Comprehensive Review of Genetic Association Studies and Meta-Analyses on miRNA Polymorphisms and Cancer Risk

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Abstract

Background: MicroRNAs (miRNAs) are small RNA molecules that regulate the expression of corresponding messenger RNAs (mRNAs). Variations in the level of expression of distinct miRNAs have been observed in the genesis, progression and prognosis of multiple human malignancies. The present study was aimed to investigate the association between four highly studied miRNA polymorphisms (mir-146a rs2910164, mir-196a2 rs11614913, mir-149 rs2292832 and mir-499 rs3746444) and cancer risk by using a two-sided meta-analytic approach.

Methods: An updated meta-analysis based on 53 independent case-control studies consisting of 27573 cancer cases and 34791 controls was performed. Odds ratio (OR) and 95% confidence interval (95% CI) were used to investigate the strength of the association.

Results: Overall, the pooled analysis showed that mir-196a2 rs11614913 was associated with a decreased cancer risk (OR = 0.846, P = 0.004; TT vs. CC) while other miRNA SNPs showed no association with overall cancer risk. Subgroup analyses based on type of cancer and ethnicity were also performed, and results indicated that there was a strong association between mir-146a rs2910164 and overall cancer risk in Caucasian population under recessive model (OR = 1.274, 95%CI = 1.096–1.481, P = 0.002). Stratified analysis by cancer type also associated mir-196a2 rs11614913 with lung and colorectal cancer at allelic and genotypic level.

Conclusions: The present meta-analysis suggests an important role of mir-196a2 rs11614913 polymorphism with overall cancer risk especially in Asian population. Further studies with large sample size are needed to evaluate and confirm this association.

Introduction

MicroRNAs (miRNAs) are a class of endogenous, small nonprotein-coding single-stranded RNA molecules of ~22 nucleotides in length that regulate a broad range of biologic and pathologic processes [1,2]. Mature miRNAs regulate the expression of approximately 30% of all human genes involved in fundamental biological processes at post-transcriptional level by sequence-specific binding to 3’ untranslated regions (UTRs) of multiple target messenger RNAs (mRNAs), leading to their degradation or translational suppression [3]. To date, more than 12000 miRNA sequences have been identified in humans, although specific functions have not yet been delineated for most of them. Cancer is eventually an outcome of chaotic expression of genes involved in developmental, cell growth and differentiation processes. Recent studies have implicated miRNAs in the genesis, progression (proliferation, migration and invasion) and prognosis of multiple human malignancies [4], including their key role in promoting cancer stem cell tumorigenicity [5]. Variations in the level of expression of distinct miRNAs (“Oncomirs”) have been observed in the development and progression of multiple human cancers and >50% of these miRNA genes are found to be located in cancer-related chromosomal regions functioning either as oncogenes or tumor suppressor genes [6–9]. Thus, variations in miRNA expression may promote carcinogenesis by modulating the expression patterns of essential genes involved in tumor growth and progression [10].

Single nucleotide polymorphisms (SNPs) are the most common form of variation present in the human genome. SNPs present in the miRNA gene regions can alter their expression and/or maturation leading to aberrant miRNA regulation. Many epidemiological studies have examined the association of SNPs in microRNAs with cancer susceptibility (Table 1). However, due to power considerations in single SNP studies with relatively small
### Table 1. Characteristics of eligible studies in meta-analysis.

| S.no. | Reference | Publication Year | Country origin | Ethnicity | Cancer Type | N (cases) | N (controls) | Control source | Genotyping method | HWE | Matching criteria |
|-------|-----------|------------------|----------------|-----------|-------------|-----------|--------------|----------------|-------------------|-----|------------------|
| 1     | Xu et al., [35] | 2008 | China | Asian | HCC | 479 | 504 | HB | PCR-RFLP | Yes | age/sex |
| 2     | Hu et al., [48] | 2009 | China | Asian | BC | 1009 | 1093 | PB | PCR-RFLP | Yes | age/area |
| 3     | Jazdzewski et al., [24] | 2008 | USA | Caucasian | PTC | 608 | 901 | PB | Sequencing | Yes | NR |
| 4     | Tian et al., [49] | 2009 | China | Asian | LC | 1058 | 1035 | PB | PCR-RFLP | Yes | age/sex/area |
| 5     | Ye et al., [23] | 2008 | USA | Caucasian | EC | 346 | 346 | HB | SNIPlex | Yes | age/sex |
| 6     | Catucci et al., [50] | 2010 | Italy | Caucasian | BC | 1894 | 2760 | PB | TaqMan and sequencing | Yes | age |
| 7     | Hoffman et al., [22] | 2009 | USA | Caucasian | BC | 441 | 479 | HB/PB | Sequenom MassARRAY | Yes | age |
| 8     | Peng et al., [51] | 2010 | China | Asian | GC | 213 | 213 | HB | PCR-RFLP | Yes | age/sex |
| 9     | Zhou et al., [52] | 2010 | China | Asian | CC | 703 | 713 | PB | PCR-RFLP | Yes | age/area |
| 10    | Yang et al., [53] | 2010 | Germany | Caucasian | BC | 1217 | 1422 | PB | TaqMan and sequencing | Yes | age |
| 11    | Xu et al., [54] | 2010 | China | Asian | PC | 251 | 280 | HB | PCR-RFLP | Yes | age |
| 12    | Qi et al., [55] | 2010 | China | Asian | HCC | 361 | 391 | HB | PCR-LDR | Yes | NR |
| 13    | Dou et al., [56] | 2010 | China | Asian | Glioma | 670 | 680 | HB | PCR-RFLP | Yes | age/sex/area |
| 14    | Xu et al., [57] | 2011 | China | Asian | HCC | 501 | 548 | PB | PCR-RFLP | Yes | age/sex/area |
| 15    | Kim et al., [58] | 2010 | Korea | Asian | LC | 654 | 640 | HB | PCR-FRET | Yes | age/sex |
| 16    | Christensen et al., [59] | 2010 | USA | Caucasian | HNSCC | 484 | 555 | PB | TaqMan | Yes | age/sex/area |
| 17    | Srivastava et al., [60] | 2010 | India | Caucasian | GBC | 230 | 230 | PB | PCR-RFLP | Yes | age/sex |
| 18    | Liu et al., [61] | 2010 | USA | Caucasian | HNSCC | 1109 | 1130 | HB | PCR-RFLP | Yes | age/sex |
| 19    | Zeng et al., [62] | 2010 | China | Asian | GC | 304 | 304 | HB | PCR-RFLP | Yes | age/sex |
| 20    | Sun et al., [63] | 2010 | China | Asian | GC | 304 | 304 | HB | PCR-RFLP | Yes | age/sex |
| 21    | Guo et al., [64] | 2010 | China | Asian | ESCC | 444 | 468 | HB | SNIPlex | Yes | age/sex/area |
| 22    | Yang et al., [65] | 2011 | Germany | Caucasian | BC | 2854 | 3188 | PB | MALDI-TOF mass spectrometry | Yes | age |
| 23    | Li et al., [66] | 2010 | China | Asian | HCC | 310 | 222 | HB | PCR-RFLP | Yes | NR |
| 24    | Okubo et al., [18] | 2010 | Japan | Asian | GC | 552 | 697 | HB | PCR-RFLP | Yes | NR |
| 25    | Chen et al., [67] | 2011 | China | Asian | CRC | 126 | 407 | HB | PCR-LDR | Yes | age/sex |
| 26    | Yue et al., [68] | 2011 | China | Asian | CC | 447 | 443 | HB | PCR-RFLP | Yes | age |
| 27    | Mittal et al., [69] | 2011 | India | Caucasian | UBC | 212 | 250 | HB | PCR-RFLP | Yes | age/sex |
| 28    | Zhou et al., [70] | 2011 | China | Asian | CC | 226 | 309 | HB | PCR-RFLP | Yes | age |
| 29    | Akkiz et al., [71] | 2011 | Turkey | Caucasian | HCC | 185 | 185 | HB | PCR-RFLP | Yes | age/sex/ smoking/alcohol |
| 30    | Zhan et al., [72] | 2011 | China | Asian | CRC | 252 | 543 | HB | PCR-RFLP | Yes | age/sex |
| 31    | Hong et al., [73] | 2011 | Korea | Asian | NSCLC | 406 | 428 | PB | TaqMan | Yes | age/sex |
| S.no. | Reference | Publication Year | Country of origin | Ethnicity | Cancer Type | N (cases) | N (controls) | Control source | Genotyping method | HWE | Matching criteria |
|-------|-----------|------------------|-------------------|-----------|-------------|-----------|--------------|-----------------|------------------|------|------------------|
| 32    | Permuth-Wey et al., [74] | 2011 | USA | Caucasian | Glioma | 593 | 614 | PB | Illumina's GoldenGate | Yes | NR |
| 33    | Akkiz et al., [75] | 2011 | Turkey | Caucasian | HCC | 222 | 222 | HB | PCR-RFLP | Yes | age/sex/smoking/alcohol |
| 34    | Zhu et al., [76] | 2011 | China | Asian | CRC | 573 | 588 | HB | TagMan | Yes | age/sex |
| 35    | Zhou et al., [25] | 2011 | China | Asian | HCC | 186 | 483 | HB | PCR-RFLP | Yes | NR |
| 36    | Schuetz et al., [77] | 2012 | Canada | Caucasian | NHL | 571 | 280 | PB | Illumina's GoldenGate | Yes | age/sex/area |
| 37    | Xiang et al., [26] | 2012 | China | Asian | HCC | 100 | 100 | HB | PCR-RFLP | Yes | NR |
| 38    | Jedlinski et al., [30] | 2011 | Australia | Caucasian | BC | 193 | 190 | HB | PCR-RFLP | Yes | age |
| 39    | Yang et al., [21] | 2011 | China | Asian | CRC | 746 | 746 | HB | SNPlex | Yes | age/sex |
| 40    | George et al., [19] | 2011 | India | Caucasian | PC | 159 | 230 | HB | PCR-RFLP | Yes | age/sex |
| 41    | Wang et al., [78] | 2010 | China | Asian | CRC | 458 | 489 | PB | SNPlex | Yes | age/sex/area |
| 42    | Zhang et al., [79] | 2011 | China | Asian | CRC | 302 | 513 | HB | PCR-RFLP | Yes | NR |
| 43    | Zhang et al., [80] | 2012 | China | Asian | BC | 252 | 248 | PB | PCR-RFLP | Yes | age/sex/area |
| 44    | Pastrello et al., [27] | 2010 | Italy | Caucasian | BC/OC | 401 | 155 | NR | Sequencing | Yes | NR |
| 45    | Vinci et al., [28] | 2011 | Italy | Caucasian | NSCLC | 101 | 129 | NR | HRMA | Yes | age/sex |
| 46    | Zhou et al., [81] | 2012 | China | Asian | HCC | 1686 | 1895 | HB | TagMan | Yes | age/sex |
| 47    | Kim et al., [29] | 2012 | Korea | Asian | CRC | 159 | 201 | PB | PCR-RFLP | Yes | NR |
| 48    | Smith et al., [82] | 2012 | Australia | Caucasian | BC | 193 | 193 | HB | HRMA | Yes | age/sex/ethnicity |
| 49    | Hishida et al., [83] | 2011 | Japan | Asian | GC | 583 | 540 | HB | PCR-CTPP | Yes | age/sex |
| 50    | Honkawa et al., [84] | 2008 | USA | Caucasian | CRC | 279 | 278 | PB | SNPlex | Yes | age/sex/ethnicity/residence |
| 51    | Lung et al., [85] | 2012 | China | Asian | NPC | 233 | 378 | PB | PCR-RFLP | Yes | age/sex |
| 52    | Chu et al., [20] | 2012 | Taiwan | Asian | OSCC | 470 | 425 | HB | PCR-RFLP | Yes | NR |
| 53    | Bae et al., [86] | 2012 | Korea | Asian | CRC | 417 | 404 | HB | TagMan | Yes | NR |

HCC: hepatocellular cancer; BC: breast cancer; GBC: gallbladder cancer; GC: gastric cancer; NSCLC: non-small cell lung carcinoma; CC: cervical cancer; LC: lung cancer; EC: esophageal cancer; PC: prostate cancer; HNSCC: head and neck squamous cell carcinoma; NHL: Non-Hodgkin lymphoma; OC: ovarian cancer; PTC: papillary thyroid carcinoma; NSCLC: non-small cell lung cancer; RCC: renal cell carcinoma; UBC: urinary bladder cancer; CRC: colorectal cancer; ESCC: esophageal squamous cell carcinoma; NPC: Nasopharyngeal Carcinoma; OSCC: oral squamous cell carcinoma; HWE: Hardy-Weinberg equilibrium; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; PCR-LDR: polymerase chain reaction–ligation detection reaction; PCR-FRET: polymerase chain reaction–fluorescent resonance energy transfer; HRMA: high-resolution melting analysis; PCR-CTPP: polymerase chain reaction with confronting two-pair primers; Tm-shift: Melting-temperature–shift allele-specific genotyping; HB: hospital based; PB: population based; NR: not reported; *Let7f-2 rs17276588 deviated from HWE in controls. **miR-499 rs2228930 and miR-149 rs2228932 deviated from HWE in controls. neither miR-499 rs2228930 and miR-149 rs2228932 deviated from HWE in controls. °miR-499 rs3746444 deviated from HWE in controls. †miR-196 rs1616413 and miR-468 rs290164 deviated from HWE in controls. ‡miRNA1-49 rs2228932 deviated from HWE in controls. doi:10.1371/journal.pone.0050966.t001
sample sizes, the outcomes of these studies remain contradictory rather than convincing. The present article applied a meta-analytic approach for relevant miRNA SNPs to better clarify potential associations between these SNPs and cancer. We also systematically reviewed published meta-analyses of observational studies investigating the association between miRNA polymorphisms and cancer risk to investigate their strengths and limitations.

**Methods**

**Publication Search**

We searched the PubMed, Medline and Embase databases using the search terms “miRNA,” “cancer/carcinoma,” and “polymorphism/variant” updated until August 25, 2012 and limited to English language papers. Identification of meta-analyses of association studies on miRNA polymorphisms and cancer was also carried out through a search of electronic databases of PubMed, Medline and Embase, up to August 2012. The Medical Subject Headings and key words used for the search were “miRNA”, “cancer”, “polymorphism”, and “meta-analysis” (with both synonymous and plural forms). The online searching was accompanied by checking reference lists from the identified articles and reviews for potentially eligible original reports.

**Inclusion and Exclusion Criteria**

All miRNA association studies were included in the present meta-analysis if they met the following criteria: 1) case-control study, 2) outcome cancer (histologically/pathologically proven), and 3) sufficient data for examining an odds ratio (OR) with 95% confidence interval (95% CI). The major exclusion criteria were as follows: 1) duplicate data, 2) case reports, series, abstract, comment, review and editorial and 3) insufficient data. Articles published in a language other than English were also excluded.

**Data Extraction**

From each study, information like: author, year of publication, country of origin, cancer type, ethnicity, number of cases and controls, source of control groups (study design) and genotyping method was extracted. In some cases, identical data were described in more than one publication; in such cases the secondary studies were not included in the meta-analysis. In a few studies, part of the data had already been reported elsewhere, therefore, only the novel data was included. We also checked for HWE in control subjects among all publications.

**Genotype and Allele Distributions**

Genotype distributions were extracted from the eligible publications for each polymorphism or computed from allele frequencies (if genotype frequencies were not reported) on the basis of sample size, assuming Hardy-Weinberg equilibrium (HWE).

**Methodological Quality Assessment**

The quality of selected studies was evaluated by scoring according to a set of predetermined criteria. The categories in scoring system used for assessing study quality are summarized in Table S1 [11]. Quality scores ranged from 0 to 10 and studies were scored as “good” if the score was 8–10, “fair” if the score was 5–7 and “poor” if the score was <4.

**Statistical Analysis**

In the present meta-analysis, we investigated the potential association between the variant allele of miRNA polymorphisms and cancer risk. Also, analysis between the heterozygote, the homozygote and also in dominant and recessive models was done to estimate cancer risk. Stratified analyses were performed by tumor site, ethnicity and source of controls (hospital or population based). Other potentially relevant sub-group analyses such as age, sex and cancer subgroup could not reliably be investigated due to limited data availability. Between-study heterogeneity was evaluated with a $\chi^2$-based Q-test among the studies [12]. Heterogeneity was considered significant when $P<0.05$. In case of no significant heterogeneity, point estimates and 95% CI was estimated using the fixed effect model (Mantel–Haenszel), otherwise, random effects model (DerSimonian Laird) was employed [13,14]. The significance of overall odds ratio (OR) was determined by the Z-test. A $\chi^2$ test with one degree of freedom was performed in controls to observe deviation from HWE. Publication bias was weighted by Begg’s funnel plot and Egger’s linear regression method with $P<0.05$ being considered statistically significant [15]. To assess the stability of the results, sensitivity analyses were performed. Each study in turn was removed from the total, and the remaining studies were reanalyzed. Moreover, sensitivity analysis was also performed, excluding studies whose allele frequencies in controls exhibited significant deviation from the HWE, given that the deviation may denote bias [16]. The type I error rate was fixed at 0.05. All the p values were two sided and all the statistical tests were implemented using the Comprehensive Meta-analysis software (Version 2.0, BIOSTAT, Englewood, NJ).

**Hardy–Weinberg Equilibrium Correction**

For evaluating impact of HWE-deviated studies on point estimates in genotype based contrasts, ORs were corrected by using the HWE-predicted genotype count in controls instead of the observed counts, as recommended by Trikalinos et al. [17]; thereafter, they were incorporated in the sensitivity analysis.

**Results**

**Study Characteristics**

Table 1 and Table S2 show the characteristics of eligible studies and genotype frequency distributions of studied miRNA SNPs included in the present meta-analysis. Fifty-three studies published between 2008 and August 2012 met our inclusion criteria with a total of 27573 cancer cases and 34791 controls. Two studies in Chinese language and 12 cohort studies were excluded from the present analysis. The total score of most studies was over 7 (Table S3). Thirty-two of the studies were conducted on subjects with Asian ethnicity (14689 cases/19894 controls) and 21 with Caucasian ethnicity (12884 cases/14997 controls). Malignancies were histologically or pathologically confirmed in 35 of the included studies, while in 11 studies it was not defined. Controls in 19 studies were population-based, while controls of 31 studies were hospital-based. Two studies included both population-based and hospital-based controls while another 2 studies did not report about the control source. Twelve out of 53 studies did not report on the matching criteria for controls while other studies recruited controls corresponding to cases by the age/sex/area. A classical polymerase chain reaction–restriction fragment length-polymerorphism (PCR–RFLP) method was adopted in 28 of the 53 studies. Seven studies used TaqMan assay; four studies used direct sequencing of the polymorphism; three studies used SNPlex; two studies used SNaPshot, Illumina’s GoldenGate, high-resolution melting analysis (HRMA) and polymerase chain reaction–ligation
Table 2). Significant heterogeneity was observed (Table 3). After the exclusion of the study by George et al. [19], whose genotypic distribution in controls deviated from HWE, the borderline significant association was lost (P = 0.066; allelic model). However, no association was found between genotype CC and cancer risk under the other models. Based on the ethnicity of study population, association was found in Asian populations under allelic and recessive models (Table 4). Removing low scoring studies did not alter the above obtained results \{18,19,25,26,28,29\} (Table S4c).

mir-149 rs2292832. Seven studies evaluated mir-149 rs2292832 and its association with cancer risk. The results of the overall meta-analysis did not suggest any association between rs2292832 and cancer susceptibility for all genetic models (Table 5). Exclusion of the study by Vinci et al. [31] and Kim et al., [29] with quality score of 2 did not altered the pooled estimate (Table S4d).

Through stratified analyses, no significant associations were found in any of the subgroups (racial descent, cancer types and study design) (Table 5).

The effect of some polymorphisms could not be evaluated due to the limited number of studies (mir-27a rs9595819 and mir-373 rs129983273 and rs10425222 = 3 studies; mir-100 rs1834306, mir-124-1 rs331564, mir-128 rs11134527, mir-155 rs229883 and rs2298903, mir-15a rs9354146 and rs2476391, mir-1792 rs17642969, mir-219 rs107822 and rs213210, mir-26a1 rs7372209, mir-30a rs1358379, mir-30c1 rs16827546, mir-335 rs3007348 and rs41272366, mir-423 rs650162, mir-499 rs2298030, mir-604 rs2368392, mir-608 rs4919510 and mir-631 rs57492532 = 2 studies; mir-618 rs2688218, mir-605 rs2043556, mir-34b/c rs4938723, mir-126 rs4636297, let-7i-2 rs17726388, let-7a3 rs731085, mir-101-2 rs7356140, mir-101-2 rs17803780 and rs12373841 and mir-338 rs62073058 = 1 study each).

Sensitivity Analysis

A single study involved in the meta-analysis was removed each time to reflect the influence of the individual data set to the pooled ORs for each of the studied miRNA polymorphisms. The corresponding pooled ORs were not significantly altered for any of the SNPs studied (Table S5a-d).

Publication Bias Analysis

Publication bias was assessed by performing funnel plot and Egger’s regression test under all models. For mir-149 rs2292832, because the number of included studies was small, we did not perform publication bias analysis. After combining all the cancer types, a little asymmetry was observed for mir-146a rs2910164, but the results of Egger’s regression test suggested no evidence for publication bias (Y axis intercept = -0.896, (95% CI) = -3.047 to 1.253; t = 0.859, p = 0.398 for allelic model) (Figure S1). Also, Begg and Mazumdar rank correlation test indicated absence of publication bias (P2tailed = 0.646). Similarly for mir-196a2 rs11614913 and mir-499 rs3746444, funnel plots were symmetrical and the Egger’s test for both models showed no significance, suggesting little evidence of publication bias (Figure S2 and S3).

mirRNAs and Cancer
| Variables          | n*  | Cases/Controls | C-allele vs. G-allele | CC vs. GG OR (95% CI) | CC vs. GG P<sub>het</sub> | CG vs. GG OR (95% CI) | CG vs. GG P<sub>het</sub> | Dominant (CC+CG vs. GG) OR (95% CI) | Dominant (CC+CG vs. GG) P<sub>het</sub> | Recessive (CC vs. CG+GG) OR (95% CI) | Recessive (CC vs. CG+GG) P<sub>het</sub> |
|-------------------|-----|---------------|----------------------|----------------------|--------------------------|----------------------|--------------------------|-------------------------------------|-------------------------------------|---------------------------------------|-------------------------------------|
| **Total**         | 27  | 12088/17340   | 0.963 (0.892–1.039)  | <0.001               |                          | 0.918 (0.777–1.086)  | <0.001                   | 1.008 (0.929–1.094)                | 0.008                               | 1.002 (0.919–1.092)                | <0.001                             |
| **Cancer type**   |     |               |                      |                      |                          |                      |                          | 1.034 (0.894–1.196)                | 0.001                               |                                      |                                    |
| Hepatocellular    | 5   | 1146/1510     | 1.102 (0.981–1.237)  | 0.242                | 0.764 (0.590–0.988)      | 0.313                | 0.126 (0.940–1.349)       | 0.229                               | 1.148 (0.939–1.403)                | 0.293                               |
| Breast            | 3   | 2669/3395     | 1.023 (0.945–1.107)  | 0.938                | 1.098 (0.912–1.322)      | 0.539                | 0.989 (0.887–1.103)       | 0.888                               | 1.007 (0.908–1.118)                | 0.955                               | 1.094 (0.920–1.300)                | 0.453                               |
| Other             | 19  | 8273/12435    | 0.934 (0.844–1.035)  | <0.001               | 0.913 (0.727–1.146)      | <0.001               | 0.999 (0.896–1.115)       | 0.002                               | 0.985 (0.877–1.106)                | <0.001                             | 1.009 (0.826–1.232)                | <0.001                             |
| **Ethnicity**     |     |               |                      |                      |                          |                      |                          | 1.002                               | 1.019                               | 1.002                                | 1.018                               |
| Asian             | 14  | 5914/10118    | 0.889 (0.785–1.007)  | <0.001               | 0.818 (0.650–1.029)      | <0.001               | 0.972 (0.891–1.060)       | 0.084                               | 0.931 (0.800–1.083)                | <0.001                             | 0.925 (0.770–1.112)                | <0.001                             |
| Caucasian         | 13  | 6174/7222     | 1.050 (0.993–1.111)  | 0.459                | 1.102 (0.886–1.370)      | <0.001               | 1.046 (0.974–1.124)       | 0.017                               | 1.055 (0.985–1.130)                | 0.114                               | 1.274 (1.096–1.481)                | 0.153                               |
| **Study design**  |     |               |                      |                      |                          |                      |                          | 1.002                               | 1.002                               | 1.002                                | 1.002                               |
| Population based  | 9*  | 5706/10226    | 1.015 (0.898–1.147)  | 0.379                | 1.185 (0.936–1.500)      | 0.008                | 1.070 (0.990–1.156)       | 0.051                               | 1.089 (1.012–1.172)                | 0.265                               | 1.331 (1.076–1.645)                | 0.002                               |
| Hospital based    | 17* | 6409/10606    | 0.893 (0.799–0.997)  | <0.001               | 0.809 (0.650–1.007)      | <0.001               | 0.950 (0.887–1.029)       | 0.117                               | 0.922 (0.813–1.044)                | 0.002                               | 0.922 (0.762–1.115)                | <0.001                             |

Random effects model was used when P value of Q for heterogeneity test (P<sub>het</sub>)<0.05; otherwise, fixed effect model was used.

*Number of studies involved.

*The study by Lung et al., [85] has both hospital based and population based controls.

OR: odds ratio; CI: confidence interval.

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| Variables              | n*   | Cases/Controls | T-allele vs. C-allele | TT vs. CC | TC vs. CC | Dominant (TT+TC vs. CC) | Recessive (TT vs. TC+CC) |
|------------------------|------|----------------|----------------------|----------|----------|------------------------|------------------------|
|                        |      |                | OR (95% CI)          | P_{meta} | OR (95% CI) | P_{meta}               | OR (95% CI)             | P_{meta}               | OR (95% CI) | P_{meta}               | OR (95% CI) | P_{meta}               | OR (95% CI) | P_{meta}               | OR (95% CI) | P_{meta}               | OR (95% CI) | P_{meta}               |
| Total                  | 30   | 13703/15439    | 0.941 (0.889–0.996)  | <0.001   | 0.846 (0.747–0.958) | <0.001   | 1.017 (0.934–1.106)    | 0.001                 | 0.972 (0.890–1.060) | <0.001   | 0.854 (0.778–0.939) | <0.001   |
| Cancer type            |      |                |                      |          |           |                        |                        |                        |          |                        |                        |          |                        |                        |
| Breast                 | 5    | 3449/4140      | 0.914 (0.804–1.040)  | 0.020    | 0.812 (0.607–1.085)  | 0.014    | 0.939 (0.850–1.037)    | 0.532                 | 0.911 (0.829–1.000) | 0.148    | 0.872 (0.703–1.080) | 0.027    |
| Lung                   | 4    | 2219/2232      | 0.893 (0.821–0.971)  | 0.149    | 0.793 (0.671–0.938)  | 0.259    | 0.927 (0.801–1.074)    | 0.059                 | 0.882 (0.768–1.013) | 0.075    | 0.842 (0.737–0.962) | 0.201    |
| Colorectal             | 3    | 951/1538       | 0.848 (0.754–0.954)  | 0.223    | 0.690 (0.543–0.876)  | 0.150    | 0.886 (0.719–1.091)    | 0.636                 | 0.813 (0.667–0.990) | 0.351    | 0.751 (0.621–0.909) | 0.209    |
| Hepatocellular         | 4    | 1015/999       | 0.862 (0.683–1.088)  | 0.019    | 0.744 (0.466–1.189)  | 0.022    | 0.897 (0.721–1.115)    | 0.631                 | 0.850 (0.692–1.043) | 0.190    | 0.809 (0.567–1.154) | 0.037    |
| Other                  | 14   | 6069/6530      | 0.994 (0.912–1.083)  | 0.001    | 0.915 (0.744–1.124)  | <0.001   | 1.125 (0.975–1.298)    | 0.001                 | 1.080 (0.941–1.240) | <0.001   | 0.873 (0.740–1.029) | <0.001   |
| Ethnicity              |      |                |                      |          |           |                        |                        |                        |          |                        |                        |          |                        |                        |
| Asian                  | 17   | 7718/8580      | 0.905 (0.845–0.969)  | 0.004    | 0.820 (0.699–0.963)  | <0.001   | 0.983 (0.866–1.116)    | 0.003                 | 0.923 (0.814–1.047) | 0.001    | 0.831 (0.748–0.923) | 0.006    |
| Caucasian              | 13   | 5985/6859      | 0.994 (0.908–1.088)  | 0.002    | 0.889 (0.729–1.084)  | 0.002    | 1.056 (0.944–1.183)    | 0.039                 | 1.033 (0.918–1.162) | 0.010    | 0.889 (0.741–1.067) | 0.002    |
| Study design           |      |                |                      |          |           |                        |                        |                        |          |                        |                        |          |                        |                        |
| Population based       | 12*  | 6520/7355      | 0.901 (0.833–0.975)  | 0.010    | 0.777 (0.651–0.928)  | 0.003    | 0.946 (0.877–1.021)    | 0.262                 | 0.905 (0.842–0.972) | 0.136    | 0.812 (0.693–0.952) | 0.001    |
| Hospital based         | 18*  | 7508/8421      | 0.945 (0.873–1.022)  | <0.001   | 0.845 (0.708–1.009)  | <0.001   | 1.032 (0.910–1.170)    | <0.001                | 0.994 (0.963–1.121) | <0.001   | 0.854 (0.755–0.965) | 0.002    |

Random effects model was used when P value of Q-test for heterogeneity test (P_{meta})<0.05; otherwise, fixed effect model was used.

*Number of studies involved.

*The study by Hoffman et al. [22] has both hospital based and population based controls.

OR: odds ratio; CI: confidence interval.

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Table 4. Meta-analysis of mir-499 rs3746444 polymorphism.

| Variables                  | n*  | Cases/Controls | C-allele vs. T-allele | CC vs. TT | CT vs. TT | Dominant (CC+CT vs. TT) | Recessive (CC vs. CT-TT) |
|----------------------------|-----|----------------|----------------------|----------|----------|------------------------|--------------------------|
|                            |     |                | OR (95% CI)          | OR (95% CI) | OR (95% CI) | OR (95% CI) | OR (95% CI) |
| Total                      | 14  | 7141/8479      | 1.130 (1.002–1.275) | <0.001   | 1.124 (0.964–1.310) | 0.055 | 1.177 (1.007–1.377) | <0.001 | 1.141 (0.985–1.322) | <0.001 | 1.091 (0.871–1.368) | 0.035 |
| Cancer type                |     |                |                      |          |          |                        |                          |
| Breast                     | 2   | 2588/3260      | 1.115 (0.878–1.417) | 0.017    | 1.257 (0.701–2.255) | 0.036 | 1.067 (0.952–1.196) | 0.163 | 1.079 (0.967–1.203) | 0.056 | 1.111 (0.869–1.421) | 0.050 |
| Hepatocellular             | 3   | 436/784        | 1.134 (0.641–2.006) | 0.001    | 1.245 (0.357–4.338) | 0.023 | 1.001 (0.762–1.314) | 0.074 | 1.116 (0.625–1.993) | 0.009 | 1.515 (0.839–2.734) | 0.062 |
| Other                      | 9   | 4117/4435      | 1.139 (0.976–1.330) | <0.001   | 1.074 (0.875–1.320) | 0.383 | 1.262 (0.992–1.606) | <0.001 | 1.175 (0.948–1.456) | <0.001 | 1.012 (0.828–1.238) | 0.130 |
| Ethnicity                  |     |                |                      |          |          |                        |                          |
| Asian                      | 8   | 3751/4343      | 1.227 (1.006–1.497) | <0.001   | 1.402 (0.941–2.088) | 0.037 | 1.210 (0.972–1.506) | <0.001 | 1.243 (0.994–1.554) | <0.001 | 1.357 (1.062–1.734) | 0.074 |
| Caucasian                  | 6   | 3390/4136      | 0.989 (0.916–1.067) | 0.343    | 0.976 (0.803–1.186) | 0.875 | 1.140 (0.895–1.451) | 0.001 | 0.967 (0.878–1.064) | 0.237 | 0.936 (0.773–1.132) | 0.394 |
| Study design               |     |                |                      |          |          |                        |                          |
| Population based           | 5   | 4026/4726      | 1.037 (0.961–1.119) | 0.021    | 1.098 (0.886–1.360) | 0.126 | 1.027 (0.935–1.128) | 0.217 | 1.036 (0.947–1.133) | 0.065 | 1.086 (0.879–1.342) | 0.200 |
| Hospital based             | 8   | 3014/3624      | 1.206 (0.926–1.570) | <0.001   | 1.246 (0.874–1.776) | 0.045 | 1.369 (1.017–1.844) | <0.001 | 1.360 (1.033–1.789) | <0.001 | 1.113 (0.763–1.624) | 0.016 |

Random effects model was used when P value of Q-test for heterogeneity test (P_{Het})<0.05; otherwise, fixed effect model was used.

*Number of studies involved.

OR: odds ratio; CI: confidence interval.

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| Variables | n* | Cases/Controls | T-allele vs. C-allele | TT vs. CC | TC vs. CC | Dominant (TT+TC vs. CC) | Recessive (TT vs. TC+CC) |
|-----------|----|----------------|----------------------|----------|----------|------------------------|-------------------------|
|           |    |                | OR (95% CI)          | P_Het    | OR (95% CI) | P_Het                  | OR (95% CI)              | P_Het                  |
| Total     | 7  | 4142/4242      | 0.994 (0.924–1.069)  | 0.345    | 1.000 (0.859–1.165) | 0.324                   | 0.982 (0.893–1.081)     | 0.526                   | 0.988 (0.902–1.083)     | 0.351                   | 1.041 (0.913–1.187)     | 0.333                   |
| Cancer type |    |                |                      |          |           |                        |                         |                         |                         |                         |                         |                         |
| Breast    | 2  | 1254/1322      | 0.949 (0.845–1.067)  | 0.167    | 0.931 (0.709–1.222) | 0.418                   | 0.919 (0.781–1.082)     | 0.106                   | 0.921 (0.789–1.077)     | 0.101                   | 0.971 (0.750–1.259)     | 0.737                   |
| Other     | 5  | 2888/2920      | 1.018 (0.938–1.103)  | 0.417    | 1.034 (0.860–1.243) | 0.205                   | 1.018 (0.904–1.146)     | 0.816                   | 1.025 (0.916–1.147)     | 0.591                   | 1.066 (0.915–1.241)     | 0.172                   |
| Ethnicity |    |                |                      |          |           |                        |                         |                         |                         |                         |                         |                         |
| Asian     | 5  | 2932/2983      | 0.991 (0.916–1.073)  | 0.418    | 1.003 (0.838–1.200) | 0.486                   | 0.968 (0.860–1.088)     | 0.314                   | 0.978 (0.875–1.094)     | 0.240                   | 1.056 (0.910–1.225)     | 0.499                   |
| Caucasian | 2  | 1210/1259      | 1.004 (0.887–1.136)  | 0.094    | 0.993 (0.742–1.328) | 0.061                   | 1.013 (0.858–1.197)     | 0.658                   | 1.009 (0.861–1.182)     | 0.297                   | 0.988 (0.746–1.310)     | 0.068                   |
| Study design |    |                |                      |          |           |                        |                         |                         |                         |                         |                         |                         |
| Population based | 4 | 2562/2558   | 0.996 (0.908–1.094)  | 0.324    | 0.980 (0.815–1.179) | 0.496                   | 0.979 (0.888–1.103)     | 0.275                   | 0.990 (0.883–1.110)     | 0.206                   | 1.088 (0.917–1.291)     | 0.409                   |
| Hospital based | 2 | 1579/1555    | 0.957 (0.853–1.074)  | 0.688    | 1.016 (0.670–1.539) | 0.234                   | 0.976 (0.826–1.154)     | 0.323                   | 0.960 (0.819–1.124)     | 0.363                   | 0.930 (0.753–1.149)     | 0.742                   |

Random effects model was used when P value of Q-test for heterogeneity test (P_Het)<0.05; otherwise, fixed effect model was used.

*a Number of studies involved.

OR: odds ratio; CI: confidence interval.

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Meta-analyses of Association Studies on miRNA SNPs

A cumulative meta-analysis was also done by sorting the studies in the sequence of largest to smallest, and analysis performed with the addition of each study. The point estimate of the study did not deviate with the addition of smaller studies, ruling out the possibility of publication bias for all the analyzed miRNA SNPs. [23, 24]

Meta-analyses of Association Studies on miRNA SNPs

Eleven meta-analyses published in 2011 and 2012 were retrieved, focusing on 2 miRNA polymorphisms (miR-146a rs2910164 and miR-196a2 rs11614913). Table 6 shows the main characteristics of individual meta-analyses included. The number of primary studies included in the meta-analyses ranged from 4 to 27 with the number of subjects included spanning from 3007 to 10569. The results of the published meta-analyses of the association between miRNA SNPs and cancer showed an overall statistically significant increased risk for mir-196a2 rs11614913 (variant C allele). In subgroup analysis, the increased risk was more prominent in digestive system cancers such as breast, colorectal and hepatocellular cancer. For mir-146a rs2910164, an overall analysis, no significant associations were found. However, in the stratified analysis, this polymorphism was associated with increased breast cancer risk among Europeans [32] and negatively associated with digestive system cancer [33]. The results are also consistent with the outcome from our present meta-analysis.

We also computed the population-attributable risk (PAR) to refer to the proportion of disease risk in Caucasians and Asians that can be attributed to the causal effects of the risk SNP (variant genotype). PAR can be assessed by using the formula [34]: PAR (%) = [(OR-1)/OR] \times 100\%, where OR is the pooled OR stratified for ethnicity and allele frequencies used for computing PAR were taken from the meta-analyses incorporating the largest number of individuals. The results showed mir-196a2 rs11614913 to be the most impacting polymorphism (which might account for approximately 15% among Asians) [PAR (%) mir-196a2 rs11614913 ‘T’ allele carriers: Asians = 14.9, Caucasians = 14.1]. Although the ORs and allele frequencies used for computing PAR were taken from the same ethnic group, the results could still be biased due to the difference in geographic areas and population stratification in individual studies. A more consistent estimation of the PAR requires additional statistics to identify population subgroups significantly affected by particular miRNA polymorphism.

Discussion

In the present study, we reviewed the available literature on genetic studies of miRNA SNPs in cancer and conducted four independent meta-analyses for association between overall cancer and mir-146a rs2910164, mir-196a2 rs11614913, mir-149

Table 6. Description of meta-analyses included in the systematic review.

| S. no. | Reference | Publication Year | Cancer Type | Cases/controls | miRNA | rs number | Phet* | OR* | 95% CI* |
|--------|-----------|------------------|-------------|----------------|--------|-----------|-------|-----|--------|
| 1      | Lian et al., [32] | 2012 | BC | 4238/4469 | miR-146a | rs2910164 | 0.757 | 1.16 | 0.98–1.36 |
| 2      | Guo et al., [11] | 2012 | HCC, CRC, GBC, PS2, OSCC, ESCC, GC | 4999/7606 | miR-196a2 | rs11614913 | 0.0003 | 1.38 | 1.13–1.67 |
| 3      | Xu et al., [41] | 2011 | BC, LC, PTC, HCC, GBC, HNSCC, PC, ESCC | 7183/7943 | miR-146a | rs2910164 | 0.03 | 0.89 | 0.75–1.05 |
| 4      | Wang et al., [87] | 2012 | LC, BC, GBC, Glioma, HCC, HNSCC | 7922/8849 | miR-196a2 | rs11614913 | 0.45 | 0.92 | 0.85–0.99 |
| 5      | Chu et al., [88] | 2011 | BC, ESCCC, LC, GC, HCC, GBC, PC, PTC, HNSCC, Glioma | 9341/10569 | miR-196a2 | rs11614913 | <0.001 | 1.22 | 1.04–1.44 |
| 6      | Gao et al., [89] | 2011 | BC | 3007/3718 | miR-146a | rs2910164 | 0.65 | 0.90 | 0.75–1.07 |
| 7      | Qiu et al., [90] | 2011 | BC, UBC, ESCC, OC, CGBC, GC, HCC, LC, PVC, HNSCC, Glioma | 10585/12183 | miR-146a | rs2910164 | <0.001 | 1.13 | 0.93–1.37 |
| 8      | Qiu et al., [91] | 2011 | BC, UBC, ESCC, OC, CGBCGC, GC, HCC, LC, PVC, HNSCC, Glioma, CRC | 10441/12353 | miR-196a2 | rs11614913 | <0.001 | 1.30 | 1.14–1.48 |
| 9      | Zhang et al., [92] | 2012 | LC, HCC, BC, CRC, GC, ESCC, GC, Glioma, UBC, PC | 10453/12075 | miR-196a2 | rs11614913 | <0.001 | 1.23 | 1.08–1.39 |
| 10     | Wang et al., [93] | 2012 | BC, GC, PC, UBC, CC, ESCC, OC, HCC, PTC, RCC, GBC | 10496/12885 | miR-146a | rs2910164 | 0.09 | 1.16 | 0.98–1.38 |
| 11     | Wang et al., [94] | 2012 | LC, GC, CRC, GBC, HCC, ESCC | 2394/2767 | miR-146a | rs2910164 | 0.02 | 1.17 | 0.95–1.44 |

BC: breast cancer; GBC: gallbladder cancer; GC: gastric cancer; LC: lung cancer; PTC: papillary thyroid carcinoma; ESCC: esophageal squamous cell carcinoma; HCC: hepatocellular cancer; CRC: colorectal cancer; OS2: oral squamous cell carcinoma; PSCC: pharynx squamous cancer. GC: ovarian cancer; CC: cervical cancer; RCC: renal cell cancer. *Homozygous wild vs. homozygous variant genotype.
One of the important concerns in every meta-analysis is publication bias. Because meta-analysis reviews quantitative data from numerous studies, the publication bias effect of the literature incorporated in the study can bias the meta-analytic outcome. In the present study, the funnel plot for overall results was symmetrical for all the analyzed miRNA SNPs, indicating negligible likelihood of publication bias. The Egger’s test and Begg and Mazumdar rank correlation test were also negative for publication bias. However, the possibility of publication bias cannot completely be ruled out [47]. Sensitivity analyses using HWE-adjusted ORs and corresponding variances also did not modify the results.

To the best of our knowledge, the present study is the most comprehensive meta-analysis to date to have assessed the relationship between the miRNA polymorphisms and cancer risk. Nevertheless, our meta-analysis had some limitations common to these types of studies. First, the present meta-analysis only included case-control studies, most of which were hospital based and excluded 12 cohort studies to avoid potential heterogeneity in comparing results. Thus, the controls may not reflect the representative element of the source population. Second, the difference in the geographic areas (environmental factors) and genetic backgrounds of the study cohort in each article could influence the results. Third, the low sample size in some of the included studies might influence the statistical power to better evaluate the association between miRNA polymorphisms and overall cancer, especially in subgroup analysis. Fourth, gene-gene and gene-environment interactions were not analyzed which might alter the associations between miRNA gene polymorphisms and cancer. Also, a more precise analysis stratified by variables such as age, sex etc. could not be performed due to limitations of the data which also restricted our ability to detect possible sources of heterogeneity.

In conclusion, the results of our meta-analysis demonstrate that mir-196a2 rs11614913 polymorphisms have significant associations with overall cancer risk, although some results are limited by the small number of studies. However, no significant association exists between mir-146a rs2910164, mir-499 rs3746444 and mir-149 rs2292832 and overall cancer. Further studies with a large sample size are needed to evaluate their association with cancer risk.

Supporting Information

Figure S1  Begg’s funnel plot of publication bias for mir-146a rs2910164. Log OR is plotted versus standard error of Log OR for each included study. Every circle dot represents a separate study for the indicated association (C versus G). (TIF)

Figure S2  Begg’s funnel plot of publication bias for mir-196a2 rs11614913. Log OR is plotted versus standard error of Log OR for each included study. Every circle dot represents a separate study for the indicated association (TT versus CC). (TIF)

Figure S3  Begg’s funnel plot of publication bias for mir-499 rs3746444. Log OR is plotted versus standard error of Log OR for each included study. Every circle dot represents a separate study for the indicated association (CC versus TT). (TIF)

Table S1  Scale for methodological quality assessment. (DOC)

Table S2  Genotype frequency distributions of miRNA SNPs studied in included studies. (DOC)
Table S3 | Quality scores for included studies. (DOC)

Table S4 | Meta-analysis of studied miRNA polymorphisms after removing low scoring studies (score ≤5). (DOC)

Table S5 | Sensitivity analysis result for studied miRNA polymorphisms. (DOC)

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Author Contributions

Conceived and designed the experiments: AS KS. Performed the experiments: AS KS. Analyzed the data: AS KS. Contributed reagents/materials/analysis tools: AS KS. Wrote the paper: AS KS.

Table S6 | Initial free energy (dG) predicted by mfold for the SNPs associated with the precursor and mature forms of human miRNAs. (DOC)
