Quantitative Assessment of the Association between Genetic Variants in MicroRNAs and Colorectal Cancer Risk

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Background. The associations between polymorphisms in microRNAs and the susceptibility of colorectal cancer (CRC) were inconsistent in previous studies. This study aims to quantify the strength of the correlation between the four common polymorphisms among microRNAs (hsa-mir-146a rs2910164, hsa-mir-149 rs2292832, hsa-mir-196a2 rs11614913, and hsa-mir-499 rs3746444) and CRC risk.

Methods. We searched PubMed, Web of Knowledge, and CNKI to find relevant studies. The combined odds ratio (OR) with 95% confidence interval (95% CI) was used to estimate the strength of the association in a fixed or random effect model.

Results. 15 studies involving 5,486 CRC patients and 7,184 controls were included. Meta-analyses showed that rs3746444 had association with CRC risk in Caucasians (OR = 0.57, 95% CI = 0.34–0.95). In the subgroup analysis, we found significant associations between rs2910164 and CRC in hospital based studies (OR = 1.24, 95% CI = 1.03–1.49). rs2292832 may be a high risk factor of CRC in population based studied (OR = 1.18, 95% CI = 1.08–1.38).

Conclusion. This meta-analysis showed that rs2910164 and rs2292832 may increase the risk of CRC. However, rs11614913 polymorphism may reduce the risk of CRC. Rs3746444 may have a decreased risk to CRC in Caucasians.

1. Introduction

Colorectal cancer (CRC) is the third leading cause of cancer death for both men and women in the USA [1]. In Europe, CRC represents one of the primary causes of cancer deaths [2], and, in Asia, CRC is the fourth leading cause of mortality by cancer, and its incidence is increasing [3]. Epidemiological evidences have suggested that the risk of colorectal neoplasm is affected by multiple factors including a positive family history, excessive meat consumption, smoking status, and alcohol consumption, as well as genetic alterations, such as genetic polymorphisms [2].

However, the mechanism of colorectal carcinogenesis is still not fully understood. Compared with other complex diseases, CRC may be caused by both genetic and environmental factors [4]. Because well-recognized genetic predisposition syndromes account for less than 3% of CRC, low-penetrance genetic factors alone or in combination with environmental factors probably contribute to the development of CRC [5].

MicroRNAs (miRNAs) are a large class of small noncoding RNAs, which participate in various biological processes and may regulate tumor suppressor genes or oncogenes [6]. Single nucleotide polymorphisms (SNPs) in miRNAs may alter the expression, processing, and transcription of miRNAs and thus contribute to cancer development [7]. Recently, many epidemiological studies have demonstrated that some SNPs in the miRNA genes could alter miRNA expression and/or maturation and also be associated with the susceptibility and progression of cancer [8]. R s2910164 in hsa-mir-146a locus resides at position +60 relative to the first nucleotide of the pre-miR-146a gene. The previous studies have shown that miR-146a plays an important role in cell proliferation and metastatic ability in cancers and the variation of miR-146a may be involved in carcinogenesis.
impact on the expression of its target gene, which may play the expression levels of mature miR-196a and may have an mature sequence of miR-196a-3P. Rs11614913 could influence (miR-196a-5P and miR-196a-3P) and rs11614913 located in the stem region of the mir-499 gene. And several studies have identified miR-499 rs3746444 as a possible biomarker for some cancers [11–13]. Compared with other three genes, there were few studies of rs2292932 in miR-149 up to now. But the T allele in miR-149 might be masked by the presence of other unidentified causal genes involved in cancer development [14]. Furthermore, several Genome-wide association studies have reported that these four common SNPs may be associated with cancer risk [8]. However, results of these studies remain inconsistent. To provide a more precise estimate of the association of miRNA polymorphisms with CRC risk, we performed a meta-analysis on all eligible published case-control studies and evaluated the effect of the four SNPs on CRC risk.

2. Methods

2.1. Publication Search. Relevant articles were independently searched by two authors (Wang M and Dai ZJ) in PubMed, Web of Science, and Chinese National Knowledge Infrastructure (CNKI). The keywords were as follows: colorectal cancer/colorectal carcinoma, microRNA 146a/149/196a2/499, and polymorphism/genotype/variant/SNP. All qualified studies prior to December 20, 2014, were included. The eligible literature must be published in English or Chinese. Furthermore, reference lists of main reports and review articles were also reviewed manually to identify additional relevant publications.

2.2. Selection Criteria. The following criteria were used to select eligible studies for further meta-analysis: (1) case-control design; (2) full-text study; (3) studies that evaluated the associations between miRNA polymorphisms and CRC risk; and (4) studies that included detailed genotyping data.

2.3. Data Extraction. Articles were reviewed independently by two reviewers and data with discrepancies were discussed by all authors. For each included study, the following information was collected: first author, year of publication, country of origin, ethnicity, source of control, total number of cases and controls, and genotyping methods as well as number of cases and controls with the different genotypes. Different ethnic groups were categorized as Caucasian, Asian, African, and “mixed.” All the case and control groups were well controlled.

2.4. Statistical Analysis. The associations between miRNA polymorphisms and CRC risk were measured by odds ratio (OR) with 95% confidence interval (CI). The significance of the pooled OR was determined by the Z test.

The meta-analysis assessed association by using 5 different genetic models: homozygous genetic model, heterozygote genetic model, dominant genetic model, recessive genetic model, and allelic model. Hardy-Weinberg equilibrium (HWE) among the control subjects was tested by the Chi-square test. Statistical heterogeneity among studies was assessed with the Q and I² statistics. If the P value of heterogeneity test was more than 0.05 (P > 0.05), the pooled OR estimate of the study was calculated by the fixed-effects model. Otherwise, the random-effects model was used. The value of the I index was used to assess the degree of heterogeneity (I² < 25%: no heterogeneity; 25% < I² < 50%: moderate heterogeneity; 50% < I² < 75%: high heterogeneity; and I² > 75%: extreme high heterogeneity). Publication bias was evaluated by the funnel plot. All statistical analyses were carried out with the review manager version 5.1 (Revman; The Cochrane Collaboration, Oxford, UK).

3. Results

3.1. Characteristics of Studies. According to the inclusion criteria defined above (Figure 1), fifteen studies on miRNA polymorphisms with CRC risk were identified [8], including 5,486 CRC patients and 7,184 cancer-free controls. All the included eligible studies were published in English or Chinese. Among the eligible fifteen studies, eleven studies were based on Asian backgrounds which were carried out in China and Korea. Only four studies were based on
Table 1: Characteristics of the studies included in the meta-analysis.

| First author | Year | Country       | Ethnicity | Genotyping method | Source of control | Number (case/control) | SNP number |
|--------------|------|---------------|-----------|-------------------|-------------------|----------------------|------------|
| Dikaiakos [28] | 2015 | Greek         | Caucasian | PCR-RFLP          | HB                | 157/299              | 1, 3, 4    |
| Kupcinskas [29] | 2014 | Lithuania, Latvia | Caucasian | TaqMan            | HB                | 193/428              | 1, 3       |
| Mao [30] | 2014 | China         | Asian     | SNPScan           | PB                | 554/566              | 1          |
| Hu [31] | 2014 | China         | Asian     | PCR-RFLP          | HB                | 276/373              | 1, 4       |
| Wu [15] | 2014 | China         | Asian     | ASA               | PB                | 175/300              | 1, 2, 4    |
| Lv [8] | 2013 | China         | Asian     | PCR-RFLP          | PB                | 353/540              | 1, 2, 3, 4 |
| Chae [21] | 2013 | Korea         | Asian     | PCR-RFLP          | PB                | 1147/1203            | 1          |
| Ma [32] | 2013 | China         | Asian     | TaqMan            | PB                | 160/178              | 1, 2, 3, 4 |
| Vinci [16] | 2013 | Italy         | Caucasian | TaqMan            | PB                | 478/477              | 2, 3       |
| Zhang [17] | 2012 | China         | Asian     | PCR-RFLP          | PB                | 197/212              | 1, 3       |
| Hezova [20] | 2012 | Czech         | Caucasian | TaqMan            | PB                | 446/502              | 1, 2, 3, 4 |
| Min [22] | 2012 | Korea         | Asian     | PCR-RFLP          | PB                | 573/588              | 3          |
| Zhu [33] | 2012 | China         | Asian     | TaqMan            | PB                | 252/543              | 3          |
| Chen [34] | 2012 | China         | Asian     | PCR-LDR           | PB                | 126/407              | 3          |
| Zhan [35] | 2011 | China         | Asian     | PCR-RFLP          | PB                | 315/510              | 3          |

HWE: Hardy-Weinberg equilibrium; PB: population based; HB: hospital based; PCR: polymerase chain reaction; RFLP: restriction fragment length polymorphism; LDR: ligation detection reaction; ASA: allele-specific amplification; SNP: single-nucleotide polymorphisms; SNP number 1: miR-146-a G>C (rs2910164); 2: miR-149 C>T (rs2292832); 3: miR-196a-2 C>T (rs11614913); 4: miR-499 A>G (rs3746444).  

Caucasian, having been carried out in Greek, Lithuania, Italy, and Czech, respectively. Simultaneously, there were seven hospital based studies and eight population based studies. Main characteristics of the included studies were listed in Table 1.

3.2. Meta-Analysis Results. As shown in Table 2, the frequencies of the minor allele varied widely across the eligible studies, ranging from 0.14 to 0.80 (rs2910164), 0.33 to 0.82 (rs2292832), 0.34 to 0.66 (rs11614913), and 0.14 to 0.68 (rs3746444). The average frequencies of the minor allele in the four polymorphisms were 0.47, 0.66, 0.51, and 0.27, respectively. The distributions of genotypes in the controls were all in agreement with HWE except four studies [8, 15–17].

The main results of this meta-analysis were presented in Table S1, in Supplementary Material available online at http://dx.doi.org/10.1155/2015/276410. There were 11 studies with 3,937 cases and 5,120 controls for rs2910164. miR-146a rs2910164 polymorphism has no association with CRC risk in the overall population (C versus G: OR = 0.96, 95% CI = 0.81–1.20, P = 0.88; CC versus GG + GC: OR = 0.99, 95% CI = 0.76–1.27, P = 0.91; GC + CC versus GG: OR = 0.99, 95% CI = 0.82–1.20, P = 0.90). When stratifying analysis by ethnicity, there was also no significant association observed between the rs2910164 and CRC susceptibility in the five genetic models. In the analysis stratified by the source of control, significant associations were observed in the hospital based studies for recessive genetic model (CC versus GG + GC: OR = 1.24, 95% CI = 1.03–1.49, P = 0.02). No associations were found in population based studies.

The association of the rs2292832 polymorphism with CRC susceptibility was investigated in 5 studies with 1,568 cases and 1,824 controls. We failed to find any significant associations in any genotype (T versus C: OR = 1.05, 95% CI = 0.95–1.17, P = 0.36; T T versus CC: OR = 1.09, 95% CI = 0.87–1.37, P = 0.44; CT versus CC: OR = 1.35, 95% CI = 0.64–2.86, P = 0.43; TT versus CC + CT: OR = 0.91, 95% CI = 0.54–1.53, P = 0.71; TT + CT versus CC: OR = 1.24, 95% CI = 0.82–1.87, P = 0.31). And there was also no association existing in any genetic models after we rejected the studies which were not in agreement with HWE. There was only one study based on Caucasian. When excluding the Caucasian study, the null association remained in Asians. We further made stratified analysis based on the source of control. And we observed significant associations in population based studies in the recessive genetic model (TT versus CC + CT: OR = 1.18, 95% CI = 1.08–1.38, P = 0.04).

Ten studies with 2,906 cases and 4,150 controls were used to evaluate the relationship between rs11614913 polymorphism and CRC risk. No significant association was detected
Table 2: MiRNA polymorphisms genotype distribution and allele frequency in cases and controls.

| First author        | Genotype (N) | Allele frequency (N) | MAF | HWE (P value) |
|---------------------|--------------|----------------------|-----|---------------|
|                     | Case Control | Case Control         |     |               |
|                     | Total AA Aa aa | Total AA Aa aa | A a |               |
| rs2910164           |              |                      |     |               |
| Dikaiakos 2015 [28] | 157 8 48 101 299 | 21 120 158 | 64 250 162 436 | 0.80 | 0.782 |
| Kupcinskas 2014 [29]| 193 140 50 2 428 | 275 134 15 | 330 54 684 164 | 0.14 | 0.789 |
| Mao 2014 [30]       | 554 70 291 186 566 | 85 271 205 | 431 663 441 681 | 0.61 | 0.768 |
| Hu 2014 [31]        | 200 34 82 84 373 | 44 187 142 | 250 150 275 471 | 0.38 | 0.137 |
| Wu 2014 [15]        | 175 22 59 80 300 | 53 120 114 | 103 219 226 348 | 0.68 | **0.035** |
| Lv 2013 [8]         | 331 54 230 47 513 | 96 274 143 | 338 324 560 466 | 0.49 | 0.080 |
| Chae 2013 [21]      | 399 61 182 156 568 | 121 282 165 | 304 494 524 612 | 0.62 | 0.980 |
| Ma 2013 [36]        | 1147 444 534 169 1203 | 397 614 192 | 1422 872 1408 998 | 0.38 | 0.075 |
| Vinci 2013 [16]     | 160 17 57 86 178 | 13 65 100 | 91 229 91 265 | 0.72 | 0.590 |
| Hezova 2012 [20]    | 197 115 70 12 212 | 124 79 9 | 300 94 327 97 | 0.24 | 0.415 |
| Min 2012 [22]       | 446 151 233 62 502 | 188 245 69 | 535 357 621 383 | 0.40 | 0.443 |
| rs2292832           |              |                      |     |               |
| Wu 2014 [15]        | 175 21 123 28 300 | 76 58 116 | 165 179 210 290 | 0.52 | **<0.001** |
| Lv 2013 [8]         | 347 30 64 253 459 | 48 103 308 | 124 570 199 719 | 0.82 | **<0.001** |
| Vinci 2013 [16]     | 160 79 58 23 178 | 86 75 17 | 216 104 247 109 | 0.33 | 0.912 |
| Zhang 2012 [14]     | 443 50 190 203 435 | 46 202 187 | 290 596 294 576 | 0.67 | 0.431 |
| Min 2012 [22]       | 446 48 177 221 502 | 51 219 232 | 273 619 321 683 | 0.69 | 0.948 |
| rs11614913          |              |                      |     |               |
| Dikaiakos 2015 [28] | 157 19 69 69 299 | 33 149 117 | 107 207 215 383 | 0.66 | 0.156 |
| Kupcinskas 2014 [29]| 193 79 87 27 428 | 199 174 54 | 245 141 572 282 | 0.37 | 0.104 |
| Lv 2013 [8]         | 374 10 223 114 531 | 109 331 91 | 243 451 549 413 | 0.60 | **<0.001** |
| Vinci 2013 [16]     | 160 62 86 12 178 | 83 84 11 | 210 110 250 106 | 0.34 | 0.087 |
| Hezova 2012 [20]    | 197 82 89 26 212 | 87 103 22 | 253 141 277 147 | 0.36 | 0.291 |
| Zhang 2012 [14]     | 455 79 204 172 463 | 81 197 185 | 362 548 359 567 | 0.60 | **0.026** |
| Min 2012 [22]       | 466 120 201 125 502 | 100 254 148 | 441 451 454 550 | 0.48 | 0.633 |
| Zhu 2012 [33]       | 573 140 303 130 588 | 121 295 172 | 583 563 537 639 | 0.49 | 0.790 |
| Chen 2012 [34]      | 126 27 64 35 407 | 94 206 107 | 118 134 394 420 | 0.53 | 0.788 |
| Zhan 2011 [35]      | 252 68 128 56 543 | 112 267 163 | 264 240 493 593 | 0.48 | 0.890 |
| rs3746444           |              |                      |     |               |
| Dikaiakos 2015 [28] | 157 85 64 8 299 | 182 99 18 | 234 80 463 135 | 0.25 | 0.361 |
| Hu 2014 [31]        | 211 157 49 5 373 | 282 81 10 | 363 59 654 101 | 0.14 | 0.162 |
| Wu 2014 [15]        | 175 96 17 38 300 | 141 44 63 | 209 93 326 170 | 0.31 | **<0.001** |
| Lv 2013 [8]         | 346 258 88 504 366 | 138 | — | — |
| Vinci 2013 [16]     | 160 35 32 93 178 | 17 56 105 | 102 218 90 266 | 0.68 | **0.026** |
| Min 2012 [22]       | 446 292 142 12 502 | 334 154 14 | 726 166 822 182 | 0.19 | 0.453 |

A: the major allele; a: the minor allele; MAF: minor allele frequencies.
| Study or subgroup | Experimental Events | Control Events | Weight | Odds ratio M-H, random, 95% CI | Odds ratio M-H, random, 95% CI |
|------------------|--------------------|----------------|--------|-------------------------------|-------------------------------|
| **2.1.1 Asian**  |                    |                |        |                               |                               |
| Chae et al. 2013 | 494                | 798            | 10.1%  | 1.39 [1.16, 1.67]              |                               |
| Hu et al. 2014  | 250                | 400            | 9.0%   | 0.97 [0.76, 1.25]              |                               |
| Lv et al. 2013  | 324                | 662            | 9.9%   | 0.76 [0.63, 0.93]              |                               |
| Ma et al. 2013  | 872                | 2294           | 11.0%  | 0.87 [0.77, 0.97]              |                               |
| Mao et al. 2014 | 663                | 1094           | 10.3%  | 1.00 [0.84, 1.18]              |                               |
| Min et al. 2012 | 357                | 892            | 10.1%  | 1.08 [0.90, 1.30]              |                               |
| Wu 2014         | 219                | 322            | 8.4%   | 1.38 [1.04, 1.84]              |                               |
| **Subtotal (95% CI)** | **6462**           | **7994**       | **69.0%** | **1.03 [0.88, 1.21]** | **1.03 [0.88, 1.21]** |
| Total events    | 3179               | 4053           |        |                               |                               |

Heterogeneity: $r^2 = 0.04; \chi^2 = 30.93, df (P < 0.0001); I^2 = 81$

Test for overall effect: $Z = 0.37 (P = 0.71)$

**2.1.2 Caucasian**

| Study or subgroup | Experimental Events | Control Events | Weight | Odds ratio M-H, random, 95% CI | Odds ratio M-H, random, 95% CI |
|------------------|--------------------|----------------|--------|-------------------------------|-------------------------------|
| Dikaiakos et al. 2015 | 250                | 314            | 7.8%   | 1.45 [1.05, 2.02]              |                               |
| Hezova et al. 2012 | 94                 | 694            | 8.0%   | 0.53 [0.39, 0.72]              |                               |
| Kupcinskas et al. 2014 | 54                 | 384            | 7.7%   | 0.68 [0.49, 0.95]              |                               |
| Vinci et al. 2013  | 229                | 320            | 7.6%   | 0.86 [0.61, 1.21]              |                               |
| **Subtotal (95% CI)** | **1712**           | **2226**       | **31.0%** | **0.82 [0.53, 1.26]** | **0.82 [0.53, 1.26]** |
| Total events      | 627                | 962            |        |                               |                               |

Heterogeneity: $r^2 = 0.16; \chi^2 = 20.39, df (P = 0.0001); I^2 = 85$

Test for overall effect: $Z = 0.91 (P = 0.36)$

Total (95% CI) | 8174 | 10220 | 100.0% | 0.96 [0.82, 1.12] |

Heterogeneity: $r^2 = 0.05; \chi^2 = 56.08, df (P < 0.00001); I^2 = 82$

Test for overall effect: $Z = 0.49 (P = 0.62)$

Test for subgroup differences: $\chi^2 = 0.97, df = 1 (P = 0.32), I^2 = 0$

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**Figure 2**: Forest plot of miR-146a rs2910164 polymorphism and CRC risk (C versus G). 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 all the genetic models (haploid model: OR = 1.10, 95% CI = 0.84–1.43, $P = 0.50$; homozygote comparison: OR = 1.18, 95% CI = 0.76–1.83, $P = 0.47$; heterozygote comparison: OR = 1.11, 95% CI = 0.82–1.50, $P = 0.49$; dominant model: OR = 1.13, 95% CI = 0.83–1.55, $P = 0.44$ and recessive model: OR = 1.07, 95% CI = 0.82–1.38, $P = 0.63$, Figure 4). When excluding the studies which were inconsistent with HWE, the results showed a significant association between rs11614913 polymorphism and CRC risk in the homozygous genetic model (TT versus CC: OR = 1.18, 95% CI = 0.76–1.83, $P = 0.47$; heterozygote comparison: OR = 1.11, 95% CI = 0.82–1.50, $P = 0.49$; dominant model: OR = 1.13, 95% CI = 0.83–1.55, $P = 0.44$ and recessive model: OR = 1.07, 95% CI = 0.82–1.38, $P = 0.63$, Figure 4). Further subgroup analysis by ethnicity showed no association between rs11614913 and CRC risk either in Caucasians or in Asians. And no significant associations were observed in population based studies and hospital based studies for all genetic models.

For miR-499 rs3746444 polymorphism, our meta-analysis contained 6 studies with 1,471 cases and 2,104 controls. Overall, the rs3746444 polymorphism has no association with CRC risk (haploid model: OR = 0.96, 95% CI = 0.84–1.10, $P = 0.58$; homozygote comparison: OR = 0.90, 95% CI = 0.67–1.22, $P = 0.50$; heterozygote comparison: OR = 0.83, 95% CI = 0.54–1.26, $P = 0.38$; dominant model: OR = 0.91, 95% CI = 0.71–1.17, $P = 0.45$ and recessive model: OR = 0.95, 95% CI = 0.73–1.25, $P = 0.73$). Further analysis of the studies which were in agreement with HWE also showed no association between the rs3746444 polymorphism and CRC. And null associations were found in hospital based studies or population based studies. However, in the stratified analysis according to ethnicity, the homozygote model demonstrated a significant decrease in the CRC risk in

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For miR-499 rs3746444 polymorphism, our meta-analysis contained 6 studies with 1,471 cases and 2,104 controls. Overall, the rs3746444 polymorphism has no association with CRC risk (haploid model: OR = 0.96, 95% CI = 0.84–1.10, $P = 0.58$; homozygote comparison: OR = 0.90, 95% CI = 0.67–1.22, $P = 0.50$; heterozygote comparison: OR = 0.83, 95% CI = 0.54–1.26, $P = 0.38$; dominant model: OR = 0.91, 95% CI = 0.71–1.17, $P = 0.45$ and recessive model: OR = 0.95, 95% CI = 0.73–1.25, $P = 0.73$). Further analysis of the studies which were in agreement with HWE also showed no association between the rs3746444 polymorphism and CRC. And null associations were found in hospital based studies or population based studies. However, in the stratified analysis according to ethnicity, the homozygote model demonstrated a significant decrease in the CRC risk in
### Study or subgroup

| Study or subgroup | Experimental Events | Total Events | Weight M-H, fixed, 95% CI | Odds ratio M-H, fixed, 95% CI |
|------------------|---------------------|-------------|---------------------------|-------------------------------|
| **1.1.1 Asian**  |                     |             |                           |                               |
| Lv et al. 2013   | 570                 | 694         | 16.3%                     | 1.27 [0.99, 1.63]             |
| Min et al. 2012  | 619                 | 892         | 28.9%                     | 1.07 [0.88, 1.29]             |
| Wu 2014          | 179                 | 344         | 16.7%                     | 0.79 [0.60, 1.04]             |
| Zhang et al. 2012| 596                 | 886         | 28.0%                     | 1.05 [0.86, 1.28]             |
| **Subtotal (95% CI)** | 2816               | 3292        | 89.8%                     | 1.05 [0.94, 1.17]             |
| **Total events** | 1964                | 2268        |                           |                               |

Heterogeneity: $\chi^2 = 6.53$, df = 3 ($P = 0.09$); $I^2 = 54\%$
Test for overall effect: $Z = 0.79$ ($P = 0.43$)

| **1.1.2 Caucasian** |                     |             |                           |                               |
| Vinci et al. 2013   | 104                 | 320         | 10.2%                     | 1.09 [0.79, 1.51]             |
| **Subtotal (95% CI)** | 320                | 356         | 10.2%                     | 1.09 [0.79, 1.51]             |
| **Total events**    | 104                 | 109         |                           |                               |

Heterogeneity: not applicable
Test for overall effect: $Z = 0.53$ ($P = 0.60$)

| **Total (95% CI)**  | 3136                | 3648        | 100.0%                    | 1.05 [0.95, 1.17]             |
| **Total events**    | 2068                | 2377        |                           |                               |

Heterogeneity: $\chi^2 = 6.59$, df = 4 ($P = 0.16$); $I^2 = 39\%$
Test for overall effect: $Z = 0.92$ ($P = 0.36$)
Test for subgroup differences: $\chi^2 = 0.06$, df = 1 ($P = 0.81$), $I^2 = 0\%$

**Figure 3**: Forest plot of miR-149 rs2292832 polymorphism and CRC risk (T versus C). 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.

Caucasians (GG versus AA: OR = 0.57, 95% CI = 0.34–0.95, $P = 0.03$, Figure 5).

#### 3.3. Publication Bias
In this meta-analysis, we performed funnel plot to access the publication bias. As showed in Figure 6, the funnel plots failed to reveal any obvious asymmetry in all genotypes in overall population. Therefore, the results indicated that publication bias was not significant in this meta-analysis.

### 4. Discussion

Allelic variants in the sequence of mature miRNAs represent a particularly interesting potential source of phenotypic diversity of genetic diseases, which may contribute directly to disease susceptibility [18]. Accumulating evidence has shown that miRNAs regulate the expression of roughly 30% of the all human genes through posttranscriptional mechanisms [19]. SNPs in miRNA genes could function through three ways: firstly, the transcription of the primary transcript; secondly, pri-miRNA and pre-miRNA processing; and thirdly, effects on miRNA-mRNA interactions [7]. Genetic effects connected to SNPs at the level of miRNA genes may have a significant relationship with the expression and clinical features in CRC [16].

A large number of studies had highlighted several associations between SNPs in miRNAs and the risk of CRC [8]. However, controversial experimental data were obtained. For example, there was no significant association between rs2910164 and CRC in Hezova et al.'s study [20], whereas Chae et al. reported that miR-146a rs2910164 polymorphism of genotype CC may contribute to a higher risk of CRC [21]. Min et al. from Korea [22] demonstrated that miR-196a2 rs11614913 polymorphism had a decreased risk to CRC, whereas Lv et al. [8] reported that T allele in rs11614913 was associated with an increased risk of CRC compared with the C allele.

The present meta-analysis, including 5,486 CRC patients and 7,184 cancer-free controls from 15 case-control studies, was conducted to evaluate the association between the four common SNPs in miRNAs (miR-146a rs2910164, miR-149 rs2292832, miR-196a2 rs11614913, and miR-499 rs3746444)
and CRC risk. In this study, we found that rs3746444 polymorphism has no association with CRC risk in the overall population. However, accumulative data from two studies based on Caucasian background (317 cases and 477 controls) showed significantly decreased cancer risks in the homozygous model. Considering the limited sample size, we need more large well-designed studies to evaluate the result. Simultaneously, we failed to find any significant correlation between the rs2910164, rs2292832, and rs11614913 polymorphisms and risk of CRC in overall studies or in different ethnic groups for all genetic models. But significant association between rs2910164 and CRC was observed in hospital based studies in the recessive model. And we found that rs2292832 polymorphisms may be associated with increased the risk of CRC in population based studies for recessive model. While T allele of rs11614913 was a protect factor of CRC in the homozygous model when we excluded the studies which were not in agreement with HWE.

Although the biological mechanism of SNPs in miRNAs contributing to the regulation of cancer susceptibility and development remains unknown, a large quantity of meta-analyses has been engaged in the newly developed field in the past few years [23]. A previous meta-analysis by K. Srivastava and A. Srivastava [23] was demonstrated that miR-196a2 rs11614913 polymorphisms have significant associations with overall cancer risk. In Xu et al’s meta-analysis [24], TT genotype of rs11614913 polymorphism was associated with decreased cancer risk. Rs2910164 C allele was associated with decreased overall cancer risk especially for cervical cancer and prostate cancer risk in Chinese population. Rs3746444 G allele was a risk factor in Chinese population, especially for breast cancer. Different results were also presented in several

| Study or subgroup | Experimental Events | Total | Control Events | Total | Weight | Odds ratio M-H, random, 95% CI | Odds ratio M-H, random, 95% CI |
|------------------|---------------------|-------|----------------|-------|--------|-------------------------------|-------------------------------|
| **4.4.1 Asian**  |                     |       |                |       |        |                               |                               |
| Chen et al. 2012 | 35                  | 126   | 107            | 407   | 9.7%   | 1.08 [0.69, 1.69]              |                               |
| Lv et al. 2013  | 114                 | 347   | 91             | 531   | 11.3%  | 3.27 [1.72, 3.25]              |                               |
| Min et al. 2012 | 125                 | 446   | 148            | 502   | 11.7%  | 0.93 [0.70, 1.23]              |                               |
| Zhan et al. 2011| 56                  | 252   | 163            | 543   | 10.9%  | 0.67 [0.47, 0.94]              |                               |
| Zhang et al. 2012| 172                | 455   | 185            | 463   | 11.9%  | 0.91 [0.70, 1.19]              |                               |
| Zhu et al. 2012  | 130                 | 573   | 172            | 588   | 11.9%  | 0.71 [0.54, 0.92]              |                               |
| **Subtotal (95% CI)** | 2199              | 8304  | 673           | 2199  | 67.3%  | 1.00 [0.70, 1.44]              |                               |
| **Total events** | 632                | 866   |                |       |        |                               |                               |

Heterogeneity: $r^2 = 0.18; \chi^2 = 40.50$, df ($P < 0.00001$); $I^2 = 88$

Test for overall effect: $Z = 0.01$ ($P = 0.99$)

| **4.4.2 Caucasian** |                     |       |                |       |        |                               |                               |
| Dikaiakos et al. 2015 | 69               | 157   | 117            | 299   | 10.4%  | 1.22 [0.82, 1.80]              |                               |
| Hezova et al. 2012  | 26                 | 197   | 22             | 212   | 7.8%   | 1.31 [0.72, 2.40]              |                               |
| Kupcinskas et al. 2014 | 27              | 193   | 54             | 427   | 9.0%   | 1.12 [0.68, 1.85]              |                               |
| Vinci et al. 2013   | 12                 | 160   | 11             | 178   | 5.5%   | 1.23 [0.53, 2.87]              |                               |
| **Subtotal (95% CI)** | 707               | 1116  |                |       | 32.7%  | 1.21 [0.93, 1.57]              |                               |
| **Total events** | 134                | 204   |                |       |        |                               |                               |

Heterogeneity: $r^2 = 0.00; \chi^2 = 0.16$, df ($P = 0.98$); $I^2 = 0$

Test for overall effect: $Z = 1.43$ ($P = 0.15$)

| **Total (95% CI)** | 2906               | 4150  | 100.0%        |       | 1.07 [0.82, 1.38]              |                               |
| **Total events** | 766                | 1070  |                |       |        |                               |                               |

Heterogeneity: $r^2 = 0.13; \chi^2 = 42.77$, df ($P < 0.00001$); $I^2 = 79$

Test for overall effect: $Z = 0.49$ ($P = 0.63$)

Test for subgroup differences: $\chi^2 = 0.69$, df ($P = 0.40$), $I^2 = 0$

**Figure 4:** Forest plot of miR-196a2 rs11614913 polymorphism and CRC risk (TT versus CC + CT). 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.
| Study or subgroup | Experimental Events | Experimental Total | Control Events | Control Total | Weight | Odds ratio M-H, fixed, 95% CI | Odds ratio M-H, fixed, 95% CI |
|------------------|---------------------|-------------------|----------------|---------------|--------|----------------------------|----------------------------|
| 3.2.1 Asian      |                     |                   |                |               |        |                            |                            |
| Hu et al. 2014   | 5                   | 162               | 10             | 292           | 7.8%   | 0.90 [0.30, 2.67]           |                            |
| Min et al. 2012  | 12                  | 304               | 14             | 348           | 14.1%  | 0.98 [0.45, 2.15]           |                            |
| Wu 2014          | 38                  | 134               | 63             | 272           | 33.4%  | 1.31 [0.82, 2.10]           |                            |
| **Subtotal (95% CI)** | **600**           | **912**           | **55.3%**      | **1.17 [0.80, 1.71]** |        |                            |                            |
| **Total events** | 55                  | 87                |                |               |        |                            |                            |
| Heterogeneity: $\tau^2 = 0.65$, df = 2 ($P = 0.72$); $I^2 = 0\%$ | | | | | | | |
| Test for overall effect: $Z = 0.82$ ($P = 0.41$) | | | | | | | |
| 3.2.2 Caucasian  |                     |                   |                |               |        |                            |                            |
| Dikaiakos et al. 2015 | 8                  | 93                | 18             | 200           | 11.7%  | 0.95 [0.40, 2.28]           |                            |
| Vinci et al. 2013 | 93                  | 128               | 105            | 322           | 33.0%  | 0.43 [0.23, 0.82]           |                            |
| **Subtotal (95% CI)** | **221**           | **322**           | **44.7%**      | **0.57 [0.34, 0.95]** |        |                            |                            |
| **Total events** | 101                 | 123               |                |               |        |                            |                            |
| Heterogeneity: $\tau^2 = 2.06$, df = 1 ($P = 0.15$); $I^2 = 52\%$ | | | | | | | |
| Test for overall effect: $Z = 2.15$ ($P = 0.03$) | | | | | | | |
| **Total (95% CI)** | **821**            | **1234**          | **100.0%**     | **0.90 [0.67, 1.22]** |        |                            |                            |
| **Total events** | 156                 | 210               |                |               |        |                            |                            |
| Heterogeneity: $\tau^2 = 7.61$, df = 4 ($P = 0.11$); $I^2 = 47\%$ | | | | | | | |
| Test for overall effect: $Z = 0.68$ ($P = 0.50$) | | | | | | | |
| Test for subgroup differences: $\tau^2 = 4.91$, df = 1 ($P = 0.03$); $I^2 = 79.6\%$ | | | | | | | |

**Figure 5:** Forest plot of miR-499 rs3746444 polymorphism and CRC risk (GG versus AA). 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.

Meta-analyses which evaluated the relationship between miRNAs and CRC risk [25]. Wan et al.’s study demonstrates that miR-196a2 rs11614913 most likely contributes to decreased risk of CRC, whereas miR-146a rs2910164 may not be associated with the susceptibility to CRC [25]. While the pooled data from Du et al.’s meta-analysis supported that the miR-196a2 rs11614913 and miR-149 rs2292832 polymorphisms may contribute to susceptibility to CRC [26]. The latest meta-analysis, which related to the associations between miRNA polymorphisms and colorectal cancer, performed by Wu et al. indicated that SNP rs11614913 but not SNP rs2910164 and SNP rs2292832 may contribute to susceptibility to CRC in an Asian-specific manner [27]. This result was unconformable with our results. Compared with Wu’s meta-analysis which included 9 relevant studies, our research totally identified 15 studies including 5,486 CRC cases and 7,184 controls. Thus, our meta-analysis contained the newest data and largest sample size on the study of the relationship between miRNAs and colorectal cancer. Additionally, we performed subgroup analyses by different data information in more detail.

Some limitations still existed in this meta-analysis. Firstly, the included studies are mainly based on Asian background. There were only five studies based on Caucasian background and no studies on African background. Secondly, some detailed information (such as sex, age, life-style, and environmental factors) was not considered. Further large scale multicenter studies based on Caucasian or African will be needed to clarify the possible roles of these polymorphisms in CRC. Though many investigators have been devoted to identify the roles of the miRNAs in carcinogenesis, the mechanisms were still unclear. Further biologically functional studies are warranted to explain the molecular mechanisms.

In summary, this meta-analysis showed that there were no associations between the four polymorphisms and CRC risk in the overall population. Further analysis stratified by the source of control showed that rs2910164 polymorphism is associated with CRC risk in hospital based studies, and rs2292832 polymorphism may contribute to the susceptibility of CRC in population based studies. In the pooled analysis from all studies which are in agreement with HWE revealed that rs11614913 TT carriers may have a decreased CRC risk. Moreover, in the stratified analysis according to ethnicity, rs3746444 polymorphism may reduce the risk of CRC in Caucasians.
Conflict of Interests

No conflict of interests exists for any of the authors.

Authors’ Contribution

Meng Wang and Dan Xu contributed equally to this work.

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