A Genetic Variant in miR-196a2 Increased Digestive System Cancer Risks: A Meta-Analysis of 15 Case-Control Studies

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Abstract

Background: MicroRNAs (miRNAs) negatively regulate the gene expression and act as tumor suppressors or oncogenes in oncogenesis. The association between single nucleotide polymorphism (SNP) in miR-196a2 rs11614913 and the susceptibility of digestive system cancers was inconsistent in previous studies.

Methodology/Principal Findings: An updated meta-analysis based on 15 independent case-control studies consisting of 4999 cancer patients and 7606 controls was performed to address this association. It was found that miR-196a2 polymorphism significantly elevated the risks of digestive system cancers (CT vs. TT, OR = 1.25, 95% CI = 1.07–1.44; CC vs. TT, OR = 1.38, 95% CI = 1.13–1.67; CC/CT vs. TT, OR = 1.29, 95% CI = 1.10–1.50; CC vs. CT/TT, OR = 1.14, 95% CI = 1.01–1.30; C vs. T, OR = 1.15, 95% CI = 1.05–1.26). We also found that variant in miR-196a2 increased the susceptibility of colorectal cancer (CRC) (CT vs. TT, OR = 1.23, 95% CI = 1.04–1.44; CC vs. TT, OR = 1.32, 95% CI = 1.08–1.61; CC/CT vs. TT, OR = 1.25, 95% CI = 1.07–1.46; C vs. T, OR = 1.15, 95% CI = 1.05–1.28), while the association in recessive model (CC vs. CT/TT, OR = 1.16, 95% CI = 0.98–1.38) showed a marginal significance. Additionally, significant association between miR-196a2 polymorphism and increased risk of hepatocellular cancer (HCC) was detected. By stratifying tumors on the basis of site of origin, source of controls, ethnicity and allele frequency in controls, elevated cancer risks were observed.

Conclusion/Significance: Our findings suggest the significant association between miR-196a2 polymorphism and increased susceptibility of digestive system cancers, especially of CRC, HCC and Asians. Besides, C allele may contribute to increased digestive cancer risks.

Introduction

MicroRNAs (miRNAs) are endogenous, small non-coding and have a length of 18–25 nucleotides RNAs. miRNAs can interact with messenger RNAs (mRNAs) by binding to 3′ un-translated regions (UTRs) and lead to the degradation or translational repression of mRNAs. Studies revealed that miRNAs played key roles in various biological processes including cell growth regulation, differentiation, apoptosis and tumorigenesis [1,2,3]. miRNAs regulate approximately 30% of human genes and exhibit a remarkable contribution to carcinogenesis [2,4]. Aberrant modulation of specific miRNAs was considered to be a crucial event of diverse diseases including cancers [5] although the detailed process of miRNAs expression and mutation are still ambiguous. Moreover, some studies detected that miRNAs participated in the etiology, progression and prognosis of cancers, such as non-small cell lung cancer [6] and hepatocellular carcinoma [7]. Several possible mechanisms, including genetic and epigenetic alternations, have been proposed. SNPs in miRNAs are marked as novel genetic variations which may modify the cancer susceptibilities [8]. Genetic variant in miR-196a2 had been demonstrated to be associated with some cancer risks, but different studies showed conflicting associations. Meta-analysis on breast cancer, lung cancer and other cancers revealed that rs11614913 was a functional SNP and had potential ability to modify the cancer risks [9,10,11,12,13]. As we know, the above-referenced meta-analysis included gastric cancer (GC), HCC and other digestive cancers for the SNP in miR-196a2. However, by the limitation of inadequate publications, they did not calculate pooled ORs of digestive system cancers comprehensively. To improve the efficiency of meta-analysis on digestive cancers and reduce the potential between-study heterogeneity which might derive from various cancers in diverse systems, we focused on digestive system cancers only and added more recent publications on CRC [14,15,16] and HCC [17] in this study. We also contacted the authors to request for genotype frequencies about oral cavity squamous cancer (OSCC) and pharynx squamous cancer (PSCC) [18,19] which were not shown in published articles. In addition, an unpublished case-control study on CRC which was performed by Mingwu Zhang...
et al at the Molecular Epidemiology Laboratory in Zhejiang University School of Medicine was collected. Overall, 9 datasets from 7 studies (including 2875 cases with digestive cancers and 5556 controls) which had not been studied in previous meta-analysis were additionally included in our study. And we performed this meta-analysis focusing on the following issues: (a) What is the association between miR-196a2 polymorphism and the susceptibility of digestive system cancers, especially of colorectal cancer? (b) Would changes in tumor sites, demographic characteristics and other factors transform this association significantly?

Materials and Methods

Identification of eligible studies

A systematic search in PubMed was conducted using a retrieving query formulation “(microrna 196a2 OR rs11614913) polymorphisms cancer” (last search updated on 20 Aug, 2011). We also searched references in published articles and reviews on this topic in PubMed. Eligible studies were selected according to the following explicit inclusion criteria: (a) Study was designed using the methodology of a case-control study. (b) The association between miR-196a2 polymorphism and digestive system cancer risks was explored. (c) There was sufficient data for the computation of odds ratios and corresponding 95% confidence intervals (ORs, 95% CIs). (d) Cases with carcinomas were diagnosed by histopathology. Moreover, we also contacted some researchers to request unpublished study outcomes and detailed datasets for pooled calculation (Figure 1).

Data extraction

Two investigators (Guo and Jin) screened titles, abstracts and full texts independently using a standardized screening guide. Data extraction was carried out independently after the concealment of authors, journals, supporting organizations and funds to avoid investigators’ bias. After data abstraction, discrepancies and differences were resolved by consensus and discussion.

Characteristics of enrolled studies were assigned to the structured form (Table 1), including first author’s name, publication time, study country origin, ethnicity, cancer type, source of controls, genotyping method, matched criteria between cases and controls, sample size, C allele frequency in controls (Table S1), genotype frequency distribution and quality scores.

Methodological quality assessment

Three reviewers (Guo, Jin and Zhang) independently evaluated the quality of selected studies by scoring according to a set of predetermined criteria (Table S2) which was extracted and modified from previous studies [20,21,22]. Quality scores ranged from 0 to 10 and the studies with higher scores presented better quality. Disagreements were resolved by discussion.

Statistical analysis

Crude ORs and corresponding 95% CIs were calculated to investigate the association strength between miR-196a2 polymorphism and the susceptibility of digestive system cancers. Pooled ORs were obtained from combination of single studies by heterozygote comparison (CT vs. TT), homozygote comparison (CC vs. TT), dominant and recessive models (CC/CT vs. TT, CC vs. CT/TT), allelic comparison (C vs. T) respectively. We used chi-square-based Q-test [23] and the I² index [24] to check the heterogeneity among different studies. When Q-test showed the existence of notable heterogeneity (P-value less than 0.10 and/or I² index more than 50%), we used the random-effects model (DerSimonian and Laird method) [25]; otherwise, the fixed-effects model (Mantel and Haenszel method) was conducted [26].

Stratification by tumor site, source of controls, ethnicity and allele frequency in controls was conducted. All cancers were categorized into two groups: digestive tract cancer and digestive gland cancer. Eligible studies were classified into population-based and hospital-based according to control source. The subjects were classified by ethnicity into Caucasian group and Asian group. We also classified the selected studies into C>T (C allele frequency more than T allele frequency) group and C≤T (C allele frequency less than or equivalent to T allele frequency) group by allele frequency in controls.

Hardy-Weinberg equilibrium (HWE) in control population was judged by the chi-square test. P-value less than 0.05 was
Results

Studies characteristics

13 eligible studies including 12 published studies [14,15,16,17,18,19,32,34,35] and 1 unpublished one were collected in this meta-analysis according to the inclusion criteria. Characteristics of these studies were presented in Table 1 and the genotype frequency distribution was shown in Table S1.

Among studies on head and neck squamous cell carcinoma (HNSCC, which included oral, pharyngeal and laryngeal cancers) [18,19], laryngeal cancer in respiratory system was not used. We considered patients with oral cancer and pharyngeal cancer as separate groups and pooled them into quantitative analysis independently. Therefore, this meta-analysis employed 15 separate case-control studies, including 4999 cases and 7606 controls, for the polymorphism of miR-196a2.

12 studies were matched for age, sex and/or residence, smoking, alcohol consumption [14,15,16,17,18,19,32,34,35]; 9 studies collected Asians as subjects and the other 6 investigated Caucasians; C allele frequency of controls was the minor allele frequency (MAF) in 7 studies and T allele frequency was MAF in the 8 studies remained; controls in 10 studies were hospital-based and controls of the other studies were population-based; 11 studies described alimentary tract cancers and 4 studies focused on tumors in digestive glands. To dilute the potential confounding bias of HBV infection in the study of Qi et al [33], we kept the HBV patients without HCC as controls and the HBV patients with HCC as cases.

Genotypes in all studies were detected with genetic DNA from blood samples using 4 genotyping methods totally. 15 out of 15 studies checked genotypes for quality control. Genotype distribution of controls in all studies was consistent with HWE, except for Mingwu Zhang’s study on CRC.

Publication bias

We found no significant evidence of publication bias (P-value>0.05) in any comparison model using Egger’s linear regression method. Furthermore, the shape of funnel plot for the allele contrast (C vs. T) showed approximately symmetric and inverted funnel-shaped (Figure S1). Begge’s funnel plot (C vs. T) did not reveal any remarkable asymmetry in the distribution of scattered points (Figure 2). Among all studies included, Wang’s study on ESCC [35] and Liu’s on PSCC [19] deviated from other symmetrically distributed studies. When these two studies were deleted, I² decreased from 63% (Ph = 0.0005) to 42% (Ph = 0.05). While the summary OR for allele contrast (C vs. T) still kept significant (OR = 1.15, 95% CI = 1.06–1.25), and this result was considered to be a state of disequilibrium. Publication bias was diagnosed with Egger’s linear regression method [27,28] and funnel plot. The P-value less than 0.05 in Egger’s linear regression indicated the presence of potential publication bias. The standard error of logarithm for OR was plotted against its OR in funnel plot. Begg’s funnel plot was also plotted to detect the publication bias and influence of individual study on pooled OR. Log OR was plotted versus standard error of Log OR for each included study in Begg’s funnel plot [29]. And asymmetric or incomplete funnel-shaped plots demonstrated publication bias also. In the one-way sensitivity analysis, we excluded one single study each time, and the new pooled results could reflect the influence of that deleted study to the overall summary OR.

The frequency distributions of C allele in Asians and Caucasians were compared using chi-square test. All statistical analysis was implemented with SAS 9.2 software (SAS Institute Inc., Cary, NC, USA), STATA 11.0 (STATA Corp, College Station, Texas) and RevMan 5.1 (http://ims.cochrane.org/revman/download). All P-values were two-sided.

Table 1. Characteristics of eligible studies in meta-analysis.

| First author | Publication Year | Country | Ethnicity | Control | Cancer | Genotyping method | Matching criteria | Quality score | HWE |
|--------------|------------------|---------|-----------|---------|--------|-------------------|-----------------|--------------|-----|
| Chao 2010    | China            | Asian   | HB        | CRC     | age/sex| PCR-RFLP          |                 | 6.5          | Y   |
| Chen 2010    | China            | Asian   | HB        | CRC     | age/sex| TaqManSNP         |                 | 7.5          | Y   |
| Zhu 2010     | China            | Asian   | HB        | CRC     | age/sex| PCR-RFLP          |                 | 7.5          | Y   |
| Zhang 2010   | China            | Asian   | PB        | CRC     | age/sex| PCR-RFLP          |                 | 7.5          | N   |
| Wang 2010    | China            | Asian   | PB        | ESCC    | age/sex/area | PCR-RFLP |                 | 7            | Y   |
| Srivastava 2010 | India        | Caucasian | PB        | GBC     | age/sex| PCR-RFLP          |                 | 6.5          | Y   |
| Okubo 2010   | Japan            | Asian   | HB        | CRC     | age/sex| PCR-RFLP          |                 | 6.5          | Y   |
| Peng 2010    | China            | Asian   | HB        | GC      | age/sex| PCR-RFLP          |                 | 6.5          | Y   |
| Li 2010      | China            | Asian   | HB        | HCC     | age/sex| PCR-RFLP          |                 | 6        | Y   |
| Qi 2010      | China            | Asian   | HB        | HCC     | age/sex| TaqManSNP         |                 | 8.5          | Y   |
| Akkiz 2010   | Turkey           | Caucasian| HB        | HCC     | age/sex| PCR-RFLP          |                 | 7            | Y   |
| Christensen 2010 | USA          | Caucasian| PB        | OSCC    | age/sex/residence| TaqManSNP     |                 | 8.5          | Y   |
| Liu 2010     | USA              | Caucasian| HB        | OSCC    | age/sex| PCR-RFLP          |                 | 7.5          | Y   |
| Liu 2010     | USA              | Caucasian| PB        | PSCC    | age/sex/residence| TaqManSNP     |                 | 8.5          | Y   |

CRC: colorectal cancer; ESCC: esophageal squamous cell carcinoma; GBC: gallbladder cancer; GC: gastric cancer; HCC: hepatocellular cancer; OSCC: oral cavity squamous cancer; PSCC: pharynx squamous cancer; UK: unknown; HWE: Hardy-Weinberg equilibrium; Y: genotype frequency distribution agreed to HWE in controls; N: genotype frequency distribution disagreed to HWE in controls; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; PCR-LDR: polymerase chain reaction-ligation detection reaction; HB: hospital-based; PB: population-based. doi:10.1371/journal.pone.0030585.t001
similar to pooled OR without deletion of any study (OR = 1.15, 95% CI = 1.05–1.26).

**Test of heterogeneity**

Between-study heterogeneities and corresponding quantitative degrees in all comparisons and subgroups, were shown in Table S3. After stratification, the heterogeneities decreased obviously in the subgroups of CRC, GC, digestive gland, HCC, hospital-based controls, and C≤T group (Ph > 0.10 and I² < 50% in most genetic comparisons).

**Sensitivity analysis**

We deleted one single study from the overall pooled analysis each time to check the influence of the removed data set to the overall ORs. Two studies (Wang [ESCC] [35] and Liu [PSCC] [19]) changed the between-study heterogeneities materially in heterozygote comparison and recessive model respectively. After the deletion of anyone of the two studies mentioned, the heterogeneity vanished, while the association still kept significant (Table S4).

**Meta-analysis results**

The association strength between miR-196a2 polymorphism and the susceptibility for digestive system cancers are shown in Table 2. Overall, there was a statistically increased risk of digestive system cancers in every genetic comparison (CT vs. TT, OR = 1.25, 95% CI = 1.07–1.45; CC vs. TT, OR = 1.38, 95% CI = 1.13–1.67; CC/CT vs. TT, OR = 1.29, 95% CI = 1.10–1.50; CC vs. CT/TT, OR = 1.14, 95% CI = 1.01–1.30; C vs. T, OR = 1.15, 95% CI = 1.05–1.26).

Tumor site, source of controls, ethnicity and allele frequency in controls were taken into consideration for subgroup analysis. The forest plots of dominant models (CC/CT vs. TT) in different subgroups were shown in Figure S2. Comparing with genotype TT, heterozygote CT (OR = 1.23, 95% CI = 1.03–1.48), homozygote CC (OR = 1.32, 95% CI = 1.05–1.65), combination of CT/CC (OR = 1.26, 95% CI = 1.04–1.51) predominantly increased incidences of cancers in alimentary tract. And we also found that C allele carriers had more risks of digestive tract cancers (C vs. T, OR = 1.13, 95% CI = 1.02–1.25), but no significant result was observed in recessive model (CC vs. CT/TT, OR = 1.12, 95% CI = 0.98–1.28).

Significant association between SNP rs11614913 and increased risks of digestive gland cancers was found in three genetic models (CT vs. TT, OR = 1.30, 95% CI = 1.02–1.65; CC vs. TT, OR = 1.64, 95% CI = 1.24–2.17; CC/CT vs. TT, OR = 1.38, 95% CI = 1.10–1.74), except for recessive model (CC vs. TT, OR = 1.24, 95% CI = 0.85–1.79) and allele contrast (C vs. T, OR = 1.20, 95% CI = 0.96–1.51). Additionally, we demonstrated that this locus polymorphism was significantly linked to higher risks for CRC (CT vs. TT, OR = 1.23, 95% CI = 1.04–1.44; CC vs. TT, OR = 1.32, 95% CI = 1.08–1.61; CC/CT vs. TT, OR = 1.25, 95% CI = 1.07–1.46; C vs. T, OR = 1.15, 95% CI = 1.05–1.28), but a marginal significance was found in recessive model (CC vs. CT/TT, OR = 1.16, 95% CI = 0.98–1.38). We also observed increased susceptibility of HCC in homozygote comparison (OR = 1.79, 95% CI = 1.31–2.43), dominant model (OR = 1.41, 95% CI = 1.11–1.79), recessive model (OR = 1.49, 95% CI = 1.16–1.91) and allele contrast (OR = 1.32, 95% CI = 1.14–1.54). We just found a marginal significance in homozygote comparison (OR = 1.27, 95% CI = 0.99–1.64) in HCC study. Compared with CRC and HCC, no significant associations were found in GC, OSCC and PSCC.

With consideration of control source, studies with hospital-based controls showed elevated risks in four genetic comparisons (CT vs. TT, OR = 1.21, 95% CI = 1.08–1.36; CC vs. TT, OR = 1.37, 95% CI = 1.12–1.66; CC/CT vs. TT, OR = 1.24, 95% CI = 1.12–1.38; C vs. T, OR = 1.16, 95% CI = 1.05–1.28) and an edge effect was obtained in recessive model (OR = 1.18, 95% CI = 1.00–1.40). However, studies with population-based controls presented no significant association.

For the Asian group, every genetic comparison produced significantly increased risks (CT vs. TT, OR = 1.26, 95% CI = 1.05–1.50; CC vs. TT, OR = 1.47, 95% CI = 1.18–1.82; CC/CT vs. TT, OR = 1.32, 95% CI = 1.10–1.57; CC vs. CT/TT, OR = 1.25, 95% CI = 1.11–1.40; C vs. T, OR = 1.20, 95% CI = 0.98–1.28).
Table 2. Pooled ORs and 95% CIs of stratified meta-analysis.

| Stratification          | N  | CT vs. TT OR (95% CI) | CC vs. TT OR (95% CI) | CC/CT vs. TT OR (95% CI) | CC vs. CT/TT OR (95% CI) | C vs. T OR (95% CI) |
|-------------------------|----|-----------------------|-----------------------|--------------------------|--------------------------|--------------------|
| Digestive cancers       | 15 | 1.25(1.07–1.45)*      | 1.38(1.13–1.67)*      | 1.29(1.10–1.50)*         | 1.14(1.01–1.30)*         | 1.15(1.05–1.26)*   |
| Tumor site              |    |                       |                       |                          |                          |                    |
| Alimentary tract        | 11 | 1.23(1.03–1.48)*      | 1.32(1.05–1.65)*      | 1.26(1.04–1.51)*         | 1.12(0.98–1.28)          | 1.13(1.02–1.25)*   |
| CRC                     | 4  | 1.23(1.04–1.44)*      | 1.32(1.08–1.61)*      | 1.25(1.07–1.46)*         | 1.16(0.98–1.38)          | 1.15(1.05–1.28)*   |
| GC                      | 2  | 1.07(0.85–1.34)       | 1.24(0.94–1.65)       | 1.12(0.90–1.39)          | 1.22(0.96–1.55)          | 1.12(0.98–1.28)    |
| ESCC                    | 1  | 2.42(1.66–3.55)*      | 2.67(1.77–4.04)*      | 2.51(1.74–3.62)*         | 1.35(1.02–1.78)*         | 1.45(1.21–1.75)*   |
| OSCC                    | 2  | 1.00(0.47–2.13)       | 1.07(0.64–1.80)       | 1.03(0.53–1.98)          | 1.05(0.87–1.29)          | 1.04(0.91–1.19)    |
| PSCC                    | 2  | 1.35(0.79–2.32)       | 1.33(0.49–3.60)       | 1.36(0.66–2.83)          | 1.00(0.59–1.68)          | 1.09(0.72–1.63)    |
| Digestive gland         | 4  | 1.30(1.02–1.65)*      | 1.64(1.24–2.17)*      | 1.38(1.10–1.74)*         | 1.24(0.85–1.79)          | 1.20(0.96–1.51)    |
| HCC                     | 3  | 1.27(0.99–1.64)       | 1.79(1.31–2.43)*      | 1.41(1.11–1.79)*         | 1.49(1.16–1.91)*         | 1.32(1.14–1.54)*   |
| GBC                     | 1  | 1.50(0.72–3.12)       | 1.04(0.51–2.11)       | 1.20(0.60–2.41)          | 0.74(0.51–1.07)          | 0.85(0.64–1.15)    |
| Source of control       |    |                       |                       |                          |                          |                    |
| HB                      | 10 | 1.21(1.08–1.36)*      | 1.37(1.12–1.66)*      | 1.24(1.12–1.38)*         | 1.18(1.00–1.40)          | 1.16(1.05–1.28)*   |
| PB                      | 5  | 1.37(0.84–2.22)       | 1.39(0.84, 2.30)      | 1.36(0.85–2.19)          | 1.09(0.94–1.27)          | 1.11(0.91–1.36)    |
| Ethnicity               |    |                       |                       |                          |                          |                    |
| Asian                   | 9  | 1.26(1.05–1.50)*      | 1.47(1.18–1.82)*      | 1.32(1.10–1.57)*         | 1.25(1.11–1.40)*         | 1.20(1.10–1.31)*   |
| Caucasian               | 6  | 1.26(0.93–1.72)       | 1.26(0.88–1.80)       | 1.25(0.91–1.72)          | 1.02(0.83–1.27)          | 1.07(0.91–1.26)    |
| Allele frequency controls|   |                       |                       |                          |                          |                    |
| C>T                     | 8  | 1.36(1.00–1.85)       | 1.45(1.01–2.07)*      | 1.39(1.02–1.90)*         | 1.11(0.91–1.36)          | 1.15(0.98–1.34)    |
| C>T                     | 7  | 1.18(1.04–1.34)*      | 1.33(1.14–1.55)*      | 1.22(1.08–1.37)*         | 1.20(1.05–1.37)*         | 1.15(1.07–1.24)*   |

N: involved studies’ number; CT vs. TT: Heterozygote comparison; CC vs. TT: Homozygote comparison; CC/CT vs. TT: Dominant model; CC vs. CT/TT: Recessive model; C vs. T: Allele contrast; Random model was chosen for data pooling when P-value<0.10 and/or I²>50%; otherwise fixed model was used.

*: OR had statistical significance with corresponding 95% CI not including 1.

rs11614913 was thought to be implicated in altered expression and function of mature miRNAs, thus contributed to modified cancer risks. Many studies demonstrated variant in rs11614913 was significantly associated with the susceptibility of various cancers. Hong et al. found that carriers with TC/CC genotype of miR-196a2 had higher risks for non-small cell lung cancer (NSCLC) comparing with TT carriers [36]. Comparing with TT genotype, Hu et al. observed that CC or CC/CT genotypes significantly increased breast cancer risks [37]. Similar results were also found in glioma [38], prostatic cancer [39] and other kinds of cancers.

Further more, SNPs in miRNAs can occasionally disturb the gene or protein expression and result in pathogenicity [40]. Zhan and his colleagues reported that the expression levels of miR-196a in CC and CC/CT genotypes were higher than those in TT genotype in CRC [15]. Li et al. also found that CC and CC/CT genotypes increased the expression level of miR-196a in HCC patients with HBV infection comparing with TT genotype [30]. Hu et al. found that the expression level of miR-196a in CC genotype carriers was significantly lower than that in CT or TT carriers with NSCLC [6]. Additionally, compared with CT/TT genotype, CC genotype of miR-196a2 predominantly decreased the survival time of NSCLC patients in Hu’s study [6]. Thus Hu and his colleagues proposed the genetic variant in this locus to be a prognostic biomarker for NSCLC.

Our study showed that the presence of C allele significantly increased the risk of digestive system cancers with the comparison to T allele. This finding indicates that the genetic variant in miR-196a2 may crucially modify the susceptibility of various cancers.
digestive system cancers. Previous meta-analysis which described cancers locating in multiple systems of organism supported our finding [9,13].

We found that miR-196a2 polymorphism, in stratified analysis by cancer site, was statistically related with elevated cancer risks in the alimentary tract group and digestive gland group. Moreover, significantly increased risks were found in CRC and HCC. However, we did not observe any significant association between the genetic variant and the susceptibility of GC, OSCC and PSCC. There are some possibilities for this discrepancy among tumor sites. Firstly, the tissue specificity leads to different cancer susceptibilities in different tissues. Secondly, the relative small amount of eligible studies in stratified analysis might induce significant/significant association by chance due to insufficient statistical power [41]. Two previous meta-analysis reported insignificant association between miR-196a2 polymorphism and HCC risks [9,13], which was inconsistent to our finding. We infer that fewer included studies and the neglect of HBV infection in controls might lead to insignificant results in previous meta-analysis.

In the subgroup of ethnicity, we found significant association between miR-196a2 polymorphism and increased risks of digestive system cancers in Asians but not in Caucasians. A former meta-analysis reported a parallel observation to us [9]. Inconsistency between the two ethnicities can be explained by the possibility that different ethnic groups live with multiple life styles and environmental factors and thus yield diverse gene-environment interactions [42]. And different populations carry different genotype and/or allele frequencies of this locus polymorphism and may lead to various degrees of cancer susceptibility [43]. Relative small sample size in Caucasians might cause the inconspicuousness also.

The majority (70%, 7/10) of studies with hospital-based controls recruited Asians as tested subjects and we found significantly increased risks in this subgroup. While most (60%, 3/5) studies with population-based controls investigated Caucasians and we did not found any significant results in this subgroup. So the mentioned ethnic interpretations are available to the inconsistency in control source stratification. And the possible selection bias in controls with different matched criteria and sample size may also be the reasons. Chu’s meta-analysis study also reported significantly increased cancer risks in Asians but not in Caucasians [9].

Disagreements in the stratification of allele frequency in controls might attribute to above interpretations for ethnic effect to some extent.

Some advantages can be highlighted in our study. On one hand, this meta-analysis shed light on the association between miR-196a2 polymorphism and increased risks of digestive system cancers, CRC and HCC comprehensively and systematically. On the other hand, the inclusion of an unpublished study on CRC and the collection of unpublished genotype frequency of OSCC and PSCC strengthened the power and persuasion of our inference. Further more, all included studies had acceptable quality (scored at least 6). Limitations of this study should be noticed at the same time. Firstly, genetic factors, tumor biological characteristics and their interactions with environmental factors produce evident influences to the cancer susceptibility and tumorigenesis. Different cancers have different risk factors and diverse sensitivities to them. For instance, Hellobacter pylori infections and smoking may increase the incidence of gastric cancer. And hepatitis B, C virus infections and exposure of aflatoxin in food are risk to liver cancer [44]. Studies included in this meta-analysis contained various cancers, ethnic populations and nations, and multifactor such as gender, age, lifestyle, culture barriers, access to health care and exposure to pathogens and carcinogens were disparate. While lacking of individual information inhibited us from controlling the possible confounding factors which might be caused by the inconsistencies above. We also could not perform more precise calculation of adjusted ORs and further analysis of potential gene-environment interactions. Secondly, included researches did not cover all kinds of digestive system cancers, such as pancreatic cancer. And thirdly, language bias might derive from the screened references of English documents only.

In summary, this meta-analysis indicated that miR-196a2 rs11614913 polymorphism may increase the susceptibility of digestive system cancers, especially of CRC and HCC. SNP in this locus may considerably act as a candidate of biomarker for cancer screening, diagnosis and therapy in the future. To confirm our findings, further well-designed studies with large sample size in diverse ethnic populations, more types of digestive system cancers along with tissue-specific biochemical, functional and expression characteristics are required.

Supporting Information

Figure S1 Funnel plot of publication bias. The standard error of log (OR) is plotted versus OR for each study. Each square represents a separate study for the indicated association by allele contrast (C vs. T). The dotted line in blue indicates the estimated OR. PLoS ONE | www.plosone.org 6 January 2012 | Volume 7 | Issue 1 | e30585

(TIF)

Figure S2 Forest plots of dominant model (CC/CT vs. TT) in different subgroups. The squares and horizontal lines correspond to OR and 95% CI of specific study, and the area of squares reflects study weight (inverse of the variance). The diamond represents the pooled OR and its 95% CI. PLoS ONE | www.plosone.org 6 January 2012 | Volume 7 | Issue 1 | e30585

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Table S1 Genotype frequency distribution of studies included. PLoS ONE | www.plosone.org 6 January 2012 | Volume 7 | Issue 1 | e30585

(DOC)

Table S2 Scale for methodological quality assessment. PLoS ONE | www.plosone.org 6 January 2012 | Volume 7 | Issue 1 | e30585

(DOC)

Table S3 Heterogeneity test. PLoS ONE | www.plosone.org 6 January 2012 | Volume 7 | Issue 1 | e30585

(DOC)

Table S4 ORs (95% CI) of sensitivity analysis. PLoS ONE | www.plosone.org 6 January 2012 | Volume 7 | Issue 1 | e30585

(DOC)

Table S5 MOOSE Checklist. PLoS ONE | www.plosone.org 6 January 2012 | Volume 7 | Issue 1 | e30585

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Author Contributions

Conceived and designed the experiments: JG KC. Performed the experiments: JG MJ MZ. Analyzed the data: JG MZ. Contributed reagents/materials/analysis tools: MJ MZ. Wrote the paper: KC MJ.
References

1. Bartel DP, Chen CZ (2004) Micromanagers of gene expression: the potentially widespread influence of metazoan microRNAs. Nat Rev Genet 5: 396–400.

2. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116: 281–297.

3. Landl D, Gemignani F, Naccara A, Pandini B, Vodicka P, et al. (2008) Polymorphisms within microRNA-binding sites and risk of sporadic colorectal cancer. Carcinogenesis 29: 579–584.

4. Carthew RW (2006) Gene regulation by microRNA.Curr Opin Genet Dev 16: 203–208.

5. Kent OA, Mendell JT (2006) A small piece in the cancer puzzle: microRNAs as tumor suppressors and oncogenes. Oncogene 25: 6180–6186.

6. Hu Z, Chen J, Tian T, Zhou X, Gu H, et al. (2008) Genetic variants of miRNA sequences and non-small cell lung cancer survival. J Clin Invest 118: 2600–2608.

7. Pineau P, Volinia S, McJunkin K, Marchio A, Battiston C, et al. (2010) miR-221 overexpression contributes to liver tumorigenesis. Proc Natl Acad Sci U S A 107: 264–269.

8. Chen K, Song F, Calin GA, Wei Q, Hao X, et al. (2008) Polymorphisms in microRNA target: a gold mine for molecular epidemiology. Carcinogenesis 29: 1306–1311.

9. Chu H, Wang M, Shi D, Ma L, Zhang Z, et al. (2011) Hsa-miR-196a2 R11614913 polymorphism contributes to cancer susceptibility: evidence from 15 case-control studies. PLoS One 6: e18108.

10. Gao LB, Bai P, Pan XM, Jia J, Li LJ, et al. (2011) The association between two polymorphisms in pre-miR-15d and breast cancer risk: a meta-analysis. Breast Cancer Res Treat 125: 571–574.

11. Tian T, Xu Y, Dai J, Wu J, Shen H, et al. (2010) Functional polymorphisms in two pre-miRNAs and cancer risk: a meta-analysis. Int J Mol Epidemiol Genet 1: 358–366.

12. Wang F, Ma YL, Zhang P, Yang JJ, Chen HQ, et al. (2011) A genetic variant in miRNA-196a2 is associated with increased cancer risk: a meta-analysis. Mol Biol Rep.

13. 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.

14. Chen H, Sun LV, Chen JJ, Zheng HQ, Zhang QF (2011) A variant in miRNA-196a2 is not associated with susceptibility to and progression of colorectal cancer in Chinese. Intern Med J.

15. Zhan JF, Chen LH, Chen ZX, Yuan YW, Xie GZ, et al. (2011) A functional variant in miRNA-196a is associated with susceptibility of colorectal cancer in a Chinese population. Arch Med Res 42: 144–148.

16. Zhu L, Chu H, Gu D, Ma L, Shi D, et al. (2011) A Functional Polymorphism in miRNA196a2 Is Associated with Colorectal Cancer Risk in a Chinese Population. DNA Cell Biol.

17. Akki H, Bayram S, Bekar A, Akgilu E, Ulger Y (2011) A functional polymorphism in pre-microRNA-196a-2 contributes to the susceptibility of hepatocellular carcinoma in a Turkish population: a case-control study. J Viral Hepat 18: e399–407.

18. Christensen BC, Avisinar-Whiting M, Ouvellet LG, Butler RA, Nelson HH, et al. (2010) Mature microRNA sequence polymorphism in MIR196A2 is associated with risk and prognosis of head and neck cancer. Clin Cancer Res 16: 3713–3720.

19. Liu Z, Li G, Wei S, Niu J, El-Naggar AK, et al. (2010) Genetic variants in selected pre-microRNA genes and the risk of squamous cell carcinoma of the head and neck. Cancer 116: 4735–4740.

20. Thakkinstian A, McEvoy M, Minelli C, Gibson P, Hancox B, et al. (2005) Systematic review and meta-analysis of the association between (beta)2-adrenergceptor polymorphisms and asthma: a HuGE review. Am J Epidemiol 162: 201–211.

21. Camargo MC, Mera R, Correa P, Peek RM, Jr., Fontham ET, et al. (2006) Interleukin-1beta and interleukin-1 receptor antagonist gene polymorphisms and gastric cancer: a meta-analysis. Cancer Epidemiol Biomarkers Prev 15: 1674–1687.

22. Gao LB, Pan XM, Li LJ, Liang WB, Zhu Y, et al. (2011) RAD51 115G/C polymorphism and breast cancer risk: a meta-analysis from 21 studies. Breast Cancer Res Treat 125: 827–835.

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

24. Higgins JP, Thompson SG, Deeks JJ, Altman DG (2003) Measuring inconsistency in meta-analyses. BMJ 327: 557–560.

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

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

27. Hayashi Y, Nozuchi Y, Fuku T (2005) Systematic evaluation and comparison of statistical tests for publication bias. J Epidemiol 15: 225–243.

28. Peters JL, Sutton AJ, Jones DR, Abrams KR, Rashid L (2006) Comparison of two methods to detect publication bias in meta-analysis. JAMA 295: 676–680.

29. Begg CB, Mazumdar M (1994) Operating characteristics of a rank correlation test for publication bias. Biometrics 50: 1088–1101.

30. Li XD, Li ZG, Song XX, Liu CF (2010) A variant in microRNA-196a2 is associated with susceptibility to hepatocellular carcinoma in Chinese patients with cirrhosis. Pathology 42: 138–142.

31. Okubo 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.

32. Peng S, Kuang Z, Sheng C, Zhang Y, Xu H, et al. (2010) Association of microRNA-196a-2 gene polymorphism with gastric cancer risk in a Chinese population. Dig Dis Sci 55: 2298–2293.

33. Qi P, Hou TH, Geng L, Zhou FG, Gu X, et al. (2010) Association of a variant of miR-196 with susceptibility to hepatocellular carcinoma in male Chinese patients with chronic hepatitis B virus infection. Hum Immunol 71: 621–628.

34. 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.

35. Wang K, Gao H, Hu H, Xiong G, Guan X, et al. (2010) A functional variation in pre-microRNA-196a is associated with susceptibility of esophageal squamous cell carcinoma risk in Chinese Han. Biomarkers 15: 614–618.

36. Hong YS, Kang HJ, Kwak JY, Park BI, You CH, et al. (2011) Association between microRNA196a2 rs11614913 genotypes and the risk of non-small cell lung cancer in Korean population. J Prev Med Public Health 44: 125–130.

37. Hu Z, Liang J, Wang Z, Tian T, Zhou X, et al. (2009) Common genetic variants in pre-miRNAs were associated with increased risk of breast cancer in Chinese women. Hum Mol Genet 18: 79–84.

38. Dou T, Wu Q, Chen X, Ribas J, Ni X, et al. (2010) A polymorphism of microRNA196a genome region was associated with decreased risk of glioma in Chinese population. J Cancer Res Clin Oncol 136: 1853–1859.

39. George GP, Gangavar R, Meisfeld KE, Sarkhosh SN, Mittal RD (2011) Genetic variation in microRNA genes and prostate cancer risk in North Indian population. Mol Biol Rep 38: 1609–1615.

40. Yu Z, Li Z, Jolicoeur N, Zhang L, Fortin Y, et al. (2007) Aberrant allele frequencies of the SNPs located in microRNA target sites are potentially associated with human cancers. Nucleic Acids Res 35: 4535–4541.

41. Tapia T, Sanchez A, Vallejos M, Alvarez C, Moraga M, et al. (2008) ATM D1853N polymorphism and breast cancer susceptibility or ethnic influences? Breast Cancer Res Treat 107: 281–288.

42. Dick DM (2011) Gene-environment interaction in psychological traits and disorders. Annu Rev Clin Psychol 7: 383–409.

43. Gao LB, Pan XM, Sun H, Wang X, Rao L, et al. (2010) The association between ATM D1853N polymorphism and breast cancer susceptibility: a meta-analysis. J Exp Clin Cancer Res 29: 117.

44. Wallace TA, Martin DN, Amb S (2011) Interactions among genes, tumor biology and the environment in cancer health disparities: examining the evidence on a national and global scale. Carcinogenesis 32: 1107–1121.