Significant association between functional microRNA polymorphisms and head and neck cancer susceptibility: a comprehensive meta-analysis

Yu-Ming Niu1,4,*, Xin-Ya Du2,*, Ming-Yi Lu3, Qiong-Li Xu1, Jie Luo4 & Ming Shen5

Molecular epidemiological studies have showed a closer association between microRNA polymorphisms with and head and neck cancer (HNC) risk. But the results of these studies were inconsistent. We performed this meta-analysis to clarify the associations between microRNA polymorphisms and HNC risk. Four electronic databases (PubMed, Embase, CNKI, and Wanfang) were searched. Odds ratios (ORs) with 95% confidence interval (CIs) were calculated to assess the association between microRNA-146a rs2910164 G>C, microRNA-196a2 rs11614913 C>T, microRNA-149 rs2292832 C>T, microRNA-499 rs3746444 A>G polymorphisms and HNC risk. Heterogeneity, publication bias and sensitivity analysis were conducted to guarantee the statistical power. Overall, 11 selected articles involving 16100 subjects were included in this meta-analysis. Significantly increased risk between microRNA-146a rs2910164 G>C polymorphism and HNC risk were observed in Caucasian population (GC vs. GG: OR = 1.31, 95%CI = 1.01–1.68; GC + CC vs. GG: OR = 1.26, 95%CI = 1.02–1.57). For microRNA-196a2 rs11614913 C>T, similarly increased risk were also found in Asian population (T vs. C, OR = 1.14, 95%CI = 1.04–1.25; TT vs. CC, OR = 1.33, 95%CI = 1.09–1.64; CT + TT vs. CC OR = 1.32, 95%CI = 0.99–1.76; TT vs. CC + CT, OR = 1.14, 95%CI = 0.99–1.33). In addition, no significant association was detected between microRNA-149 rs2292832 C>T and microRNA-499 rs3746444 A>G polymorphism and HNC risk. This meta-analysis demonstrates that microRNA polymorphisms are associated with HNC development based on ethnicity diversity.

Head and neck cancer (HNC) is the sixth most common malignancy worldwide and comprises a variety of epithelial malignancies involving the oral cavity, nasal cavity, thyroid, pharynx, and larynx1. Approximately 633,000 new cases and 355,000 deaths were reported in 2008, resulting in severe disability,

1Department of Stomatology and Center for Evidence-Based Medicine and Clinical Research, Taihe Hospital, Hubei University of Medicine, 32 South Renmin Road, Shiyan 442000, China. 2Department of Stomatology, People’s Hospital of New District Longhua Shenzhen, 2 East Jianshe Road, Shenzhen 518109, China. 3Department of Oral and Maxillofacial Surgery, Chung Shan Medical University Hospital, No. 119, Sec. 1, Chien-Kuo N. Rd, Taichung 40201, China. 4Department of Neurosurgery and Evidence-Based Medicine Center, Taihe Hospital, Hubei University of Medicine, 32 South Renmin Road, Shiyan 442000, China. 5Jiangsu Key Laboratory of Oral Diseases, Nanjing Medical University; Department of Dental Implant, Affiliated Hospital of Stomatology, Nanjing Medical University, No. 140 Hanzhong Road, Nanjing 210029, China. *These authors contributed equally to this work. Correspondence and requests for materials should be addressed to J.L. (email: taihehospital@yeah.net) or M.S. (email: mingshen85@yahoo.com)
reduced the quality of life, and a poor survival rate, as well as an increased economic burden on individuals and society. Various factors, such as lifestyle habits (tobacco and alcohol consumption), viral infection (human papillomavirus (HPV)) and oral hygiene have been proven to contribute to the development of HNC. However, the factors contributing to susceptibility are still being explored. Progress has been made recently, but the treatment and prognosis for HNC are not yet satisfactory.

To date, many molecular epidemiological studies have shown that genetic factors may play an important role in tumorigenesis, and the genetic predisposition is gaining increasing attention. MicroRNAs are short, single-stranded, noncoding RNAs that are 20–22 nucleotides long and they participate in the post-transcriptional regulation of gene expression. They are critical regulators of various fundamental biological processes such as proliferation, differentiation, apoptosis, and disease susceptibility. SNPs have been reported to be associated with HNC risk, but the results have been conflicting. Therefore, a comprehensive meta-analysis involving the related publications was performed to assess the possible association between microRNA polymorphisms and HNC susceptibility.

Methods

Search strategy. Four electronic databases (Pubmed, Embase, CNKI, and Wanfang) were searched using the following terms: “microRNA”, “miRNA”, “head and neck cancer”, “polymorphism”, and “variant”, up to December 1, 2014. The combined phrases for all genetic studies on the association between HNC and microRNA polymorphisms were also used. Only studies written in English and Chinese were selected.

Study selection. All selected studies fulfilled the following inclusion criteria: (1) case-control design focus on HNC; (2) research on microRNA polymorphisms; and (3) adequate genotype data (or data available to calculate) to assess the odds ratio (OR) and 95% confidence interval (CI). The exclusion criteria included: (1) review articles; (2) case reports; (3) results without the research polymorphisms or outcome data; (4) animal model research; and (5) repeated or overlapping publications with the same author or team were deleted according to the publication date or sample size.

Data extraction. Two reviewers (Niu and Du) independently collected the data for analysis, including the first author’s name, publication year, sources of controls, study country/region, ethnicity of participants (such as Asian or Caucasian), genotyping method, and number of genotypes in HNC cases and controls. A third reviewer was introduced (Lu) to adjust all discrepancies during the analysis for consistency. The Hardy-Weinberg equilibrium (HWE) was calculated based on the genotypes of the controls.

Statistical analysis. ORs with 95% CIs were calculated to evaluate the strength of the association between the four polymorphisms and HNC risk. For the microRNA-146a rs2910164 G>C polymorphism, the pooled ORs were obtained for the allele contrast (C vs. G), co-dominant model (GC vs. GG), dominant model (GC + CC vs. GG), and recessive model (CC vs. GG + GC). Similar genetic models were also assessed for the microRNA-149 rs2292832 C>T, microRNA-196a2 rs11614913 C>T and microRNA-499 rs3746444 A>G variants. Subgroup analyses of ethnicity, study design, cancer location (type) and genotyping methods were also submitted to statistical testing. Heterogeneity was assessed with the Cochran’s Q statistic and F method. ORs estimation was calculated with a fixed-effects model (the Mantel-Haenszel method) when the P value was more than 0.10 or F was less than 40%; otherwise, a random-effects model (DerSimonian and Laird method) was adopted. A further-more meta-regression was conducted to analyze the existed heterogeneity. Cumulative meta-analyses
and sensitivity analyses were conducted to evaluate the stability of the results by removal of each study sequentially for each polymorphism. The potential publication bias of the literature was analyzed by Egger’s linear regression and Begg’s funnel plots. Statistical analysis was performed using STATA version 11.0 (Stata Corporation, College Station, TX, USA) with two-sided P values and $P < 0.05$ considered statistically significant.

Results

Study characteristics. A total of 134 relevant studies were identified from a systematic literature search. The search procedure is shown in Fig. 1. Following the study selection criteria, 107 studies were excluded in the first step of title and duplicate screening step, and 16 studies were subsequently excluded from our research due to various deficiencies (3 were reviews, 5 were not on the research polymorphism locus, and 8 were focused on cell line and other no-related article). In total, 11 eligible articles were selected with adequate data, including seven studies on microRNA-146a rs2910164 G $>$ C, five studies on microRNA-196a2 rs11614913 C $>$ T, three publications on microRNA-149 rs2292832 C $>$ T, and two studies on microRNA-499 rs3746444 A $>$ G, respectively. Four studies involved Caucasian populations, and seven studies involved Asian populations. Regarding the genotyping method, eight studies used the Applied Biosystems, two studies adopted polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), one study used PCR with two-primer method and another was conducted with the MassARRAY iPLEX platform. Only one study deviated from the HWE analysis in microRNA-149 rs2292832 C $>$ T polymorphism. The detailed characteristics of the selected studies were summarized in Table 1.

Quantitative analysis. For microRNA-146a rs2910164 G $>$ C polymorphism. Seven eligible studies with 3,841 cases and 7,900 controls focused on microRNA-146a rs2910164 G $>$ C. The results of
the combined analyses revealed a significantly increased risk for HNC risk in the genotype mutation
genetic models (C vs. G: OR = 1.20, 95%CI = 1.04–1.39, \( P = 0.01 \), \( I^2 = 77.9\% \); GC vs. GG: OR = 1.27, 95%CI = 1.05–1.55, \( P = 0.02 \), \( I^2 = 68.4\% \); GC + CC vs. GG: OR = 1.30, 95%CI = 1.07–1.58, \( P = 0.01 \), \( I^2 = 70.0\% \), Fig. 2) (Table 2). Heterogeneities existed in all five models. Meta-regression analyses and
stratified analyses were conducted, but no critical factors were found to explain these heterogeneities. In
the subgroup analyses by ethnicity and control design, significantly increased risks were also found in
the Caucasian population (GC vs. GG: OR = 1.31, 95%CI = 1.01–1.68, \( P = 0.04 \), \( I^2 = 75.3\% \); GC + CC vs. GG: OR = 1.26, 95%CI = 1.02–1.57, \( P = 0.03 \), \( I^2 = 68.7\% \) and with some others gene models (Table 2).
Furthermore, some significantly increased risks were also observed in the subgroup analysis with geno-
typing method of Applied Biosystems (Table 2). Sensitivity analysis showed that no single study qualita-
tively changed the pooled ORs, indicating that the results of this meta-analysis were highly stable (Fig. 3
fordominant model). A cumulative analysis by publication date showed that the results gradually showed
a positive association beginning with a study by Lung et al. published in 2013 (Fig. 4 for dominant
model). Funnel plot and Egger’s test were performed to estimate the publication bias of the literature,
which did not reveal any asymmetrical evidence (Fig. 5 fordominant model). The results were further

| First author | Year | Country/ Region | Racial | Source of controls | Case | Control | Genotype distribution | Genotyping methods | \( P \) for HWE | Location |
|--------------|------|----------------|--------|-------------------|------|---------|----------------------|-------------------|-------------|----------|
| Jin          | 2018 | China          | Asian   | Hospital          | 500  | 500     | GC: 300, GC: 200     | Applied Biosystems | <0.01       | Laryngeal |
| Liu          | 2010 | USA            | Caucasian | Population       | 1000 | 1000    | GC: 500, GC: 500     | PCR-RFLP          | 0.06        | HN       |
| Chu          | 2012 | China          | Asian   | Hospital          | 450  | 450     | GC: 225, GC: 225     | Applied Biosystems | 0.30        | NP       |
| Roy          | 2014 | India          | Asian   | Hospital          | 500  | 500     | GC: 250, GC: 250     | PCR-RFLP          | <0.01       | Oral     |
| Li           | 2014 | China          | Asian   | Population        | 1000 | 1000    | GC: 500, GC: 500     | Applied Biosystems | 0.27        | NP       |

**Table 1.** Characteristics of case-control studies on microRNA polymorphisms and HNC risk included in the meta-analysis. MAF: Minor allele frequency in control group. NP: Nasopharyngeal; HN: head and neck. Population: Population controls Hospital: Hospital controls Healthy: Healthy controls. *HWE in control.
supported by the analysis of the data with Egger’s test (C vs. G: $P = 0.05$; GC vs. GG: $P = 0.57$; CC vs. GG: $P = 0.57$; GC + CC vs. GG: $P = 0.24$; CC vs. GG + GC: $P = 0.87$).

For microRNA-196a2 rs11614913 C $>$ T. Five publications with 3,534 cases and 3,564 controls reported the association between microRNA-196a2 rs11614913 C $>$ T polymorphisms and HNC risk. Overall, significant results were observed in the allele contrast model (T vs. C, OR = 1.10, 95%CI = 1.03–1.19, $P = 0.01$, $I^2 = 0$%) and co-dominant model (TC vs. CC, OR = 1.21, 95%CI = 1.04–1.41, $P = 0.01$, $I^2 = 2.5$%) (Table 2). Subsequent stratified analysis according to ethnicity and increased risks were found in an Asian population (T vs. C, OR = 1.14, 95%CI = 1.04–1.25, $P = 0.01$, $I^2 = 0$%; TT vs. CC, OR = 1.33, 95%CI = 1.09–1.61, $P < 0.01$, $I^2 = 0$%; CT + TT vs. CC OR = 1.32, 95%CI = 0.99–1.76, $P = 0.06$, $I^2 = 69.7$%; TT vs. CC + CT, OR = 1.14, 95%CI = 0.99–1.33, $P = 0.08$, $I^2 = 44.8$%) (Table 2). Moreover, the similarly increased cancer risks were found in the allele contrast, co-dominant (TT vs. CC) and recessive (CC vs. GG + GC) models with genotyping method of Applied Biosystems. Publication bias analysis was also conducted, and the funnel plots were symmetric with Egger’s test approved (T vs. C: $P = 0.26$; TC vs. CC: $P = 0.19$; TT vs. CC: $P = 0.25$; TC + TT vs. CC: $P = 0.36$; TT vs. CC + TC: $P = 0.82$).

For microRNA-149 rs2292832 C $>$ T and microRNA-499 rs3746444 A $>$ G. Three studies involving 1,852 cases and 1,677 controls and two studies with 1,579 cases and 1,555 controls were included in the microRNA-149 rs2292832 C $>$ T polymorphism, microRNA-499 rs3746444 A $>$ G and HNC risk research, respectively. No significant associations were found in all models for the two SNPs loci (Table 2). The subgroup analyses based on ethnicity, control design and genotyping methods were conducted and no significant associations were found.

Discussion

HNC is one of the most common malignant diseases in the world. Many treatment measures have been conducted in recent decades. However, morbidity and mortality are still high, and the prognosis is still poor. To date, with elucidation of the pathogenesis mechanism for interactions between microRNAs and cancer development, an increasing amount of attention has been paid to the association between the SNPs of microRNAs and HNC risks.

In 2008, Jazdzewski et al.\textsuperscript{27} reported the first significant increased association between the GC heterozygous and PTC risk recessive model (OR = 1.62, 95%CI = 1.3–2.0). Since then, a series of molecular epidemiological studies have been conducted, but the conclusions were inconsistent. In this meta-analysis, we investigated the associations between microRNA-146a rs2910164 G $>$ C, microRNA-196a2 rs11614913 C $>$ T, microRNA-149 rs2292832 C $>$ T, and microRNA-499 rs3746444 A $>$ G polymorphisms and HNC susceptibility on the basis of eleven selected case-control studies. Both microRNA-146a rs2910164 G $>$ C and microRNA-196a2 rs11614913 C $>$ T polymorphisms showed a significant association with HNC risk.
Table 2. Summary ORs and 95% CI of microRNA polymorphisms and HNC risk. Population: Population controls Hospital: Hospital controls Healthy: Healthy controls. HN: head and neck. ABI: Applied Biosystems. *Numbers of comparisons. †Test for heterogeneity.

based on a large sample size and greater number of studies. In the subgroup analysis based on ethnic diversity, we observed an increased risk for the microRNA-146a rs2910164 G > C polymorphism and HNC in the Caucasian population. Moreover, similar results also indicated that the microRNA-196a2 rs11614913 C > T may play a risk role in the development of HNC in the Asian population. In the past few decades, some studies have shown that different distribution of genotype existed in different ethnicity and influenced the disease susceptibility. Our meta-analysis also indicated that the ethnicity differences may be the most critical factor resulting in HNC susceptibility among the Asian and Caucasian
populations. Furthermore, it is worth noting that some significantly increased risks were observed in these analyzed results with the genotyping method of Applied Biosystems, but not PCR-RFLP. This consistency of results indicated that the genotyping method of Applied Biosystems was more useful to improve the accuracy of an experiment and to reduce some possible errors.

To our knowledge, this is the first quantitative assessment focused on the association between microRNA polymorphisms and HNC risk specially. Eleven articles involving 6,069 cases of HNC cases and 10,031 controls were included. Even though number of studies included in this meta-analysis was

![Figure 3](image-url) Sensitivity analysis through deleting each study to reflect the influence of the individual dataset to the pooled ORs in GC + CC vs. GG model of microRNA-146a rs2910164 G > C polymorphism.

![Figure 4](image-url) Cumulative meta-analyses according to publication year in GC + CC vs. GG model of microRNA-146a rs2910164 G > C polymorphism.

| Study          | OR (95% CI) |
|----------------|-------------|
| Jazdzewski (2008) | 1.39 (1.13, 1.71) |
| Liu (2010)     | 1.20 (0.91, 1.59) |
| Chu (2012)     | 1.18 (0.96, 1.45) |
| Lung (2013)    | 1.19 (1.01, 1.41) |
| Orsos (2013)   | 1.24 (1.07, 1.44) |
| Wei (2013)     | 1.20 (1.04, 1.37) |
| Lin (2014)     | 1.30 (1.07, 1.58) |
small, we believe that the findings can help to explain the association between microRNA polymorphisms and HNC risk. First, the genotype distributions in the controls of four selected SNP loci were all mostly consistent with HWE. Second, all five analysis comparison patterns were conducted, and the significant association were always consistent. Third, Egger's test and Begg's funnel plots proved that there was no apparent publication bias was existence in our meta-analysis. All these data would guarantee the strength of our results.

However, there were some limitations in this meta-analysis. First, heterogeneity existed and was especially high for the microRNA-146a rs2910164 G>C polymorphism. To our knowledge, much variability among studies in a systematic review is termed heterogeneity. There were three most important sources of variability between the studies; i.e., clinical diversity (sometimes called clinical heterogeneity), methodological diversity (sometimes called methodological heterogeneity) and statistical heterogeneity. We followed convention and referred to statistical heterogeneity simply as heterogeneity. In regards to the statistical heterogeneity in our analysis, factors such as the diversity of cancer type, classification of disease severity, environment and personal habits could influence the results. Furthermore, the diversity of genotyping methods among the included studies could bring about the existence of heterogeneity, which also would partly change the analyzed results. Second, the number of studies describing each polymorphism was limited, influencing the statistical power of our meta-analysis. Third, environmental factors such as smoking, drinking, and HPV infection have been shown to influence the development of HNC, and the status of local tumor invasion and lymph node metastasis may be influenced by the genetic mutation. However, in our meta-analysis, the interaction between the genetic mutation, environment factors, disease stage, and HNC susceptibility could not be conducted due to the data deficiency.

In conclusion, this meta-analysis indicated that two functional polymorphisms of microRNA-146a rs2910164 G>C and microRNA-196a2 rs11614913 C>T may play an important role in the development of HNC, especially considering ethnicity diversity. Further investigation into the relationship between microRNA polymorphisms, environmental factors, and HNC susceptibility is still needed.

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Author Contributions
N.Y.M., D.X.Y. and L.M.Y. performed the literature search, data extraction, and statistical analysis and wrote the manuscript. X.Q.L. and N.Y.M. supervised the literature search, data extraction, analysis, L.J. and S.M. reviews the manuscript.

Additional Information
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