Rs4938723 Polymorphism Is Associated with Susceptibility to Hepatocellular Carcinoma Risk and Is a Protective Factor in Leukemia, Colorectal, and Esophageal Cancer

AB 1 Bin Xu
C 1 Ya Zhu
D 2 Yu Tang
E 1 Zhenyong Zhang
FG 1 Qiaxian Wen

Background: Growing evidence indicates that a non-coding RNA named miR-34b/c plays crucial roles in carcinogenesis, and its common polymorphism, pri-miR-34b/c rs4938723, also participates in this process and is associated with cancer susceptibility. However, this association was previously undefined and ambiguous. Therefore, we carried out an updated analysis to evaluate this relationship between rs4938723 polymorphism and cancer susceptibility.

Material/Methods: PubMed, EMBase, Web of Science and Chinese language (WanFang, CNKI and VIP) databases were searched for relevant studies until Sep 10, 2018. Odds ratios and 95% confidence interval were applied to assess this relationship.

Results: Thirty case-control studies were retrieved. No positive association was found in either the overall study population or in the subgroups, based on ethnicity, source of group, sex, smoking, and drinking status. The main results were observed in the stratified analysis subgroups in cancer type subgroup: rs4938723 polymorphism may be a protective factor in leukemia, colorectal cancer, and esophageal cancer; however, C-allele was a risk factor in carriers for hepatocellular carcinoma. Last but not the least, poor positive results were discovered in the age subgroup.

Conclusions: Current meta-analysis suggested that rs4938723 polymorphism was potentially associated with hepatocellular carcinoma risk, but this polymorphism had a decreased association for susceptibility to esophageal cancer, leukemia, and colorectal cancer. Furthermore, studies with larger sample sizes and including gene-gene or gene-environment interactions should be carried out to elucidate the role of rs4938723 polymorphism in cancer risk.

MeSH Keywords: Early Detection of Cancer • Ethnic Groups • Polymorphism, Genetic

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/912534
Background

Cancer is a leading cause of death worldwide. To make things worse, the number of cancer cases and deaths is expected to grow rapidly with increase in populations, age, and adaptation to lifestyle behaviors that increase cancer risk [1]. One of the major reasons for variability among individuals is the presence of single-nucleotide polymorphisms (SNPs), which makes individuals more susceptible to cancer [2]. Several explorations related to genome-wide associations have suggested there are many loci in the genome that have signs of low tumor susceptibility for common tumors [3–5].

MicroRNAs (miRNAs) are a type of single-stranded non-encoded small RNA that can inhibit the transcription of mRNA or promote its degradation at the post-transcription level by binding to the target mRNA 3' UTR region to regulate gene expression [6,7]. There is growing evidence that misalignment of miRNA expression affects tumorigenesis based on activation of either tumor suppressor or oncogene [8–12]. miRNA gene polymorphism affects tumor susceptibility by destroying miRNA biosynthesis and target gene expression, changing mature miRNA, or by affecting its interaction with target genes [13–16]. The relationship between miRNA gene polymorphisms is complicated. For example, in each case, the rs11614913 variant homozygote CC was associated with increased cancer risk. Risk of developing oesophageal cancer in Caucasian males and never-smokers was significantly associated with the rs11614913 variant homozygote TT, the minor allele in this population [17]. Rs11614913 is located on the 3′ passenger (3p) strand mature sequence of mir-196a-2, thereby possibly affecting both maturation and the repertoire of target miRNAs with which it interacts. Indeed, previous studies have shown that sequence variations in mature and precursor miRNA sequences affect miRNA biogenesis [18,19], and levels of mature miR-196a-2 were lower in CC carriers than in TT carriers [20,21]. Notably, this SNP has also been associated with poor survival in patients with lung cancer.

The miR-34 family members include miR-34a, miR-34b, and miR-34c. miR-34a is encoded by its own transcription, while the miR-34b and miR-34c share a primary transcription (pri-miR-34b/c) [22]. In the promoter region of Pri-miR-34b/c, a potentially functional rs4938723 T/C variant may affect the binding of transcription factor Gata-X, thereby changing the expression of pri-miR-34b/c [23–25]. The rs4938723 T>C variant may potentially influence the expression of miR-34b/c via genetic and epigenetic mechanisms, leading to increased or decreased risk of cancer. Previous studies proposed that miR-34b/c is dysregulated in various cancers [26–28].

Figure 1. Flowchart illustrating the search strategy used to identify association studies for pri-miR-34b/c rs4938723 polymorphism and whole cancer risk.
| First author | Origin | Ethnicity | Design | Case | Control | Cancer type (1) | Method | Cancer type (2) |
|--------------|--------|-----------|--------|------|---------|----------------|--------|----------------|
| Bensen (2013) [34] | USA | African | PB | 742 | 658 | 63 | 317 | 362 | 58 | 257 | 343 | 0.32 | Illumina | Breast cancer | Female specific cancer |
| Sanaei (2016) [47] | Canada | Caucasian | PB | 263 | 221 | 23 | 115 | 125 | 15 | 106 | 100 | 0.06 | PCR-RFLP | Breast cancer | Female specific cancer |
| Bensen (2013) [34] | USA | Caucasian | PB | 1203 | 1088 | 144 | 563 | 496 | 155 | 303 | 430 | 0.68 | Illumina | Breast cancer | Female specific cancer |
| Gao (2013) [38] | China | Asian | HB | 347 | 488 | 28 | 144 | 175 | 62 | 210 | 216 | 0.33 | PCR-RFLP | Colorectal cancer | Digestive cancer |
| Oh (2014) [45] | South Korea | Asian | HB | 545 | 428 | 40 | 233 | 272 | 41 | 171 | 216 | 0.40 | PCR-RFLP | Colorectal cancer | Digestive cancer |
| Yin (2013) [52] | China | Asian | HB | 600 | 673 | 45 | 278 | 277 | 73 | 290 | 310 | 0.67 | PCR-LDR | Esophageal cancer | Digestive cancer |
| Zhu (2015) [31] | China | Asian | PB | 237 | 274 | 25 | 99 | 113 | 30 | 122 | 122 | 0.95 | PCR-RFLP | Esophageal cancer | Digestive cancer |
| Zhang (2) (2014) [55] | China | Asian | HB | 1109 | 1275 | 84 | 536 | 489 | 133 | 573 | 569 | 0.33 | PCR-LDR | Esophageal cancer | Digestive cancer |
| You (2011) [53] | China | Asian | PB | 251 | 189 | 28 | 103 | 120 | 15 | 86 | 88 | 0.34 | MALDI-TOF-MS | Esophageal cancer | Digestive cancer |
| Yang (2014) [51] | China | Asian | HB | 419 | 402 | 40 | 186 | 193 | 62 | 184 | 156 | 0.52 | PCR-RFLP | Gastric cancer | Digestive cancer |
| Pan (2015) [46] | China | Asian | HB | 197 | 289 | 19 | 76 | 102 | 31 | 137 | 121 | 0.39 | PCR-RFLP | Gastric cancer | Digestive cancer |
| Son (2013) [49] | South Korea | Asian | HB | 157 | 201 | 13 | 75 | 60 | 17 | 74 | 110 | 0.37 | PCR-RFLP | Hepato-cellular carcinoma | Digestive cancer |
| Han (2013) [39] | China | Asian | HB | 1013 | 999 | 118 | 444 | 451 | 119 | 424 | 456 | 0.18 | fluorescent-probe real-time quantitative PCR | Hepato-cellular carcinoma | Digestive cancer |
| Xu (2011) [25] | China | Asian | HB | 502 | 549 | 62 | 236 | 204 | 54 | 229 | 266 | 0.65 | PCR-RFLP | Hepato-cellular carcinoma | Digestive cancer |
| Chen (2016) [30] | China | Asian | HB | 286 | 572 | 38 | 146 | 102 | 33 | 267 | 272 | 0.00 | PCR-RFLP | Hepato-cellular carcinoma | Digestive cancer |
| Tong (2016) [29] | China | Asian | HB | 570 | 673 | 35 | 281 | 254 | 76 | 296 | 301 | 0.80 | TaqMan | Leukemia | Other cancers |
| Hashemi (2017) [41] | Iran | Caucasian | PB | 110 | 120 | 2 | 31 | 77 | 6 | 52 | 62 | 0.24 | PCR-RFLP | Leukemia | Other cancers |
| Yuan (2016) [54] | China | Asian | HB | 328 | 568 | 36 | 175 | 117 | 68 | 258 | 242 | 0.95 | PCR-RFLP | Cervical cancer | Female specific cancer |
| Li (2013) [43] | China | Asian | PB | 217 | 360 | 31 | 104 | 82 | 37 | 155 | 168 | 0.89 | PCR-RFLP | Nasopharyngeal carcinoma | Other cancers |
Similar to other kinds of polymorphisms, miR-34b/c rs4938723 polymorphism may influence its own expression, then affect its target genes’ expression, finally promoting or inhibiting translation of target proteins to act on several biological functions. For example, Tong (2016) [29] reported rs4938723 CC genotype was significantly associated with reduced lymphoblastic leukemia risk, and C-allele may increase the transcription activity of miR-34b/c. However, Chen (2016) [30] found that TC+CC genotype was correlated with an increased risk of hepatocellular carcinoma compared to the TT genotype, which disagrees with Tong’s results. In addition, Zhu (2015) [31] indicated no association between this polymorphism and esophageal squamous cell carcinoma.

A number of meta-analyses with respect to association between this polymorphism and cancer susceptibility have been reported, but with some limitations and false-positive conclusions. Li (2017) [32] indicated a rs4938723 polymorphism

Table 1 continued. Basic information for included studies of the association between pri-miR-34b/c rs4938723 polymorphism site and whole cancer susceptibility.

| First author (year) [ref no.] | Origin | Ethnicity | Design | Case | Control | Case | Control | Method | Cancer type (1) | Cancer type (2) |
|-----------------------------|--------|-----------|--------|------|---------|------|---------|--------|----------------|----------------|
| tian (2014) [50]            | China  | Asian     | PB     | 133  | 133     | 30   | 62      | 41     | 18  | TaqMan         | Osteosarcoma    | Other cancers |
| hasheimi (2016) [40]        | Iran   | Caucasian | HB     | 152  | 152     | 10   | 56      | 85     | 5   | PCR-RFLP       | Prostate cancer | Other cancers |
| Zhang (1) (2014) [22]       | China  | Asian     | HB     | 710  | 760     | 84   | 324     | 302    | 64  | TaqMan         | Renal cell cancer | Other cancers |
| carvalho (2017) [36]        | Brazil | Mixed     | PB     | 130  | 105     | 14   | 64      | 52     | 16  | sequencing     | Retinoblastoma  | Other cancers |
| liu (2017) [44]             | China  | Asian     | HB     | 164  | 305     | 26   | 80      | 58     | 22  | PCR-RFLP       | Hepatocellular carcinoma | Digestive cancer |
| Chen (2015) [37]            | China  | Asian     | HB     | 784  | 1006    | 111  | 402     | 271    | 99  | PCR-RFLP       | Thyroid carcinoma | Other cancers |
| Bulibu (2018) [35]          | China  | Asian     | PB     | 175  | 186     | 37   | 74      | 64     | 53  | PCR-DHPLC      | Esophageal cancer | Digestive cancer |
| Wu (2017) [56]              | China  | Asian     | PB     | 893  | 990     | 92   | 396     | 405    | 84  | MassARRAY      | Gastric cancer | Digestive cancer |
| Singh (2017) [48]           | China  | Asian     | HB     | 324  | 598     | 44   | 148     | 132    | 66  | PCR-LDR        | Gastric cancer | Digestive cancer |
| He (2018) [42]              | China  | Asian     | HB     | 377  | 810     | 49   | 107     | 221    | 75  | TaqMan         | Neuroblastoma   | Other cancers |
| Pu (2012) [57]              | China  | Asian     | HB     | 1013 | 999     | 118  | 444     | 451    | 119 | Fluorescent Probe-Real-time Quantitative PCR | Hepatocellular carcinoma | Digestive cancer |

HWE – Hardy-Weinberg equilibrium; HB – hospital-based; PB – population-based; PCR-RFLP – polymerase chain reaction and restrictive fragment length polymorphism; MALDI-TOF-MS – matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; DHPLC – denaturing high performance liquid chromatography; LDR – ligation detection reaction.

Similar to other kinds of polymorphisms, miR-34b/c rs4938723 polymorphism may influence its own expression, then affect its target genes’ expression, finally promoting or inhibiting translation of target proteins to act on several biological functions. For example, Tong (2016) [29] reported rs4938723 CC genotype was significantly associated with reduced lymphoblastic leukemia risk, and C-allele may increase the transcription activity of miR-34b/c. However, Chen (2016) [30] found that TC+CC genotype was correlated with an increased risk of hepatocellular carcinoma compared to the TT genotype, which disagrees with Tong’s results. In addition, Zhu (2015) [31] indicated no association between this polymorphism and esophageal squamous cell carcinoma.

A number of meta-analyses with respect to association between this polymorphism and cancer susceptibility have been reported, but with some limitations and false-positive conclusions. Li (2017) [32] indicated a rs4938723 polymorphism
had a significant relationship with the whole cancer risk. In addition, this polymorphism played an increased risk in hepatocellular carcinoma, but a decreased risk for colorectal, gastric, and esophageal squamous cell cancer. Furthermore, Wang (2014) [33] suggested that this polymorphism may be associated with the risk of various types of cancers, including nasopharyngeal cancer, osteosarcoma, and renal cell cancer, especially in Asians. In addition to these 2 meta-analyses, some novel studies were also reported. We considered that it was necessary to re-analyze all case-control studies to assess the association between rs4938723 variant and tumor susceptibility [22,25,29–31,34–57].

Figure 3. Forest plot of cancer risk associated with pri-miR-34b/c rs4938723 polymorphism (C-allele vs. T-allele) in the whole. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR and 95% CI.
**Table 2. Total and stratified subgroup analysis for pri-miR-34b/c rs4938723 polymorphism site and cancer susceptibility.**

| Variables | N   | Case/ control | C-allele vs. T-allele | CT vs. TT | CC vs. TT | CC+CT vs. TT | CC vs. TT+TT |
|-----------|-----|---------------|----------------------|-----------|-----------|-------------|--------------|
|           |     |               | OR (95%CI) | P   | OR (95%CI) | P   | OR (95%CI) | P   | OR (95%CI) | P   |
| Total     | 30  | 13950/16071   | 1.04 (0.97–1.13) | <0.001 | 1.07 (0.98–1.17) | <0.001 | 1.07 (0.91–1.27) | <0.001 | 1.03 (0.89–1.19) | <0.001 |
| HWE       | 29  | 13664/15499   | 1.03 (0.95–1.12) | <0.001 | 1.09 (0.99–1.20) | <0.001 | 0.99 (0.83–1.19) | <0.001 | 1.07 (0.97–1.19) | <0.001 |
| Ethnicity |     |               |           |     |            |     |            |     |            |     |
| Asian     | 24  | 10351/13727   | 1.06 (0.97–1.15) | <0.001 | 1.08 (0.97–1.19) | <0.001 | 1.10 (0.91–1.32) | <0.001 | 1.08 (0.97–1.20) | <0.001 |
| Caucasian | 4   | 1727/1581     | 0.97 (0.69–1.35) | 0.001 | 0.95 (0.64–1.40) | 0.004 | 1.00 (0.56–1.78) | 0.95 (0.63–1.43) | 0.001 | 0.88 (0.71–1.10) | 0.151 |
| African   | 1   | 742/658       |               |     |            |     |            |     |            |     |
| Mixed     | 1   | 130/105       |               |     |            |     |            |     |            |     |
| China     | 22  | 10649/13098   | 1.05 (0.96–1.15) | <0.001 | 1.06 (0.96–1.18) | <0.001 | 1.11 (0.91–1.36) | <0.001 | 1.07 (0.96–1.20) | <0.001 |
| Non-China | 8   | 3301/2973     | 1.02 (0.87–1.18) | 0.004 | 1.09 (0.89–1.33) | 0.006 | 0.90 (0.75–1.07) | 0.286 | 1.06 (0.87–1.30) | <0.001 |
| Source of control | | | | | | | | | | |
| HB        | 17  | 7985/9923     | 1.07 (0.95–1.20) | <0.001 | 1.09 (0.94–1.27) | <0.001 | 1.10 (0.85–1.43) | <0.001 | 1.10 (0.95–1.28) | <0.001 |
| PB        | 13  | 5965/6149     | 1.02 (0.92–1.14) | <0.001 | 1.05 (0.93–1.18) | 0.022 | 1.05 (0.84–1.31) | 0.002 | 1.04 (0.91–1.19) | 0.002 |
| Cancer type | | | | | | | | | | |
| Hepatocellular carcinoma | 6   | 5421/6411 | 1.23 (1.06–1.44) | 0.001 | 1.19 (1.07–1.32) | 0.156 | 1.53 (1.04–2.23) | <0.001 | 1.29 (1.08–1.53) | 0.019 |
| Leukemia  | 2   | 680/793       | 0.71 (0.42–1.20) | 0.031 | 0.76 (0.33–1.75) | 0.006 | 0.52 (0.34–0.79) | 0.411 | 0.71 (0.33–1.52) | 0.009 |
| Colorectal cancer | 2   | 892/96 | 0.87 (0.75–1.01) | 0.154 | 0.97 (0.79–1.17) | 0.222 | 0.66 (0.47–0.92) | 0.342 | 0.90 (0.75–1.09) | 0.157 |
| Gastric cancer | 4   | 1833/2279 | 0.94 (0.75–1.18) | 0.001 | 0.93 (0.75–1.17) | 0.381 | 0.92 (0.58–1.47) | 0.400 | 0.92 (0.71–1.20) | 0.007 |
| Breast cancer | 3   | 2208/1967 | 0.97 (0.89–1.07) | 0.304 | 1.02 (0.90–1.16) | 0.289 | 0.90 (0.73–1.10) | 0.386 | 1.00 (0.88–1.13) | 0.287 |
| Esophageal cancer | 5   | 2372/2597 | 0.93 (0.85–1.01) | 0.475 | 1.02 (0.90–1.14) | 0.049 | 0.76 (0.62–0.92) | 0.346 | 0.97 (0.86–1.08) | 0.500 |
| Digestive cancer | 17  | 8232/9417 | 1.02 (0.92–1.13) | <0.001 | 1.05 (0.96–1.16) | 0.019 | 1.02 (0.80–1.29) | <0.001 | 1.04 (0.93–1.17) | <0.001 |
| Female specific cancer | 4   | 2535/2536 | 1.00 (0.92–1.09) | 0.228 | 1.09 (0.91–1.31) | 0.098 | 0.93 (0.77–1.12) | 0.473 | 1.05 (0.93–1.17) | 0.109 |
| Other cancers | 8   | 2830/3894 | 1.00 (1.04–1.24) | <0.001 | 1.24 (0.92–1.71) | <0.001 | 1.29 (1.26–1.77) | <0.001 | 1.37 (1.29–1.78) | <0.001 |

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0).
Material and Methods

Identification strategy

We searched in PubMed, EMbase, Web of Science, CNKI, VIP, and WanFang databases (updated on Sep 10, 2018) using ‘polymorphism’ or ‘variant’ or ‘single-nucleotide polymorphism (SNP)’ or ‘mutation’, ‘cancer’ or ‘tumor’, and ‘miR-34b/c’ or ‘pri-miR-34’. Each publication that assessed the relationship between rs4938723 polymorphism and cancer risk was collected.

Search criterion

The selection criteria were: (1) evaluation of pri-miR-34b/c rs4938723 and all types of cancer risks, (2) case-control design, and (3) available genotype frequency. Exclusion criteria were: (1) studies with duplicate data (the most recent or complete study with the largest number of cases and controls were included), and (2) studies that have not yet been published.

Data extraction

The following data were collected: first author, year of publication, race of origin, cancer type by traditional classification, cancer type by our own standard, sample size (cases/controls), each kind of genotype both for case and control groups, study design (HB: hospital-based and PB: population-based), source of control, Hardy-Weinberg equilibrium (HWE) of controls, and genotyping method.

Statistical analysis

Odds ratio (OR) with 95% confidence interval (CI) was used to measure the strength of the association between pri-miR-34b/c rs4938723 and cancers. We analyzed this correlation by using 5 different genetic models: C-allele vs. T-allele, CC vs. TT, CT vs. TT, CC+CT vs. TT, and CC vs. CT+TT. Ethnicity subgroup were categorized as Caucasian, Asian, African, or mixed (if study population was not a pure race). We divided the control group into 4 classes based on source: HB or PB. In the cancer type subgroup, we included hepatocellular carcinoma, leukemia, colorectal cancer, gastric cancer, breast cancer, esophageal...
cancer, digestive cancer, female specific cancer, and other cancers (if not in the above types).

Heterogeneity assumption was assessed with a chi-square-based Q-test. The statistical significance was calculated with the Z-test. When P for the heterogeneity test (Ph) was more than 0.10, the fixed-effects model was applied; otherwise, the random-effects model was used [58,59]. The funnel plot asymmetry and publication bias were evaluated by both Egger’s test and Begg’s test [60,61]. The departure of frequencies of rs4938723 from expected values was estimated with the Z-test.

Figure 4. Forest plot of hepatocellular carcinoma associated with pri-miR-34b/c rs4938723 polymorphism (CC vs. TT). The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR and 95% CI.
under HWE was evaluated in controls using the Pearson chi-square test. All these statistical tests were performed using Stata software (version 11.0; StataCorp LP, College Station, TX).

### Results

#### Study characteristics

After reviewing the title, abstract, and full text, we excluded meta-analyses, reviews, case-only studies, and other gene polymorphisms. The flowchart illustrating the search strategy is shown in Figure 1. Finally, 29 different papers describing 30 case-control studies [22,25,29–31,34–57] evaluating the relationship between rs4938723 polymorphism and cancer were identified. Study characteristics are shown in Table 1. The HWE in controls was consistent with 0.05, except for 1 study by Chen (2016) [30]. To observe a representation of our analysis, we investigated the minor allele frequency from 5 main worldwide populations in the 1000 Genomes Browser: East Asian, 0.305; European, 0.365; African, 0.276; American, 0.219; and South Asian, 0.244 (Figure 2).

![Forest plot of leukemia risk associated with pri-miR-34b/c rs4938723 polymorphism (CC vs. TT). The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR and 95% CI.](image)
Figure 6. Forest plot of colorectal and esophageal cancer risk associated with pri-miR-34b/c rs4938723 polymorphism (CC vs. CT+TT). The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR and 95% CI.

None of the control populations had a history of malignant diseases. Genotyping methods are listed in Table 1.

**Quantitative synthesis**

**Total analysis**

In the total group, no vital relationship was found in all comparisons (e.g., C-allele vs. T-allele: OR=1.04; 95% CI=0.97–1.13; \( P_{(heterogeneity)} < 0.001 \), Figure 3). At the same time, if we excluded 1 paper that was not consistent with HWE, a similar association was detected (Table 2). In addition, no association was detected in subgroup analysis based on ethnicity and source of control (Table 2).

**Subgroup analysis by cancer type**

Detailed results are shown in Table 2. Statistically significant relationships were observed between pri-miR-34b/c rs4938723 and risk of 4 types of cancers: as a risk factor for hepatocellular carcinoma (e.g., CC vs. TT: OR=1.53; 95% CI=1.04–2.23; \( P_{(heterogeneity)} < 0.001 \), Figure 4), but as a protective factor for
| Study ID | OR (95% CI) | % Weight |
|----------|-------------|-----------|
| Male     |             |           |
| Zhang (1) (2014) | 1.58 (1.05, 2.40) | 4.12 |
| Yin (2013) | 0.69 (0.43, 1.11) | 4.67 |
| Zhang (2) (2014) | 0.72 (0.52, 1.00) | 9.90 |
| Subtotal (I-squared=80.1%, p=0.007) | 0.90 (0.72, 1.13) | 18.89 |
| Female   |             |           |
| Zhang (1) (2014) | 1.23 (0.66, 2.26) | 2.13 |
| Yin (2013) | 0.62 (0.31, 1.25) | 2.40 |
| Zhang (2) (2014) | 0.64 (0.35, 1.16) | 3.21 |
| Subtotal (I-squared=31.1%, p=0.234) | 0.80 (0.56, 1.14) | 7.73 |
| Smoker   |             |           |
| Zhang (1) (2014) | 1.89 (1.08, 3.30) | 2.13 |
| Yin (2013) | 0.62 (0.33, 1.16) | 2.77 |
| Zhang (2) (2014) | 0.64 (0.08, 0.98) | 8.43 |
| Subtotal (I-squared=80.5%, p=0.006) | 0.86 (0.66, 1.13) | 13.33 |
| Nonsmoker|             |           |
| Zhang (1) (2014) | 1.21 (0.78, 1.88) | 4.11 |
| Yin (2013) | 0.67 (0.40, 1.11) | 4.38 |
| Zhang (2) (2014) | 0.70 (0.43, 1.13) | 6.68 |
| Subtotal (I-squared=49.0%, p=0.141) | 0.85 (0.56, 1.11) | 13.18 |
| Drinking |             |           |
| Zhang (1) (2014) | 1.66 (0.89, 3.08) | 1.82 |
| Yin (2013) | 0.81 (0.41, 1.57) | 2.19 |
| Zhang (2) (2014) | 0.68 (0.45, 1.03) | 6.09 |
| Subtotal (I-squared=64.1%, p=0.062) | 0.88 (0.65, 1.19) | 10.10 |
| Nondrinking|             |           |
| Zhang (1) (2014) | 1.36 (0.90, 2.05) | 4.44 |
| Yin (2013) | 0.57 (0.35, 0.93) | 5.09 |
| Zhang (2) (2014) | 0.68 (0.45, 1.02) | 6.85 |
| Subtotal (I-squared=76.9%, p=0.013) | 0.83 (0.65, 1.06) | 16.36 |
| < 62     |             |           |
| Yin (2013) | 0.63 (0.36, 1.11) | 3.58 |
| Zhang (2) (2014) | 0.74 (0.48, 1.14) | 5.62 |
| Subtotal (I-squared=0.0%, p=0.654) | 0.70 (0.50, 0.98) | 9.20 |
| ≥ 62     |             |           |
| Yin (2013) | 0.69 (0.41, 1.19) | 3.73 |
| Zhang (2) (2014) | 0.68 (0.46, 0.99) | 7.48 |
| Subtotal (I-squared=0.0%, p=0.942) | 0.68 (0.50, 0.93) | 11.21 |
| Overall (I-squared=53.1%, p=0.002) | 0.82 (0.75, 0.91) | 100.00 |

Subgroup analysis by age and other kinds of analysis

Interestingly, in the age subgroup, decreased associations were found both in <62 (OR=0.70; 95% CI=0.50–0.98; $P_{\text{heterogeneity}}=0.519$ for heterogeneity, Figure 6), and esophageal cancer (CC vs. CT+TT: OR=0.76; 95% CI=0.63–0.91; $P_{\text{heterogeneity}}=0.251$ for heterogeneity, Figure 6) (Table 2).
Relationships between rs4938723 polymorphism and prognosis of cancer

To our regret, no association between this polymorphism and cancer prognosis in 2 models (localized and advanced) (CC+CT vs. TT: OR=1.15; 95% CI=0.91–1.46; $P_{\text{heterogeneity}}=0.735$ for heterogeneity, $P=0.237$ for Z-test) was found (Table 3).

**Table 3.** Relationship between pri-miR-34b/c rs4938723 polymorphism and cancer prognosis.

| Genotype       | Localised | Advanced | OR (95%CI) | $P_{\text{h}}$ | $P$ |
|----------------|-----------|----------|------------|----------------|-----|
| CC+CT          | 446       | 261      |            |                |     |
| TT             | 317       | 242      | 1.15 (0.91–1.46) | 0.735          | 0.237 |
| CC             | 156       | 83       |            |                |     |
| CT+TT          | 947       | 605      | 1.71 (0.79–3.71) | 0.001          | 0.174 |

$P_{\text{h}}$ – value of Q-test for heterogeneity test

Both Begg’s funnel plot and Egger’s test were applied to assess the publication bias. No publication bias was detected [for example (C-allele vs. T-allele) ($z=0.27$, $P=0.789$ for Begg’s test; $t=0.24$, $P=0.809$ for Egger’s test, Figures 8, 9)] (Table 4). Despite the above results, each study reflected the influence of the individual dataset on the pooled OR, and observed that the corresponding pooled OR was not significantly altered, indicating that our results were statistically robust (for example: allelic contrast, Figure 10).

**Table 4.** Publication bias tests (Begg’s funnel plot and Egger’s test for publication bias test) for pri-miR-34b/c rs4938723 polymorphism.

- **Figure 8.** Begg’s funnel plot for publication bias test (C-allele vs. T-allele). Each point represents a separate study for the indicated association. Log [OR], natural logarithm of OR. Horizontal line, mean effect size.
- **Figure 9.** Egger’s publication bias plot (C-allele vs. T-allele).

**Relationships between rs4938723 polymorphism and prognosis of cancer**

To our regret, no association between this polymorphism and cancer prognosis in 2 models (localized and advanced) (CC+CT vs. TT: OR=1.15; 95% CI=0.91–1.46; $P_{\text{heterogeneity}}=0.735$ for heterogeneity, $P=0.237$ for Z-test) was found (Table 3).

Both Begg’s funnel plot and Egger’s test were applied to assess the publication bias. No publication bias was detected [for example (C-allele vs. T-allele) ($z=0.27$, $P=0.789$ for Begg’s test; $t=0.24$, $P=0.809$ for Egger’s test, Figures 8, 9)] (Table 4). Despite the above results, each study reflected the influence of the individual dataset on the pooled OR, and observed that the corresponding pooled OR was not significantly altered, indicating that our results were statistically robust (for example: allelic contrast, Figure 10).
Discussion

mir-34b/c gene is part of the p53 pathway and enhances its tumor suppressor activities [62, 63]; it transcribes microRNA-34 b and c, which inhibit p53 antagonists [64], cyclin-dependent kinases, and pro-apoptotic proteins [65]. The deregulation of miR-34b/c was observed in several carcinoma cells, and cell proliferation, apoptosis, migration, and invasion were involved. Recently, a SNP located at the promoter region of mir-34b/c gene (rs4938723T/C) was identified, and its role in tumorigenesis has been widely investigated, as it can alter miR-34b/c transcription levels, because it can affect GATA-X binding. Presence of C in this location leads to binding to the GATA-X [33].

Our meta-analysis explored the association between pri-miR-34b/c rs4938723 and overall cancer susceptibility, involving 13,950 cancer cases and 16,071 controls. The main results of our analysis are that this polymorphism has different associations with different types of cancer: increased association for hepatocellular carcinoma, but decreased association for leukemia, colorectal, and esophageal cancer. The following reasons may explain these results. First, differences in the distribution of various cancers between cases and controls might be a source of variability during pooling. Second, rs4938723 polymorphism might carry out different functions in different types of cancers. Third, because cancer is a multi-factorial disease caused by the complex interactions between many genetic and environmental factors, there is no single gene or environmental factor that has a significant effect on cancer susceptibility [66]. The present study differs from previous meta-analyses in that we included some environmental and clinical factors, such as sex, smoking status, age, drinking, and prognosis of cancer. Of note, a positive association was found in the age subgroup. Our results were also different from those of previous meta-analyses [32,33] because previously there had been no association between this polymorphism and the whole cancer risk, as well as no association for Asians and gastric cancer risk. This was because the relatively small samples in previous analysis resulted in false-positive results. So, it made sense to recombine all studies to gain a comprehensive and credible conclusion, and to correct error at the same time.

Some limitations should be considered. First, sample sizes varied widely in the different studies (range of the number of cases/controls: 110/120 to 1109/1275), which may increase the publication bias. Second, there were only 2 case-control studies regarding leukemia, colorectal, and gastric cancer; future studies should also focus on these types of cancers. Third, few studies used mixed, Caucasian, or African populations; future studies should also focus on these races. Fourth,
additional studies are needed to address the effects of race and sample size on the predicted associations, and more attention must be placed on gene-gene and gene-environment interactions. Fifth, other environmental factors, such as dietary factors and infectious agents, increase the load of carcinogenic substances humans are exposed to.

## References:

1. Torre LA, Siegel RL, Ward EM, Jemal A: Global Cancer Incidence and Mortality Rates and Trends – An Update. Cancer Epidemiol Biomarkers Prev, 2016; 25: 16–27
2. Dong LM, Potter JD, White E et al: Genetic susceptibility to cancer: The role of polymorphisms in candidate genes. JAMA, 2008; 299: 2423–36
3. Harismendy O, Frazer KA: Elucidating the role of 8q24 in colorectal cancer. Nat Genet, 2009; 41: 868–69
4. Esquela-Kerscher A, Slack FJ: Oncomirs – microRNAs with a role in cancer. Nat Rev Cancer, 2004; 6: 259–69
5. Zintzaras E: The generalized odds ratio as a measure of genetic risk effects in association studies with multiple alleles. Stat Appl Genet Mol Biol, 2010; 9: 21
6. Bartel DP: MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell, 2004; 116: 281–97
7. Bensen JT, Tse CK, Nyante SJ et al: Association of germline microRNA SNPs in African-Americans with melanoma and cutaneous melanoma. J Invest Dermatol, 2016; 136: 1292–8
8. Glover AR, Zhao JT, Gill AJ et al: MicroRNA-7 as a tumor suppressor and novel therapeutic for adrenocortical carcinoma. Oncotarget, 2015; 6: 36675–88
9. He L, Thomson JM, Hermann MT et al: A microRNA polycistron as a potential human oncogene. Nature, 2003; 425: 828–33
10. Li YQ, Lu JH, Bao XM et al: MiR-24 functions as a tumor suppressor in nasopharyngeal carcinoma through targeting FSCN1. J Exp Clin Cancer Res, 2015; 34: 130
11. Luqiu Q, Lu JH, Bao XM et al: MiR-24 functions as a tumor suppressor in nasopharyngeal carcinoma through targeting FSCN1. J Exp Clin Cancer Res, 2015; 34: 130
12. Luo W, Qin P, Liu S et al: A potential role for microRNA-196a2 in the pathogenesis of breast cancer. J Exp Clin Cancer Res, 2014; 33: 175
13. Buscaglia LE, Li Y: [Apoptosis and the target genes of microRNA-21.] Chinese Journal of Cancer Research, 2011; 30: 371–80 [in Chinese]
14. Hashemi M, Sheybani-Nasab M, Naderi M et al: Association of functional polymorphism at the miR-502-binding site in the 3' untranslated region of the SETD8 gene with risk of childhood acute lymphoblastic leukemia, a preliminary report. Tumour Biol, 2014; 35: 10375–79
15. Ryan BM, Robles AI, Harris CC: Genetic variation in microRNA networks: The implications for cancer research. Nat Rev Cancer, 2010; 10: 389–402
16. Zhu S, Wu W, Wu F et al: MicroRNA-21 targets tumor suppressor genes in invasion and metastasis. Cell Res, 2008; 18: 350–59
17. Ye K, Wang KK, Gu J et al: Genetic variations in microRNA-related genes are novel susceptibility loci for esophageal cancer risk. Cancer Prev Res (Phila), 2008; 1: 460–69
18. Duan R, Pak C, Jin P: Single nucleotide polymorphism associated with mature miR-125a alters the processing of pri-miRNA. Hum Mol Genet, 2007; 16: 1124–31
19. Gottwein E, Cai X, Cullen BR: Expression and function of microRNAs encoded by Kaposi's sarcoma-associated herpesvirus. Cold Spring Harb Symp Quant Biol, 2006; 71: 357–64
20. Hu Z, Chen J, Tian T et al: Genetic variants of miRNA sequences and non-small cell lung cancer survival. J Clin Invest, 2008; 118: 2600–8
21. Tian T, Shu Y, Chen J et al: A functional genetic variant in microRNA-196a2 is associated with increased susceptibility of lung cancer in Chinese. Cancer Epidemiol Biomarkers Prev, 2009; 18: 1183–87
22. Zang S, Qia J, Cao et al: A potentially functional polymorphism in the promoter region of miR-34b/c is associated with renal cell cancer risk in a Chinese population. Mutagenesis, 2014; 29: 149–54
23. Bossard P, Zaret KS: GATA transcription factors as potentiators of gut endoderm differentiation. Development, 1998; 125: 4909–17
24. Chou, J, Provot S, Werb Z: GATA3 in development and cancer differentiation. Cells GATA have III! Cell Physiol, 2010; 222: 42–49
25. Xu Y, Liu L, Liu J et al: A potentially functional polymorphism in the promoter region of miR-34b/c is associated with increased an risk for primary hepatocellular carcinoma. Int J Cancer, 2011; 128: 412–17
26. Cheung TH, Man KN, Yu MY et al: Dysregulated microRNAs in the pathogenesis and progression of cervical neoplasia. Cell Cycle (Georgetown, TX), 2012; 11: 2876–84
27. Hagman Z, Larn, Edsj A et al: miR-34c is downregulated in prostate cancer and exerts tumor suppressive functions. Int J Cancer, 2010; 127: 2768–76
28. Hiyoshi Y, Schetter AJ, Okayama H et al: Increased microRNA-34b and -34c predominantly expressed in stromal tissues is associated with poor prognosis in human colon cancer. PLoS One, 2015; 10: e0124899
29. Tong N, Chu H, Wang et al: Pri-miR-34b/c rs4938723 polymorphism contributes to acute lymphoblastic leukemia susceptibility in Chinese children. Leuk Lymphoma, 2016; 57: 1436–41
30. Chen L, Shen Y, Zhang JB et al: Association between polymorphisms in the promoter region of pri-miR-34b/c and risk of hepatocellular carcinoma. Genet Mol Res, 2016; 15(4)
31. Zhu J, Yang L, You W et al: Genetic variation in miR-100 rs1834306 is associated with decreased risk for esophageal squamous cell carcinoma in Kazakh patients in northwest China. Int J Clin Exp Pathol, 2015; 8: 7332–40
32. Li Y, Liao S, Li J et al: An updated meta-analysis of 23 case-control studies on the association between miR-34b/c polymorphism and cancer risk. Oncotarget, 2017; 8: 28888–96
33. Wang A, Lu X, Fang Y et al: Association between miR-34b/c polymorphism rs4938723 and cancer risk: A meta-analysis of 11 studies including 6169 cases and 6337 controls. Med Sci Monit, 2014; 20: 1977–82
34. Bensen JT, Tse CK, Nyante SJ et al: Association of germline microRNA SNPs in pre-miRNA flanking region and breast cancer risk and survival. The Carolina Breast Cancer Study. Cancer Causes Control, 2013; 24: 1099–109
35. Bulbul I, Wang W, Tang Y et al: Association between polymorphisms in the promoter region of microRNA-34b/c and the chemoradiotherapy efficacy for locally advanced esophageal squamous cell carcinoma in Chinese Han population. Pathol Oncol Res, 2017 [Epub ahead of print]

### Conclusions

Our present analysis found novel evidence that the pri-miR-34b/c rs4938723 polymorphism had 2-tier effects on the risk of different types of cancers: rs493723 polymorphism was associated increased risk of hepatocellular carcinoma and decreased risk of leukemia, colorectal and esophageal cancer. Further studies with larger samples are needed to evaluate associations between rs4938723 polymorphism and each type of cancer.

### Conflict of Interest

None.

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)
36. Carvalho IN, Reis AH, Dos Santos AC, Vargas FR: A polymorphism in miR-34b/c as a potential biomarker for early onset of hereditary retinoblastoma. Cancer Biomark, 2017; 18: 313–17
37. Chen P, Sun R, Pu Y et al: Pri-miR-34b/c and TP-53 polymorphisms are associated with the susceptibility of papillary thyroid carcinoma: a case-control study. Medicine, 2015; 94: e1536
38. Gao LB, Li LJ, Pan XM et al: A genetic variant in the promoter region of miR-34b/c is associated with a reduced risk of colorectal cancer. Bioc Chem, 2013; 394: 415–20
39. Han Y, Pu R, Han X et al: Assocations of pri-miR-34b/c and pre-miR-196a2 polymorphisms and their multiplicative interactions with hepatitis B virus mutations with hepatocellular carcinoma risk. PLoS One, 2013; 8: e58564
40. Hashemi M, Bahari G, Nader M et al: Pri-miR-34b/c rs4938723 polymorphism is associated with the risk of childhood acute lymphoblastic leukemia. Cancer Genet, 2016; 209: 493–96
41. Hashemi M, Danesh H, Bihani F et al: Pri-miR-34b/c rs4938723 polymorphism increased the risk of prostate cancer. Cancer Biomark, 2017; 18: 155–59
42. He J, Zou Y, Liu X et al: Association of common genetic variants in pre-miRNA genes and neuroblastoma susceptibility: A two-center study in Chinese children. Mol Ther Nucleic Acids, 2018; 11: 1–8
43. Li L, Wu J, Sima X et al: Interactions of miR-34b/c and TP-53 polymorphisms and their multiplicative interactions with hepatitis B virus mutations with hepatocellular carcinoma risk. Cancer Biomark, 2017; 18: 155–59
44. Liu CJ, Ma XW, Zhang XJ, Shen SQ: pri-miR-34b/c rs4938723 polymorphism is associated with hepatocellular carcinoma risk: A case-control study in a Chinese population. Int J Mol Epidemiol Genet, 2017; 8: 1–7
45. Oh J, Kim JW, Lee BE et al: Polymorphisms of the pri-miR-34b/c promoter and TP53 codon 72 are associated with risk of colorectal cancer. Oncol Rep, 2014; 31: 995–1002
46. Pan XM, Su, RF, Li ZH et al: Pri-miR-34b/c rs4938723 polymorphism is associated with a decreased risk of gastric cancer. Genet Test Mol Biomarkers, 2015; 19: 198–202
47. Sanaei S, Hashemi M, Rezaei M et al: Evaluation of the pri-miR-34b/c rs4938723 polymorphism and its association with breast cancer risk. Biomed Rep, 2016; 5: 125–29
48. Singh DK, Zhang WB, Xu Y et al: Hsa-miR-34b/c rs4938723 T>C, pri-miR-124-1 rs331564 C>G, pre-miR-125a rs12975333 G>T and hsa-miR-423 rs6505162 C>A polymorphisms and the risk of gastric cardia adenocarcinoma. Int J Clin Exp Med, 2017; 10: 14919–26
49. Son MS, Jang MJ, Jeon Y et al: Promoter polymorphisms of pri-miR-34b/c are associated with hepatocellular carcinoma. Gene, 2013; 524: 156–60
50. Tian Q, Jia J, Ling S et al: A causal role for circulating miR-34b in osteosarcoma. Eur J Surg Oncol, 2014; 40: 67–72
51. Yang C, Ma X, Liu D et al: Promoter polymorphisms of miR-34b/c are associated with risk of gastric cancer in a Chinese population. Tumour Biol, 2014; 35: 12545–54
52. Yin J, Wang X, Zheng L et al: Hsa-miR-34b/c rs4938723 T>C and hsa-miR-423 rs6505162 C>A polymorphisms are associated with the risk of esophageal cancer in a Chinese population. PLoS One, 2013; 8: e80570
53. You WY: [A case control-study on the association between polymorphisms of microRNA genes and susceptibility for Kazakh’s esophageal cancer.] Shihzei University. A Dissertation for Master’s Degree in China, 2011 [in Chinese]
54. Yuan F, Sun R, Chen P et al: Combined analysis of pri-miR-34b/c rs4938723 and TP53 Arg72Pro with cervical cancer risk. Tumour Biol, 2016; 37: 6267–73
55. Zhang J, Huang X, Xiao J et al: Pri-miR-124 rs331564 and pri-miR-34b/c rs4938723 polymorphisms are associated with decreased risk of esophageal squamous cell carcinoma in Chinese populations. PLoS One, 2014; 9: e100055
56. Wu Y, Jia Z, Cao D et al: Predictive value of miR-219-1, miR-938, miR-34b/c, and miR-218 polymorphisms for gastric cancer susceptibility and prognosis. Dis Markers, 2017; 201: 4731891
57. Pu R: [The role of a polymorphism in promoter region of miR-34b/c and hepatitis B virus mutations in HBV-positive hepatocellular carcinoma.] Second Military Medical University Master’s thesis, 2012 [in Chinese]
58. DerSimonian R, Laird N: Meta-analysis in clinical trials. Controll Clin Trials, 1986; 7: 177–88
59. Mantel N, Haenszel W: Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst, 1959; 22: 719–48
60. Egger M, Davey Smith G, Schneider M Mindr C: Bias in meta-analysis detected by a simple, graphical test. BMJ, 1997; 315: 629–34
61. Hayashino Y, Noguchi Y, Fukui T: Systematic evaluation and comparison of statistical tests for publication bias. J Epidemiol, 2005; 15: 235–43
62. He L, He X, Lim LP et al: A microRNA component of the p53 tumour suppressor network. Nature, 2007; 447: 1130–34
63. He X, He L Hannon GI: The guardian’s little helper: MicroRNAs in the p53 tumor suppressor network. Cancer Res, 2007; 67: 11099–101
64. Mandke P, Wyatt N, Fraser J et al: MicroRNA-34a modulates MDM4 expression via a target site in the open reading frame. PLoS One, 2012; 7: e42034
65. Feng Z, Zhang C, Wu R, Hu W: Tumor suppressor p53 meets microRNAs. J Mol Cell Biol, 2011; 3: 44–50
66. Pharoah PD, Dunning AM, Ponder BA, Easton DF: Association studies for finding cancer-susceptibility genetic variants. Nat Rev Cancer, 2004; 4: 850–60