Association of microRNA-499 rs3746444 Polymorphism with Cancer Risk: Evidence from 7188 Cases and 8548 Controls

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Abstract

Background: Owing to inconsistent and inconclusive results, we performed a meta-analysis to derive a more precise estimation of the association between miR-499 rs3746444 polymorphism and cancer risk.

Methodology/Principal Findings: A systematic search of the Pubmed, Excerpta Medica Database (Embase) and Chinese Biomedical Literature Database (CBM) databases was performed with the last search updated on May 6, 2012. The odds ratio (OR) and its 95% confidence interval (95%CI) were used to assess the strength of the association. A total of 15 independent studies including 7,188 cases and 8,548 controls were used in the meta-analysis. In the present meta-analysis, we found a significant association between miR-499 rs3746444 polymorphism and cancer risk in the overall analysis (G versus A: OR=1.10, 95%CI 1.01–1.19, P=0.03; GG+AG versus AA: OR=1.15, 95%CI 1.02–1.30, P=0.02; GG versus AG+AA: OR=1.07, 95%CI 0.89–1.28, P=0.50; GG versus AA: OR=1.13, 95%CI 0.98–1.31, P=0.09; AG versus AA: OR=1.16, 95%CI 1.02–1.33, P=0.03). In the subgroup analysis by ethnicity, miR-499 rs3746444 polymorphism was significantly associated with cancer risk in Asian population. In the subgroup analysis by cancer types, miR-499 rs3746444 polymorphism was significantly associated with breast cancer.

Conclusions/Significance: This meta-analysis suggests a significant association between miR-499 rs3746444 polymorphism and cancer risk. Large-scale and well-designed case-control studies are necessary to validate the risk identified in the present meta-analysis.

Introduction

Cancer remains a major cause of mortality worldwide [1]. Based on a new edition of the World Cancer Report from the International Agency for Research on Cancer, about 12.7 million cancer cases and 7.6 million cancer deaths are estimated to have occurred in 2008 [2]. So far, much remains to be learned about the mechanism of carcinogenesis. The increased incidence rate and mortality rate lead researchers to speculate that dietary, infectious, cultural, environmental and/or genetic factors might be implicated in the etiology of the disease. Especially, there is clear evidence that genetic factors play an important role in individual predisposition to cancer [3].

MicroRNAs (miRNAs) are a subset of short, endogenous non-coding RNAs that regulate gene expression at the post-transcriptional level via either translational repression or mRNA degradation [4]. MiRNAs are considered as key regulatory element in gene expression networks, which can influence many biological processes including cell differentiation, proliferation, apoptosis and tumorigenesis [5]. Single nucleotide polymorphism (SNP) is the most common type of genetic variation in human genome. SNPs residing within the miRNA genes could potentially alter various biological processes by influencing the miRNA biogenesis and altering target selection [6]. Furthermore, previous studies have demonstrated that altered expressions of miRNAs play critical roles in cancer development [7–8]. Thus, SNPs in miRNAs may in turn influence the individual susceptibility to cancers.

An important polymorphism in the miR-499 with an A to G change (rs3746444) was identified. The miR-499 rs3746444 polymorphism involves an A>G nucleotide substitution which leads to a change from A:U pair to G:U mismatch in the stem structure of miR-499 precursor [9]. To date, a number of case-control studies have been conducted to investigate the association between this polymorphism and cancer risk in diverse populations and multiple types of cancer [9–22]. However, these reported results were inconsistent and inconclusive. As far as we know, there is no meta-analysis aimed at investigating the association of miR-499 rs3746444 polymorphism with cancer risk. Hence, we performed a meta-analysis to derive a more precise estimation of the association to help us better understand the relationship between this polymorphism and cancer risk.
Materials and Methods

Identification of eligible studies
To examine the association between miR-499 rs3746444 polymorphism and cancer risk, a systematic search of the US National Library of Medicine’s Pubmed database, Excerpta Medica Database (Embase) and Chinese Biomedical Literature Database (CBM) was performed with the last search updated on May 6, 2012. Keywords used in searches included: “microRNA OR mir OR miRNA”, “cancer OR carcinoma tumor OR neoplasma”, “gene OR polymorphism OR allele OR variation”, and “499 OR rs3746444”. Searching was done without restriction on language or publication years.

Inclusion and exclusion criteria
The inclusion criteria were: 1) evaluation of miR-499 rs3746444 polymorphism and cancers; 2) a case-control design; 3) sufficient published data for estimating an odds ratio (OR) with 95% confidence interval (CI); 4) only full-text manuscripts were included. Exclusion criteria included: 1) duplication of the previous publications; 2) abstract, comment, review and editorial. When there were multiple publications from the same population, only the largest study was included. When a study reported the results on different ethnicities, we treated them as separate studies. When a study included subjects of different countries, we extracted data separately.

Data extraction
Information was carefully extracted from all eligible publications independently by two of the authors according to the inclusion criteria listed above. Disagreement was resolved by discussion between the two authors. If these two authors could not reach a consensus, then a third author was consulted to resolve the dispute. Articles identified for this meta-analysis included a case-control study and complete data, including the first author’s name, the subjects’ region/country, year of publication, cancer types, definition and numbers of cases and controls, allele as well as genotype frequencies in both case and control groups. Their reference lists were searched manually to identify additional eligible studies. If original genotype frequency data were unavailable in relevant articles, a request for additional data was sent to the corresponding author.

Statistical methods
We used the PRISMA checklist as protocol of the meta-analysis and followed the guideline (Table S1) [23]. Hardy-Weinberg equilibrium (HWE) was evaluated for each study using Chi-square test in control groups. \( P < 0.05 \) was considered representative of statistically significant in the study, and all the \( P \) values were two sided.

To examine the association between miR-499 rs3746444 polymorphism and cancers, we conducted analyses using the fixed effect models. Repeated random effects models were performed. When the Q-test of heterogeneity was not significant, we conducted analyses using the fixed effect models. The random effect models were conducted when we detected significant between-study heterogeneity.

Overall effects for meta-analysis. In the overall analysis, we found a significant association between miR-499 rs3746444 polymorphism and cancer risk in the allelic contrast, dominant model and heterozygote comparison (G versus A: \( OR = 1.10, 95\% CI 1.01–1.19, P = 0.03; \) GG+AG versus AA, recessive model (GG versus AG+AA), homzygote comparison (GG versus AA) and heterozygote comparison (GG versus AA), respectively. The significance of the pooled OR was determined by the \( \chi^2 \)-test. Heterogeneity among studies was assessed by using the Chi-square test based Q-statistic, and, when not statistically significant (based on \( P > 0.10 \)), a fixed-effects model (using the Mantel-Haenszel method) was used for the meta-analysis [24–25]. Otherwise, the random effect model (using the DerSimonian and Laird method) was used to estimate the summary OR and 95% CI [26]. Heterogeneity was also quantified by using the \( I^2 \)-squared statistic, \( I^2 = 100\% \times (Q-df)/Q \)

Evaluation of publication bias
Funnel plots were created to graphically display evidence of publication bias, in which the standard error of logarithm for OR was plotted against its OR. An asymmetric plot suggested a possible publication bias. Funnel plot asymmetry was further assessed by the method of Egger’s linear regression test [28]. The significance of the intercept was determined by the \( t \)-test (\( P < 0.05 \) was considered representative of statistically significant publication bias). The intercept \( a \) provides a measure of asymmetry, and the larger its deviation from zero the more pronounced the asymmetry.

Analyses were performed using the software Review Manager 4.2 (Cochrane Collaboration, http://www.cc-ims.net/RevMan/renotes.htm/) and Stata version 10 (StataCorp LP, College Station, Texas, USA). A \( P \) value less than 0.05 was considered statistically significant in the study, and all the \( P \) values were two sided.

Results

Characteristics of studies
There were 104 articles relevant to the searching words (Pubmed:27; Embase:60; CBM:17). The flow chart in Figure 1 summarizes the study selection process. Among these, 14 publications met the inclusion criteria [9–22]. In the study of Catucci et al. [20], the ORs were presented separately according to different countries, Germany and Italy. Therefore, we treated them as separate studies. Thus, a total of 15 independent studies including 7,108 cases and 9,548 controls were used in the meta-analysis. Table 1 lists the studies identified and their main characteristics. There were eleven studies of Asian descent [9–14,16,17,19,21–22] and four studies of Caucasian descent [15,18,20]. The results of Hardy-Weinberg equilibrium test for the distribution of the genotype in control population are shown in Table 1. The genotypes distribution in the controls in 11 of 15 studies was in agreement with HWE [9–12,16,18–22].

Main results
The main results of this meta-analysis and the heterogeneity test are shown in Table 2. We first analyzed the association in the overall population. Then in order to obtain the exact consequence of the relationship between miR-499 rs3746444 polymorphism and cancer susceptibility, stratified analyses by ethnicity and cancer types were performed. When the \( Q \)-test of heterogeneity was not significant, we conducted analyses using the fixed effect models. The random effect models were conducted when we detected significant between-study heterogeneity.

Subgroup analysis for ethnicity. Subgroup analysis was stratified by ethnicity. The meta-analysis included 11 studies (4,278 cases and 5,029 controls) in Asian population and 4 studies (2,910 cases and 3,519 controls) in Caucasian population.
In Asian population, miR-499 rs3746444 polymorphism was significantly associated with an increased cancer risk in all genetic models except for recessive model (G versus A: OR = 1.16, 95% CI 1.04–1.28, \( P = 0.005 \); GG+AG versus AA: OR = 1.25, 95% CI 1.08–1.45, \( P = 0.003 \); GG versus AG+AA: OR = 1.05, 95% CI 0.78–1.41, \( P = 0.75 \); GG versus AA: OR = 1.23, 95% CI 1.01–1.50, \( P = 0.04 \); AG versus AA: OR = 1.28, 95% CI 1.08–1.52, \( P = 0.004 \)). In Caucasian population, no significant association was observed between miR-499 rs3746444 polymorphism and cancer risk in any genetic model (G versus A: OR = 0.98, 95% CI 0.90–0.99).

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

| ID | Study          | Year | Ethnic group | Cancer type       | Sample size | P for HWE |
|----|----------------|------|--------------|-------------------|-------------|-----------|
| 1  | Alshatwi et al.[10] | 2012 | Asian        | Breast cancer     | 100         | 100       | 0.227     |
| 2  | Xiang et al. [9]  | 2012 | Asian        | Liver cancer      | 100         | 100       | 0.284     |
| 3  | Zhou et al. [11]  | 2012 | Asian        | Liver cancer      | 186         | 483       | 0.100     |
| 4  | Min et al. [12]   | 2011 | Asian        | Colorectal cancer | 446         | 502       | 0.453     |
| 5  | Mittal et al. [13] | 2011 | Asian        | Bladder cancer    | 212         | 250       | 0.020     |
| 6  | Zhou et al. [14]  | 2011 | Asian        | CSCC              | 226         | 309       | 0.005     |
| 7  | Akkiz et al. [15] | 2011 | Caucasian    | Liver cancer      | 222         | 222       | 0.036     |
| 8  | George et al. [16] | 2011 | Asian        | Prostate cancer   | 159         | 230       | 0.073     |
| 9  | Okubo et al. [17] | 2010 | Asian        | Gastric cancer    | 552         | 697       | 0.048     |
| 10 | Liu et al. [18]   | 2010 | Caucasian    | SCCHN             | 1109        | 1130      | 0.441     |
| 11 | Srivastava et al. [19] | 2010 | Asian        | Gallbladder cancer| 230         | 230       | 0.566     |
| 12 | Catucci et al. [20] | 2010 | Caucasian (Germany) | Breast cancer | 823         | 925       | 0.893     |
| 13 | Catucci et al. [20] | 2010 | Caucasian (Italy) | Breast cancer | 756         | 1242      | 0.250     |
| 14 | Tian et al. [21]  | 2009 | Asian        | Lung cancer       | 1058        | 1035      | 0.404     |
| 15 | Hu et al. [22]    | 2009 | Asian        | Breast cancer     | 1009        | 1093      | 0.057     |

*CSCC, cervical squamous cell carcinoma; SCCHN, squamous cell carcinoma of head and neck; HWE, Hardy-Weinberg equilibrium.

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In the present meta-analysis with 7,188 cases and 9,548 controls, we found a significant association between miR-499 rs3746444 polymorphism and cancer risk. In the subgroup analysis of Asian population, miR-499 rs3746444 polymorphism was significantly associated with an increased risk of liver cancer (OR = 1.29, 95%CI 0.89–1.87, P = 0.08; GG+AG versus AA: OR = 1.23, 95%CI 0.94–1.60, P = 0.12; GG versus AG+AA: OR = 1.34, 95%CI 0.97–1.85, P = 0.08; GG versus AA: OR = 1.56, 95%CI 0.69–3.48, P = 0.28; AG versus AA: OR = 1.15, 95%CI 0.86–1.52, P = 0.34).

### Evaluation of publication bias

Funnel plot and Egger’s test were performed to assess the publication bias of included studies. The results of Egger’s linear regression test are shown in Table 3. Egger’s test was used to provide statistical evidence of funnel plot symmetry. In the overall analysis, Egger’s test detected evidence of publication bias in Asian population for dominant model (P = 0.023) and heterozygote comparison (P = 0.008). In the subgroup analysis, Egger’s test only detected evidence of publication bias in Asian population for dominant model (P = 0.023) and heterozygote comparison (P = 0.019). The shape of the funnel plots revealed similar results.

### Discussion

In the present meta-analysis with 7,188 cases and 9,548 controls, we found a significant association between miR-499 rs3746444 polymorphism and cancer risk. In the subgroup analysis of Asian population, miR-499 rs3746444 polymorphism was significantly associated with an increased cancer risk. Similarly in the subgroup analysis of breast cancer, our data also indicated that this polymorphism might be a risk factor.

In recent few years, several meta-analyses have focused on genetic variants of miR-146a and miR-196a2 genes in the overall.
risk, some limitations should be addressed. Firstly, the results of these meta-analyses have all identified that the miR-196a2 C allele is a low-penetrant risk factor for cancer development, especially with breast cancer and in Asian populations [29,30,32,33]. This finding is similar to that of our meta-analysis, indicating that the two genetic variants (miR-196a2 rs1614913 and miR-499 rs3746444) may be functional polymorphisms with potential value in cancer development.

The SNP variation within the miRNA sequence may either weaken or reinforce the binding between miRNA and its target. Therefore, this would probably lead to a corresponding regulation in the target mRNA translation [5,34]. In a previous study carried out by Jazdzewski et al. [35], the data suggested that a common G/C polymorphism within the pre-miR-146a sequence decreased the generation of pre- and mature miR-146a expression, leading to less efficient inhibition of target genes, and contributed to the genetic predisposition to papillary thyroid carcinoma. Furthermore, it has been shown that aberrant expression of miRNA genes could influence the regulation of target genes and involved in tumorigenesis. Recent evidence showed that the cluster of miR-143 and miR-145 affected the risk of esophageal squamous cell carcinoma through regulating oncogenic Fasclin Homolog 1 (FSCN1) [36]. Alshatwi et al. [10] have explored miRNA expression levels in blood and found that miR-499 could discriminate breast cancer patients from healthy individuals in postmenopausal patients, which may represent novel biomarker. Based on the above reasons, it can be hypothesized that rs3746444 polymorphism could contribute to cancer risk.

In spite of the considerable efforts to explore the possible association between miR-499 rs3746444 polymorphism and cancer risk, some limitations should be addressed. Firstly, the results should be interpreted with caution as a result of obvious heterogeneity in some comparisons. Secondly, the controls for several studies did not conform to Hardy-Weinberg equilibrium expectations, which may distort the results. However, when these studies that had evidence of departure from HWE were excluded from the analysis, a significant association can still be observed. Thirdly, publication bias existed in some comparisons, which may potentially influence the results of our meta-analysis. Fourthly, lacking sufficient eligible studies limited our further stratified analysis on more types of cancer, such as lung cancer, colorectal cancer and gastric cancer. Fifthly, for each selected case-control study, our results were based on unadjusted estimates, whereas a more precise analysis could be performed if individual data were available.

In conclusion, our meta-analysis suggests a significant association between miR-499 rs3746444 polymorphism and cancer risk. In the future, large-scale and well-designed case-control studies are necessary to validate the risk identified in the present meta-analysis.

**Supporting Information**

**Table S1** Checklist of items to include in this meta-analysis.

(See Table S1 in the Supporting Information for detailed information.)

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**Author Contributions**

Conceived and designed the experiments: WF SGP ZYF. Performed the experiments: WF SGP ZYF. Analyzed the data: WF ZYF PFM. Contributed reagents/materials/analysis tools: ZYF LYY HL. Wrote the paper: WF SGP ZYF LYY HL PFM.

**References**

1. Kanavos P (2006) The rising burden of cancer in the developing world. Ann Oncol 17:viii15–viii23.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, et al. (2011) Global cancer statistics. CA Cancer J Clin 61:69–90.
3. Foulkes WD (2006) Inherited susceptibility to common cancers. N Engl J Med 359:2143–2153.
4. Cai Y, Yu X, Hu S, Yu J (2009) A brief review on the mechanisms of miRNA regulation. Genomics Proteomics Bioinformatics 7:147–154.
5. Pritchard CC, Cheng HH, Tewari M (2012) MicroRNA profiling: approaches and considerations. Nat Rev Genet 13:330–340.
6. Landi D, Moreno V, Guino E, Vodicka P, Pardini B, et al. (2011) Polymorphisms affecting micro-RNA regulation and associated with the risk of dietary-related cancers: a review from the literature and new evidence for functional role of rs17281995 (CD86) and rs1051690 (INSR), previously associated with colorectal cancer. Mutat Res 717:109–115.
7. Calin GA, Croce CM (2006) MicroRNA signatures in human cancers. Nat Rev Cancer 6:557–566.
8. Esquela-Kerscher A, Slack FJ (2006) Oncomirs – microRNAs with a role in cancer. Nat Rev Cancer 6:259–269.
9. Xiang Y, Fan S, Cao J, Huang S, Zhang LP (2012) Association of the miRNA-499 variants with susceptibility to hepatocellular carcinoma in a Chinese population. Mol Biol Rep 39:7019–7023.
10. Alshatwi AA, Shafi G, Hasan TN, Syed NA, Al-Hazzani AA, et al. (2012) Differential expression profile and genetic variants of microRNAs sequences in breast cancer patients. PLoS One 7:e30049.
11. Zhou J, Lv R, Song X, Li D, Hu X, et al. (2012) Association Between Two Genetic Variants in miRNA and Primary Liver Cancer Risk in the Chinese Population. DNA Cell Biol 31:524–530.

12. Min KT, Kim JW, Jeon YJ, Jang MJ, Chong SY, et al. (2011) Association of the miR-146aC>G, 149C>T, 196a2C>T, and 499A>G polymorphisms with colorectal cancer in the Korean population. Mol Carcinog doi: 10.1002/mc.21489.

13. Mittal RD, Gangwar R, George GP, Mittal T, Kapoor R (2011) Investigative role of pre-microRNAs in bladder cancer patients: a case-controlled study in North India. DNA Cell Biol 30:401–406.

14. Zhou B, Wang K, Wang Y, Xi M, Zhang Z, et al. (2011) Common genetic polymorphisms in pre-microRNAs and risk of cervical squamous cell carcinoma. Mol Carcinog 50:499–505.

15. Akkiz H, Bayram S, Bekar A, Akgo¨llu¨E , U¨ sku¨dar O (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 12:3107–3112.

16. George GP, Gangwar R, Mandal RK, Sankhwar SN, Mittal RD (2011) Genetic variation in microRNA genes and prostate cancer risk in North Indian population. Mol Biol Rep 38:1609–1615.

17. Okabo M, Tahara T, Shibata T, Yamashita H, Nakamura M, et al. (2010) Association between common genetic variants in pre-microRNAs and gastric cancer risk in Japanese population. Helicobacter 15:524–531.

18. Liu Z, Li G, Wei S, Niu J, El-Naggar AK, et al. (2010) Genetic variants in selected pre-microRNA genes and prostate cancer risk in the Chinese population. Cancer 116:4753–4760.

19. 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 55:495–499.

20. Cateuci I, Yang R, Verderio P, Pizzamiglio S, Heesen L, et al. (2010) Evaluation of SNPs in miR-146a, miR146a2 and miR-499 as low-penetration alleles in German and Italian familial breast cancer cases. Hum Mutat 31:E1052–1057.

21. Tian T, Shu Y, Chen J, Hu Z, Xu L, et al. (2009) A functional genetic variant in microRNA-196a2 is associated with increased susceptibility of lung cancer in Chinese. Cancer Epidemiol Biomarkers Prev 18:1183–1187.

22. Hu Z, Liang J, Wang Z, Tian T, Zhou X, 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.

23. Mober D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 6:e1000097.

24. Cochran WG (1954) The combination of estimates from different experiments. Biometrics 10:101–129.

25. Mantel N, Haenszel W (1959) Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 22:719–748.

26. DerSimonian R, Laird N (1986) Meta-analysis in clinical trials. Control Clin Trials 7:177–188.

27. Higgins JP, Thompson SG (2002) Quantifying heterogeneity in a meta-analysis. Stat Med 21:1539–1558.

28. Egger M, Davey SG, Schneider M, Minder C (1997) Bias in meta-analysis detected by a simple, graphical test. Br Med J 315:629–634.

29. Xu W, Xu J, Liu S, Chen B, Wang X, et al. (2011) Effects of common polymorphisms rs11614913 in miR-196a2 and rs2910164 in miR-146a on cancer susceptibility: a meta-analysis. PLoS One 6:e20471.

30. Chu H, Wang M, Shi D, Ma L, Zhang Z, et al. (2011) Has-miR-196a2 Rs11614913 polymorphism contributes to cancer susceptibility: evidence from 15 case-controlled studies. PLoS One 6:e18108.

31. Qin LX, He J, Wang MY, Zhang RX, Shi T, et al. (2011) The association between common genetic variant of microRNA-146a and cancer susceptibility. Cytokine 56:695–698.

32. Qin LX, Wang Y, Xia ZG, Xi B, Mao C, et al. (2011) miR-196a2 C allele is a low-penetrant risk factor for cancer development. Cytokine 56:589–592.

33. Wang F, Ma YL, Zhang P, Yang JJ, Chen HQ, et al. (2012) A genetic variant in microRNA-196a2 is associated with increased cancer risk: a meta-analysis. Mol Biol Rep 39:269–275.

34. Tan Z, Randall G, Fan J, Camoretti-Mercado B, Brockman-Schneider R, et al. (2007) Allele-specific targeting of microRNAs to HLA-G and risk of asthma. Am J Hum Genet 81:829–834.

35. Jazdzewski K, Murray EL, Franssila K, Jarzab B, Schoenberg DR, et al. (2008) Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. Proc Natl Acad Sci U S A 105:7269–7274.

36. Liu R, Liao J, Yang M, Sheng J, Yang H, et al. (2012) The cluster of miR-143 and miR-145 affects the risk for esophageal squamous cell carcinoma through co-regulating fascin homolog 1. PLoS One 7:e39308.