of Asian descent. The genotype distributions in the controls of fourteen studies were not conforming to HWE (p<0.05) (Okubo et al., 2010; Akkiz et al., 2011; Ling et al., 2011; Mittal et al., 2011; Zhou et al., 2011; Xiang et al., 2012; Shan et al., 2013; Vinci et al., 2013; Wei et al., 2013; Zou and Zhao, 2013; Bansal et al., 2014; Ma et al., 2014; Omrani et al., 2014; Wang et al., 2014).

Evidence Synthesis

The main results of present meta-analysis including the heterogeneity test are shown in Table 2. The results of overall meta-analysis suggested a borderline association between hsa-mir-499 rs3746444 polymorphism and cancer susceptibility (AG+GG *vs* AA: OR=1.15, 95% CI= 1.04-1.26, corrected *p* value=0.04). After removing studies not conforming to HWE, however, this association disappeared (AG+GG *vs* AA: OR=1.18, 95% CI= 1.03-1.34, corrected *p* value=0.21). When stratified analysis by ethnicity, cancer type, or HWE in controls, although some associations between hsa-mir-499 rs3746444 polymorphism and cancer susceptibility were detected, these associations no longer exited after adjustment using BH method.

Sensitivity Analysis

From the results of the leave-one-out sensitivity analysis, all the results above were not materially altered (data not shown). When limiting the meta-analysis to the 25 studies conforming to HWE (Table 1), all the results without Benjamini-Hochberg correction were not materially affected, but the several significance associations found in Asian subgroup (G *vs* A: OR=1.15, 95% CI= 0.98-1.34 and AG+GG *vs* AA: OR=1.17, 95% CI= 0.99-1.38) and other cancer subgroup (G *vs* A: OR=1.10, 95% CI= 0.94-1.30; AG *vs* AA: OR=1.34, 95% CI= 0.83-2.18; and AG+GG *vs* AA: OR=1.27, 95% CI= 0.88-1.84) no longer existed (Table 2). We further explored the source of heterogeneity by sample size, ethnicity, HWE in controls, cancer type, publication year and genotyping method with meta-regression. However, the results revealed that none of them contributed to the source of heterogeneity.

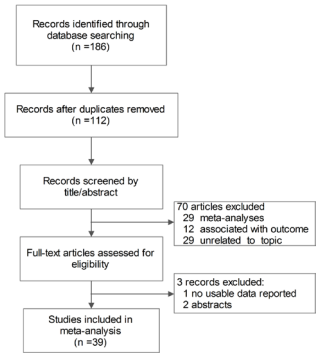

Figure 1. Flow Chart of The Selection of Publications Included in The Meta-Analysis.

Table 1. Characteristics of Studies Included in The Meta-Analysis

First author	Year	Country	Ethnicity	Cancer type	Source of control	Genotyping method	Cases			Controls			P_{HWE}
							AA	AG	GG	AA	AG	GG	
Hu	2009	China	Asian	Breast	PB	PCR-RFLP	707	258	44	816	248	29	0.06
Tian	2009	China	Asian	Lung	PB	PCR-RFLP	781	253	24	755	254	26	0.40
Catucci	2010	Italy Germany	Caucasian	Breast	HB	Sequencing	950	545	84	1305	742	120	0.28
Liu	2010	USA	Caucasian	SCCHN	HB	PCR-RFLP	745	309	55	710	366	54	0.44
Okubo	2010	Japan	Asian	Gastric	HB	PCR-RFLP	364	151	37	466	198	33	0.05
Srivastava	2010	India	Caucasian	Gallbladder	PB	PCR-RFLP	112	97	21	121	94	15	0.57
Akkiz	2011	Turkey	Caucasian	HCC	HB	PCR-RFLP	45	87	90	47	93	82	0.04
George	2011	India	Caucasian	Prostate	HB	PCR-RFLP	48	98	13	104	92	34	0.07
Ling	2011	China	Asian	Lung	HB	PCR-RFLP	284	131	111	349	120	57	0.00
Mittal	2011	India	Caucasian	Bladder	HB	PCR-RFLP	95	92	25	121	94	35	0.02
Vinci	2011	Italy	Caucasian	Lung	HB	HRM	53	41	7	70	48	11	0.50
Zhou	2011	China	Asian	CSCC	HB	PCR-RFLP	134	84	8	223	71	15	0.01
Alshatwi	2012	Saudi	Caucasian	Breast	HB	TaqMan	30	62	8	45	40	15	0.23
Chu	2012	China	Asian	Oral	HB	PCR-RFLP	339	119	12	356	66	3	0.98
Kim	2012	Korea	Asian	HCC	HB	PCR-RFLP	109	47	3	120	74	7	0.28
Min	2012	Korea	Asian	CRC	HB	PCR-RFLP	292	142	12	334	154	14	0.45
Xiang	2012	China	Asian	HCC	HB	PCR-RFLP	36	40	24	106	71	23	0.04
Zhou	2012	China	Asian	HCC	HB	PCR-RFLP	141	41	4	371	100	12	0.10
Ahn	2013	Korea	Asian	Gastric	HB	PCR-RFLP	323	123	15	299	134	14	0.83
Lv	2013	China	Asian	CRC	HB	PCR-RFLP	258	86	2	366	121	17	0.08
Shan	2013	China	Asian	HCC	HB	PCR-RFLP	128	37	7	123	48	14	0.01
Song	2013	USA	Caucasian	OSCC	HB	PCR-RFLP	184	141		214	121		0.49
Umar	2013	India	Caucasian	ESCC	HB	PCR-PFLP	155	122	12	149	140	20	0.09
Vinci	2013	Italy	Caucasian	CRC	HB	HRM	93	32	35	105	56	17	0.03
Wei	2013	China	Asian	ESCC	HB	MassARRAY	291	60	7	289	76	11	0.04
Wu	2013	China	Asian	Gastric	PB	PCR-RFLP	149	47	4	166	42	3	0.85
Zou	2013	China	Asian	HCC	HB	PCR-RFLP	136	44	5	123	48	14	0.01
Bansal	2014	India	Caucasian	Breast	HB	PCR-RFLP	80	30	11	106	43	15	0.00
Chu	2014	China	Asian	HCC	HB	PCR-RFLP	119	60	9	281	55	1	0.32
Du	2014	China	Asian	RCC	HB	TaqMan	251	94	9	255	96	11	0.59
Gutierrez-Camino	2014	Spain	Caucasian	ALL	HB	PCR-RFLP	138	56	6	206	117	24	0.19
Hasani	2014	Iran	Caucasian	ALL	HB	TARMS-PCR	35	28	12	61	42	12	0.25
Hou	2014	China	Asian	OSCC	HB	TaqMan	111	39	5	152	51	1	0.13
Hu	2014	China	Asian	CRC	HB	PCR-RFLP	157	49	5	282	81	10	0.16
Ma	2014	China	Asian	HCC	HB	MassARRAY	724	241	19	765	179	25	0.00
Omrani	2014	Iran	Caucasian	Breast	PB	TARMS-PCR	131	44	61	130	48	25	0.00
Pu	2014	China	Asian	Gastric	HB	PCR-RFLP	141	50	5	366	121	17	0.08
Qi	2014	China	Asian	HCC	HB	HRM	195	117	2	301	101	4	0.16
Wang	2014	China	Asian	HCC	HB	PCR-RFLP	98	32	22	218	62	24	0.00

*SCCHN squamous cell carcinoma of the head and neck, HCC hepatocellular carcinoma, CSCC cervical squamous cell carcinoma, CRC colorectal cancer, OSCC oral squamous cell carcinoma ESCC esophageal cancer, RCC renal cell carcinoma, All acute lymphoblastic leukemia, PB population-based, HB hospital-based, PCR-RFLP polymerase chain reaction-restriction fragment length polymorphism, HRM high-resolution melting analysis, TARMS-PCR tetra-primer amplification refractory mutation system-polymerase chain reaction

Table 2. Stratified Analyses of The Hsa-Mir-499 rs3746444 A>G Polymorphism on Cancer Risk.

Variable	Study, n	G *vs* A				GG *vs* AA				AG *vs* AA				GG *vs* AA+AG				AG+GG *vs* AA			
		OR (95% CI)	P	P_{BH}	P_h	OR (95% CI)	P	P_{BH}	P_h	OR (95% CI)	P	P_{BH}	P_h	OR (95% CI)	P	P_{BH}	P_h	OR (95% CI)	P	P_{BH}	P_h
Overall	38	1.12(1.02-1.23)[a]	0.01	0.07	0.00	1.12(0.92-1.35)	0.27	1.00	0.00	1.12(1.01-1.23)	0.03	0.14	0.00	1.07(0.89-1.30)	0.47	1.00	0.00	1.15(1.04-1.26)	0.01	0.03	0.00
Ethnicity																					
Asian	24	1.16(1.02-1.31)[a]	0.03	0.13	0.00	1.14(0.85-1.52)	0.39	1.00	0.00	1.16(1.03-1.31)	0.01	0.07	0.00	1.10(0.84-1.45)	0.49	1.00	0.00	1.18(1.03-1.34)[a]	0.01	0.07	0.00
Caucasian	14	1.05(0.94-1.18)	0.37	1.00	0.00	1.08(0.85-1.38)	0.51	1.00	0.01	1.03(0.88-1.22)	0.70	1.00	0.00	1.03(0.80-1.34)	0.81	1.00	0.00	1.08(0.94-1.24)	0.25	1.00	0.00
HWE																					
Yes	25	1.08(0.97-1.20)	0.14	0.70	0.00	1.00(0.80-1.24)	0.98	1.00	0.02	1.14(1.00-1.29)	0.04	0.22	0.00	0.95(0.76-1.18)	0.64	1.00	0.02	1.14(1.01-1.28)	0.04	0.21	0.00
No	13	1.18(1.01-1.39)	0.04	0.21	0.00	1.27(0.93-1.74)	0.13	0.65	0.00	1.09(0.92-1.28)	0.33	1.00	0.00	1.25(0.92-1.68)	0.15	0.77	0.00	1.17(0.99-1.38)	0.06	0.31	0.00
Cancer type																					
ALL	2	0.91(0.46-1.81)	0.80	1.00	0.01	0.81(0.18-3.69)	0.78	1.00	0.02	0.85(0.54-1.35)	0.22	1.00	0.20	0.83(0.22-3.20)	0.79	1.00	0.03	0.89(0.46-1.71)	0.72	1.00	0.06
Breast	5	1.19(0.98-1.46)	0.09	0.44	0.01	1.32(0.86-2.04)	0.20	1.00	0.02	1.12(0.91-1.38)	0.28	1.00	0.06	1.22(0.76-1.97)	0.41	1.00	0.00	1.19(0.98-1.45)	0.08	0.41	0.05
Lung	3	1.21(0.77-1.90)	0.42	1.00	0.00	1.30(0.59-2.85)	0.51	1.00	0.01	1.11(0.88-1.40)	0.37	1.00	0.19	1.26(0.61-2.59)	0.54	1.00	0.01	1.21(0.80-1.83)	0.37	1.00	0.00
HCC	10	1.19(0.93-1.54)	0.17	0.85	0.00	1.10(0.65-1.85)	0.73	1.00	0.00	1.21(0.94-1.55)	0.13	0.67	0.00	1.06(0.67-1.68)	0.81	1.00	0.00	1.23(0.93-1.61)	0.14	0.72	0.00
CRC	4	1.04(0.85-1.26)	0.72	1.00	0.14	0.90(0.36-2.26)	0.82	1.00	0.01	0.99(0.83-1.18)	0.89	1.00	0.38	0.91(0.34-2.45)	0.85	1.00	0.00	1.01(0.85-1.19)	0.94	1.00	0.89
SCCHN	4	1.28(0.75-2.18)	0.37	1.00	0.00	2.37(0.64-8.79)	0.20	0.99	0.03	1.16(0.65-2.06)	0.61	1.00	0.00	2.22(0.69-7.20)	0.18	0.91	0.05	1.26(0.82-1.93)	0.30	1.00	0.00
Gastric	4	1.03(0.90-1.18)	0.66	1.00	0.49	1.21(0.84-1.75)	0.32	1.00	0.66	0.98(0.83-1.15)	0.79	1.00	0.54	1.22(0.85-1.76)	0.29	1.00	0.66	1.01(0.86-1.17)	0.95	1.00	0.53
ESCC	2	0.80(0.66-0.98)	0.03	0.15	0.75	0.60(0.33-1.08)	0.09	0.44	0.88	0.81(0.64-1.04)	0.10	0.52	0.80	0.64(0.36-1.15)	0.13	0.66	0.93	0.79(0.62-1.00)	0.05	0.25	0.83
Other	5	1.14(1.00-1.31)[a]	0.05	0.23	0.36	0.98(0.71-1.36)	0.90	1.00	0.76	1.42(1.04-1.96)[a]	0.03	0.15	0.01	0.82(0.59-1.15)	0.19	0.97	0.34	1.33(1.03-1.72)[a]	0.03	0.15	0.05

*OR odds ratio, CI confidence interval, P *p* value of Z-test for pooled OR, PBH *p* value adjusted for multiple testing using Benjamini–Hochberg method, Ph P-value of heterogeneity test, ALL acute lymphoblastic leukemia, HCC hepatocellular carcinoma, CRC colorectal cancer, SCCHN squamous cell carcinoma of the head and neck, ESCC esophageal cancer, [a] The significant association without Benjamini–Hochberg correction disappeared after removing the studies not in Hardy-Weinberg equilibrium. Values in bold font indicate statistical significance before Benjamini–Hochberg correction (P<0.05).

Publication bias

Funnel plot and Egger's test were used to assess the publication bias of included studies. The graphical funnel plots for all genetic models appeared to be symmetrical. Then, Egger's test was used to provide statistical evidence of funnel plot symmetry. The results still did not show any evidence of publication bias in the overall meta-analysis (G *vs* A: t= 0.89, P= 0.38; GG *vs* AA: t= -1.06, P= 0.30; AG *vs* AA: t= 1.15, P= 0.26; GG *vs* AG+AA: t= -1.29, P= 0.20; AG+GG *vs* AA: t= 1.34, P= 0.19).

Discussion

The role of SNPs in miRNAs and the influence on cancer susceptibility have attracted much attention. Recently, the role of mutation presenting in hsa-miR-499 gene in the etiology of cancer development have drawn increasing attention. An important SNP (rs3746444) located in the seed site (nucleotides 2-8) of hsa-miR-499 has been extensively studied. The seed site at the 5' end of the miRNA is important in miRNA-mRNA binding (Lewis et al., 2005), which may be affected by the SNP thus influencing cancer susceptibility. Up to present, many epidemiologic studies have explored the role of this SNP in cancer risk, but the results of these studies remain conflicting rather than conclusive. Therefore, we performed a meta-analysis of the previously published researches to derive a more precise estimation of the association between hsa-mir-499 rs3746444 A>G polymorphism and cancer risk.

The summary results, as derived from thirty-eight case-control studies, indicated that hsa-mir-499 rs3746444 G allele showed little harmful effect on cancer risk. When limiting the meta-analysis to the 25 studies conforming to HWE, however, this significance association no longer existed (Table 2). Deviation from HWE can be due to laboratory/genotyping errors, population stratification, selection bias in the choice of controls and confounding factors unaccounted for (Zintzaras, 2010). Currently, no consensus is achieved for whether or not to include the studies departing from HWE. But if the results are different before and after removing studies not in HWE, it is suggested that the analysis without studies not conforming to HWE would be more valid (Thakkinstian et al., 2005). When stratified analysis by ethnicity, cancer type, or HWE in controls, no significance association was found in any subgroup after adjustment using BH method. Although a number of meta-analyses have explored the association between hsa-mir-499 rs3746444 A>G polymorphism and cancer risk, none of them adjusted *p* value for multiple tests. The statistical significance results found in the former meta-analyses might be obtained by accident as a result of multiple

comparisons. Taking account of all the above-mentioned aspects, we concluded that hsa-mir-499 rs3746444 A>G polymorphism was not associated with risk of cancer based on current evidence.

Some limitations likely affect the objectivity of the conclusions and they should be considered when interpreting the results. First, there is significant heterogeneity among included studies. Although sources of heterogeneity were explored by subgroup analysis and meta-regression, the results showed that sample size, ethnicity, HWE in controls, cancer type, publication year, source of controls and genotyping method did not contribute to the source of heterogeneity. Second, the effect of gene-gene and gene-environment interactions was not addressed in the analysis. Third, in the subgroup analysis, the number of each subgroup was relatively small, not having enough statistical power to explore the real association. Furthermore, the data was not stratified by age, nutrient intake and other suspected factors. Only based well-designed studies with the above factors taken into account, a better, comprehensive understanding of the relationship between the rs3746444 polymorphism and cancer risk is obtained.

In conclusion, our meta-analysis suggests that hsa-mir-499 rs3746444 A>G polymorphism was not associated with risk of cancer based on current evidence. Regarding the significant heterogeneity among included studies, large well-designed epidemiological studies will be necessary to validate the risk identified in the current meta-analysis. Moreover, further studies estimating the effect of gene-environment interactionsmay eventually provide a better, comprehensive understanding of the associations between the hsa-mir-499 rs3746444 polymorphism and cancer risk.

References

Ahn DH, Rah H, Choi YK, et al (2013). Association of the miR-146aC>G, miR-149T>C, miR-196a2T>C and miR-499A>G polymorphisms with gastric cancer risk and survival in the Korean population. *Mol Carcinog*, **52**, 39-51.

Akkiz H, Bayram S, Bekar A, et al (2011). 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 Pac J Cancer Prev*, **12**, 3107-12.

Alshatwi AA, Shafi G, Hasan TN, et al (2012). Differential expression profile and genetic variants of microRNAs sequences in breast cancer patients. *PLoS One*, **7**, 30049.

Anglicheau D, Muthukumar T, Suthanthiran M (2010). MicroRNAs: small RNAs with big effects. *Transplantation*, **90**, 105-12.

Bansal C, Sharma KL, Misra S, et al (2014). Common genetic variants in pre-microRNAs and risk of breast cancer in the North Indian population. *Ecancermedicalscience*, **8**, 473.

Benjamini Y, Hochberg Y (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B*, **57**, 289-300.

Catucci I, Yang R, Verderio P, et al (2010). Evaluation of SNPs in miR-146a, miR196a2 and miR-499 as low-penetrance alleles in German and Italian familial breast cancer cases. *Hum Mutat*, **31**, 1052-7.

Chu YH, Hsieh MJ, Chiou HL, et al (2014). MicroRNA gene polymorphisms and environmental factors increase patient susceptibility to hepatocellular carcinoma. *PLoS One*, **9**, 89930.

Chu YH, Tzeng SL, Lin CW, et al (2012). Impacts of microRNA gene polymorphisms on the susceptibility of environmental factors leading to carcinogenesis in oral cancer. *PLoS One*, **7**, 39777.

Du M, Lu D, Wang Q, et al (2014). Genetic variations in microRNAs and the risk and survival of renal cell cancer. *Carcinogenesis*, **35**, 1629-35.

Egger M, Davey Smith G, Schneider M, et al (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ*, **315**, 629-34.

George GP, Gangwar R, Mandal RK, et al (2011). Genetic variation in microRNA genes and prostate cancer risk in North Indian population. *Mol Biol Rep*, **38**, 1609-15.

Gutierrez-Camino A, Lopez-Lopez E, Martin-Guerrero I, et al (2014). Noncoding RNA-related polymorphisms in pediatric acute lymphoblastic leukemia susceptibility. *Pediatr Res*, **75**, 767-73.

Hasani SS, Hashemi M, Eskandari-Nasab E, et al (2014). A functional polymorphism in the miR-146a gene is associated with the risk of childhood acute lymphoblastic leukemia: a preliminary report. *Tumour Biol*, **35**, 219-25.

He L, Hannon GJ (2004). MicroRNAs: small RNAs with a big role in gene regulation. *Nat Rev Genet*, **5**, 522-31.

Hou YY, Lee JH, Chen HC, et al (2014). The association between miR-499a polymorphism and oral squamous cell carcinoma progression. *Oral Dis*,

Hu X, Li L, Shang M, et al (2014). Association between microRNA genetic variants and susceptibility to colorectal cancer in Chinese population. *Tumour Biol*, **35**, 2151-6.

Hu Z, Liang J, Wang Z, et al (2009). Common genetic variants in pre-microRNAs were associated with increased risk of breast cancer in Chinese women. *Hum Mutat*, **30**, 79-84.

Hu Z, Zhao Y, Tian T, et al (2009b). Association between microRNA polymorphisms, expressions, lung cancer development and prognosis. *Biomedicine Pharmacotherapy*, **63**, 322.

Hwang SG, Kim W, Rim KS, et al (2010). Association of genetic polymorphisms of microrna with risk of hepatocellular carcinoma. *Hepatology*, **52**, 1189-90.

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

Kupcinskas J, Wex T, Steponaitiene R, et al (2012). Gene polymorphisms of micrornas in helicobacter pyloriinduced high risk atrophic gastritis and gastric cancer. *Helicobacter*, **17**, 67.

Kutanzi KR, Yurchenko OV, Beland FA, et al (2011). MicroRNA-mediated drug resistance in breast cancer. *Clin Epigenetics*, **2**, 171-85.

Lewis BP, Burge CB, Bartel DP (2005). Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell*, **120**, 15-20.

Ling X, Li Y, Yang L, et al (2011). Genetic variant in seed region of hsa-miR-499-3p(rs3746444 A>G) increases risk of lung cancer. *Chin J Public Health*, **27**,

Liu Z, Li G, Wei S, et al (2010). Genetic variants in selected pre-microRNA genes and the risk of squamous cell carcinoma of the head and neck. *Cancer*, **116**, 4753-60.

Lv M, Dong W, Li L, et al (2013). Association between genetic variants in pre-miRNA and colorectal cancer risk in a Chinese population. *J Cancer Res Clin Oncol*, **139**, 1405-10.

Ma XP, Zhang T, Peng B, et al (2013). Association between microRNA polymorphisms and cancer risk based on the findings of 66 case-control studies. *PLoS One*, **8**, 79584.

Ma Y, Wang R, Zhang J, et al (2014). Identification of miR-423 and miR-499 polymorphisms on affecting the risk of

hepatocellular carcinoma in a large-scale population. *Genet Test Mol Biomarkers*, **18**, 516-24.
Min KT, Kim JW, Jeon YJ, et al (2012). Association of the miR-146aC>G, 149C>T, 196a2C>T and 499A>G polymorphisms with colorectal cancer in the Korean population. *Mol Carcinog*, **51**, 65-73.
Mittal RD, Gangwar R, George GP, et al (2011). Investigative role of pre-microRNAs in bladder cancer patients: a case-control study in North India. *DNA Cell Biol*, **30**, 401-6.
Okubo M, Tahara T, Shibata T, et al (2010). Association between common genetic variants in pre-microRNAs and gastric cancer risk in Japanese population. *Helicobacter*, **15**, 524-31.
Omrani M, Hashemi M, Eskandari-Nasab E, et al (2014). hsa-mir-499 rs3746444 gene polymorphism is associated with susceptibility to breast cancer in an Iranian population. *Biomark Med*, **8**, 259-67.
Pu JY, Dong W, Zhang L, et al (2014). No association between single nucleotide polymorphisms in pre-mirnas and the risk of gastric cancer in Chinese population. *Iran J Basic Med Sci*, **17**, 128-33.
Qi JH, Wang J, Chen J, et al (2014). High-resolution melting analysis reveals genetic polymorphisms in MicroRNAs confer hepatocellular carcinoma risk in Chinese patients. *BMC Cancer*, **14**, 643.
Raudenbush SW (2009). Analyzing effect sizes: Random-effects models. *Handbook Research Synthesis Meta-Analysis*, **2**, 295-316.
Shan YF, Huang YH, Chen ZK, et al (2013). miR-499A>G rs3746444 and miR-146aG>C expression and hepatocellular carcinoma risk in the Chinese population. *Genet Mol Res*, **12**, 5365-71.
Sharp S (1998). sbe23: Meta-analysis regression. *Stata Technical Bulletin*, **42**, 16-22.
Siegel R, Ma J, Zou Z, et al (2014). Cancer statistics, 2014. *CA Cancer J Clin*, **64**, 9-29.
Song X, Sturgis EM, Liu J, et al (2013). MicroRNA variants increase the risk of HPV-associated squamous cell carcinoma of the oropharynx in never smokers. *PLoS One*, **8**, 56622.
Srivastava K, Srivastava A, Mittal B (2010). Common genetic variants in pre-microRNAs and risk of gallbladder cancer in North Indian population. *J Hum Genet*, **55**, 495-9.
Thakkinstian A, McElduff P, D'Este C, et al (2005). A method for meta-analysis of molecular association studies. *Stat Med*, **24**, 1291-306.
Tian T, Shu Y, Chen J, et al (2009). A functional genetic variant in microRNA-196a2 is associated with increased susceptibility of lung cancer in Chinese. *Cancer Epidemiol Biomarkers Prev*, **18**, 1183-7.
Umar M, Upadhyay R, Prakash G, et al (2013). Evaluation of common genetic variants in pre-microRNA in susceptibility and prognosis of esophageal cancer. *Mol Carcinog*, **52**, 10-8.
Viechtbauer W (2005). Bias and efficiency of meta-analytic variance estimators in the random-effects model. *J Educational Behavioral Statistics*, **30**, 261-93.
Vinci S, Gelmini S, Mancini I, et al (2013). Genetic and epigenetic factors in regulation of microRNA in colorectal cancers. *Methods*, **59**, 138-46.
Vinci S, Gelmini S, Pratesi N, et al (2011). Genetic variants in miR-146a, miR-149, miR-196a2, miR-499 and their influence on relative expression in lung cancers. *Clin Chem Lab Med*, **49**, 2073-80.
Wang XH, Wang FR, Tang YF, et al (2014). Association of miR-149C>T and miR-499A>G polymorphisms with the risk of hepatocellular carcinoma in the Chinese population. *Genet Mol Res*, **13**, 5048-54.
Wei J, Zheng L, Liu S, et al (2013). MiR-196a2 rs11614913 T > c polymorphism and risk of esophageal cancer in a Chinese population. *Hum Immunol*, **74**, 1199-205.
Wu XJ, Mi YY, Yang H, et al (2013). Association of the hsa-mir-499 (rs3746444) polymorphisms with gastric cancer risk in the Chinese population. *Onkologie*, **36**, 573-6.
Xiang Y, Fan S, Cao J, et al (2012). Association of the microRNA-499 variants with susceptibility to hepatocellular carcinoma in a Chinese population. *Mol Biol Rep*, **39**, 7019-23.
Zhou B, Wang K, Wang Y, et al (2011). Common genetic polymorphisms in pre-microRNAs and risk of cervical squamous cell carcinoma. *Mol Carcinog*, **50**, 499-505.
Zhou J, Lv R, Song X, et al (2012). Association between two genetic variants in miRNA and primary liver cancer risk in the Chinese population. *DNA Cell Biol*, **31**, 524-30.
Zintzaras E (2010). Impact of Hardy-Weinberg equilibrium deviation on allele-based risk effect of genetic association studies and meta-analysis. *Eur J Epidemiol*, **25**, 553-60.
Zou HZ, Zhao YQ (2013). Positive association between miR-499A>G and hepatocellular carcinoma risk in a Chinese population. *Asian Pac J Cancer Prev*, **14**, 1769-72.