Association of microRNA polymorphisms with the risk of head and neck squamous cell carcinoma in a Chinese population: a case–control study

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Abstract

Background: MicroRNA (miRNA) polymorphisms may alter miRNA-related processes, and they likely contribute to cancer susceptibility. Various studies have investigated the associations between genetic variants in several key miRNAs and the risk of human cancers; however, few studies have focused on head and neck squamous cell carcinoma (HNSCC) risk. This study aimed to evaluate the associations between several key miRNA polymorphisms and HNSCC risk in a Chinese population.

Methods: In this study, we genotyped five common single-nucleotide polymorphisms (SNPs) in several key miRNAs (miR-149 rs2292832, miR-146a rs2910164, miR-605 rs2043556, miR-608 rs4919510, and miR-196a2 rs11614913) and evaluated the associations between these SNPs and HNSCC risk according to cancer site with a case–control study including 576 cases and 1552 controls, which were matched by age and sex in a Chinese population.

Results: The results revealed that miR-605 rs2043556 [dominant model: adjusted odds ratio (OR) 0.71, 95% confidence interval (CI) 0.58–0.88; additive model: adjusted OR 0.74, 95% CI 0.62–0.89] and miR-196a2 rs11614913 (dominant model: adjusted OR 1.36, 95% CI 1.08–1.72; additive model: adjusted OR 1.28, 95% CI 1.10–1.48) were significantly associated with the risk of oral squamous cell carcinoma (OSCC). Furthermore, when these two loci were evaluated together based on the number of putative risk alleles (rs2043556 A and rs11614913 G), a significant locus-dosage effect was noted on the risk of OSCC (P_trend < 0.001). However, no significant association was detected between the other three SNPs (miR-149 rs2292832, miR-146a rs2910164, and miR-608 rs4919510) and HNSCC risk.

Conclusion: Our study provided the evidence that miR-605 rs2043556 and miR-196a2 rs11614913 may have an impact on genetic susceptibility to OSCC in Chinese population.

Keywords: Head and neck cancer, microRNA, Polymorphism, Squamous cell carcinoma, Susceptibility

Background

Head and neck cancer, predominantly head and neck squamous cell carcinoma (HNSCC), represents a common cancer worldwide and has been considered a serious and growing public health problem in many countries [1, 2]. It was estimated that 45,780 new patients would be diagnosed with cancer of the oral cavity and the pharynx, and 8650 deaths from these diseases occurred in 2015 in the United States alone [3]. Environmental carcinogens and carcinogenic viruses have been identified as the main etiologic factors for HNSCC [4]. Furthermore, genetic variants play a risk-modulating role in the etiology of HNSCC [5].

MicroRNAs (miRNAs) are 20–24 nt single-stranded RNA molecules that repress the expression of specific
target genes by binding to the 3′-untranslated regions (UTRs) of messenger RNA (mRNA) [6]. A single miRNA may regulate the expression of many genes, and it has been proposed that more than one-third of all protein-coding genes are under translational control by miRNAs [7]. Numerous studies have demonstrated that aberrant expression of miRNAs is closely associated with the cell proliferation, invasion, metastasis, and prognosis of various cancers [8, 9]. Given that small variations in the expression of a specific miRNA may affect thousands of target mRNAs and result in diverse functional consequences [10], miRNAs have been considered ideal candidate genes for cancer predisposition.

Studies have demonstrated that potentially functional single nucleotide polymorphisms (SNPs) located in several key miRNAs may influence the function of mature miRNAs and then affect the process of carcinogenesis [11–13]. For example, rs2292832 in miR-149 and rs2043556 in miR-605 were associated with the modified expression level of these two miRNAs [14]. rs2910164 in miR-146a altered the mature miR-146a expression level that was involved in the regulation of cell differentiation and cancer formation [15, 16]. rs4919510 in miR-608 has been predicted by in silico algorithms to exhibit differential capacities to bind to the potential target genes of miR-608, such as the insulin receptor (IR) and tumor protein 53 (TP53) [17]. Furthermore, rs11614913 in miR-196a2 affects the expression of miR-196a, and aberrant regulation of miR-196a is involved in the development and progression of several cancers, including oral cancer [18]. To date, some population studies and meta-analyses have been performed to investigate the associations between polymorphisms of the above important miRNAs and the risk of multiple types of malignant tumors [19, 20]. However, the results were inconsistent, and few studies focused on the associations of these SNPs with HNSCC risk in Chinese population.

Thus, we performed a case-control study on associations of five common SNPs in key miRNAs (rs2292832 in miR-149, rs2910164 in miR-146a, rs2043556 in miR-605, rs4919510 in miR-608, and rs11614913 in miR-196a2) with HNSCC risk in China.

**Methods**

**Study subjects**

This study is a hospital-based case–control study. All newly diagnosed HNSCC patients historically confirmed by two pathologists were consecutively recruited from Jiangsu Stomatological Hospital and the First Affiliated Hospital of Nanjing Medical University, Nanjing, China between January 2009 and May 2013. Exclusion criteria included secondary HNSCC or metastasized cancer from other organs. None of the patients received neo-adjuvant chemotherapy or radiotherapy before surgery. Cancer-free controls matched to the cases by age (±5 years) and sex were randomly selected from a cohort of more than 30,000 participants in a community-based screening program for non-infectious diseases in the Jiangsu Province, China. All participants were genetically unrelated and of the ethnic Han Chinese population. Each participant was scheduled for a face-to-face interview to answer a structured questionnaire that elicited information on demographic characteristics and environmental exposure history, such as age, sex, smoking status, and drinking status. Written informed consent was obtained from each participant, and the study was approved by the Institutional Review Boards of all relevant institutes.

**SNP selection and genotyping**

Based on previous reports about miRNA polymorphisms and cancer risk [14–18], we chose five most investigated and potentially functional SNPs (rs2292832 in miR-149, rs2910164 in miR-146a, rs2043556 in miR-605, rs4919510 in miR-608, and rs11614913 in miR-196a2) for genotyping. Venous blood was collected from all subjects and centrifuged at a speed of 4000 round/min for 10 min. The centrifuged blood was stored at −40 °C for use. Genomic DNA was isolated from leukocyte pellets of venous blood by proteinase K digestion, and this process included secondary HNSCC or metastasized cancer from other organs. None of the patients received neo-adjuvant chemotherapy or radiotherapy before surgery. Cancer-free controls matched to the cases by age (±5 years) and sex were randomly selected from a cohort of more than 30,000 participants in a community-based screening program for non-infectious diseases in the Jiangsu Province, China. All participants were genetically unrelated and of the ethnic Han Chinese population. Each participant was scheduled for a face-to-face interview to answer a structured questionnaire that elicited information on demographic characteristics and environmental exposure history, such as age, sex, smoking status, and drinking status. Written informed consent was obtained from each participant, and the study was approved by the Institutional Review Boards of all relevant institutes.

**Statistical analysis**

The Hardy–Weinberg equilibrium was tested by a goodness-of-fit χ² test to compare the observed genotype frequencies with the expected ones among the control subjects. Distributions of selected demographic variables, risk factors, and frequencies of variant genotypes between the cases and controls were evaluated by using the Pearson’s Chi squared test (uncorrected). The
associations of variant genotypes with HNSCC risk were estimated by computing odds ratios (ORs) and 95% confidence intervals (CIs) from both univariate and multivariate logistic regression analyses according to cancer site. The heterogeneity between subgroups was assessed with the Chi square-based Q test. All statistical analyses were performed with Statistical Analysis System software (v.9.1 SAS Institute, Cary, NC, USA). *P* < 0.05 was considered as the level of statistical significance.

Additionally, we used another data-mining tool, the non-parametric multifactor dimensionality reduction (MDR) software (version 2.0 beta 8.4, Norris-Cotton Cancer Center, Geisel School of Medicine, Dartmouth College, Hanover, NH, USA) to identify the potential locus–locus and gene-environment interactions with tri-chotomies genotypes, age (dichotomized into ≥60 years and <60 years), sex, smoking status, and drinking status. The fitness of the MDR model was assessed by estimating the testing accuracy and the cross-validation consistency (CVC). Models that were true positive would have estimating the testing accuracy of >0.5. The best model with the highest CVC and the highest testing accuracy was selected [21].

**Results**

**Selected characteristics of studied subjects**

A total of 576 HNSCC patients and 1552 cancer-free controls were included in the study. Distributions of physiological characteristics in the case and control groups are presented in Table 1. No significant difference in the distributions of age, sex, and smoking status were noted between the case and control groups. Expectedly, more drinkers were found in the case group than in the control group (44.3 vs. 32.8%, *P* < 0.001). Further, logistic regression suggested that drinking status was associated with an increased HNSCC risk (β = 0.493, OR 1.64, 95% CI 1.35–1.99, *P* < 0.001). Although the proportion of smokers was a bit higher in the case group (45.3%) than in the control group (42.6%), the association between smoking and HNSCC risk was not significant (β = 0.111, OR 1.12, 95% CI 0.92–1.35, *P* = 0.260). In the 576 cases, 462 (80.2%) had oral squamous cell carcinoma (OSCC), and 114 (19.8%) had HNSCC at other sites [9 (1.6%) had oropharyngeal tumor, 102 (17.7%) had laryngeal tumor, 1 had nasal sinus cancer, 1 had parotid carcinoma, and 1 had salivary gland carcinoma].

**Primary information of selected SNPs**

The position, minor allele frequencies (MAFs), and other primary information of five selected SNPs are presented in Table 2. The Hardy–Weinberg equilibrium was not severely violated judging from the goodness-of-fit χ² test (all *P* > 0.05). Among the five loci, the genotype distributions of two SNPs were significantly different between the case and control groups (*P* = 0.004 for miR-605 rs2043556 and *P* = 0.019 for miR-196a2 rs11614913).

**Associations between selected SNPs and HNSCC risk**

Logistic regression analyses revealed that variant genotypes of miR-605 rs2043556 significantly decreased the risk of OSCC (AG vs. AA: adjusted OR 0.74, 95% CI 0.59–0.93; GG vs. AA: adjusted OR 0.56, 95% CI 0.35–0.89; dominant model: adjusted OR 0.71, 95% CI 0.58–0.88; recessive model: adjusted OR 0.63, 95% CI 0.40–1.00; additive model: adjusted OR 0.74, 95% CI 0.62–0.89), whereas variant genotypes of rs11614913 in miR-196a2 significantly increased the risk of OSCC (GG vs. AA: adjusted OR 1.64, 95% CI 1.22–2.11; dominant model: adjusted OR 1.36, 95% CI 1.08–1.72; recessive model: adjusted OR 1.42, 95% CI 1.11–1.83; additive model: adjusted OR 1.28, 95% CI 1.10–1.48) (Table 3). After false discovery rate (FDR) adjustment, the above associations remained significant for rs2043556 in miR-605 (AG vs. AA: *P* = 0.045; GG vs AA: *P* = 0.038; dominant model: *P* = 0.010; additive model: *P* = 0.005) and rs11614913 in miR-196a2 (GG vs. AA: *P* = 0.005; dominant model: *P* = 0.025; recessive model: *P* = 0.030; additive model: *P* = 0.003). We also performed logistic regression analysis conditioning on all selected miRNAs and SNPs, and the results indicated that the effects of rs2043556 in miR-605 and rs11614913 in miR-196a2 on OSCC risk were independent (*P* = 0.001 for both miR-605 rs2043556 and miR-196a2 rs11614913 in additive model).

### Table 1 Selected characteristics of head and neck squamous cell carcinoma (HNSCC) patients and cancer-free controls

| Variable       | Patients [cases (%)] | Controls [cases (%)] | *P*  |
|----------------|----------------------|----------------------|------|
| Total          | 576                  | 1552                 |      |
| Age (years)    |                      |                      |      |
| <60            | 265 (46.0)           | 719 (46.3)           | 0.895|
| ≥60            | 311 (54.0)           | 833 (53.7)           |      |
| Gender         |                      |                      | 0.750|
| Female         | 214 (37.2)           | 565 (36.4)           |      |
| Male           | 362 (62.8)           | 987 (63.6)           |      |
| Smoking status |                      |                      | 0.260|
| No             | 315 (54.7)           | 891 (57.4)           |      |
| Yes            | 261 (45.3)           | 661 (42.6)           |      |
| Drinking status|                      |                      | <0.001|
| No             | 321 (55.7)           | 1043 (67.2)          |      |
| Yes            | 255 (44.3)           | 509 (32.8)           |      |

*Italic value indicate significance of *p* value (*p* < 0.05)  
*a* Two-sided Chi squared test
Table 2 Primary information and minor allele frequencies (MAFs) of selected single-nuclide polymorphisms (SNPs)

| Gene        | Chromosome | SNP     | Base change | Call rates (%) | HWE     | MAF in controls | \( p^a \) | \( p^b \) |
|-------------|------------|---------|-------------|----------------|---------|----------------|----------|----------|
| Has-miR-149 | 2q37.3     | rs2292832 | A>G         | 99.77          | 0.092   | 0.322          | 0.349    | 0.436    |
| Pre-miR-146a| 5q34       | rs2910164 | G>C         | 99.81          | 0.468   | 0.429          | 0.558    | 0.558    |
| Has-miR-605 | 10q21.1    | rs2043556 | A>G         | 99.77          | 0.753   | 0.281          | 0.004    | 0.020    |
| Has-miR-608 | 10q25.1    | rs4919510 | G>C         | 99.85          | 0.835   | 0.425          | 0.245    | 0.408    |
| Pre-miR-196a| 12q13.13   | rs11614913| A>G         | 99.91          | 0.796   | 0.432          | 0.019    | 0.048    |

Italic value indicate significance of \( p \) value (\( p < 0.05 \))
HWE: Hardy–Weinberg equilibrium, MAF: minor allele frequency

\( a \) Two-sided Chi squared test for the comparison of the allele frequency between HNSCC patients and cancer-free controls
\( b \) \( p \) values adjusted by false discovery rate (FDR) method

Combined effects of the two significant SNPs on OSCC risk

When these two loci were evaluated together by the number (0–4) of putative risk alleles (miR-605 rs2043556 A, A and miR-196a2 rs11614913 G, G), a significant locus-dosage effect was detected on HNSCC risk between the groups with 0–2 risk alleles and 3–4 risk alleles (\( P_{\text{trend}} < 0.001 \)). Compared with the group with 0–1 risk allele, the groups with 3 and 4 risk alleles had significantly increased risk of OSCC with adjusted ORs of 1.51 (95% CI 1.10–2.09) and 2.23 (95% CI 1.51–3.29) (Table 4). Compared with the risk in the groups with 0–2 risk alleles, the increase in OSCC risk remained significant for the group with 3–4 risk alleles (adjusted OR 1.48, 95% CI 1.20–1.83). Logistic regression analyses identified no association between the other three SNPs and OSCC risk (data not shown).

Stratification analysis for association between variant genotypes and OSCC risk

We further conducted a stratification analysis by age, sex, smoking status, drinking status, and tumor site on the associations between rs2043556 in miR-605 and rs11614913 in miR-196a2 and OSCC risk. As presented in Table 5, the association of decreased OSCC risk with miR-605 rs2043556 was more notable in males, whereas the association of increased risk with miR-196a2 rs11614913 was more pronounced in females, non-smokers, and non-drinkers than in their counterparts. The combined effect of rs2043556 in miR-605 and rs11614913 in miR-196a2 on OSCC risk was stronger in patients of ≥60 years old than in those of <60 years old.

MDR analysis for OSCC risk predicition

In addition, the MDR method was used to assess potential locus–locus and gene-environment interactions with five SNPs and age, sex, smoking status, and drinking status. As shown in Table 6, age was the strongest factor for predicting HNSCC risk with the highest CVC (100%) and testing accuracy (55.70%). We also observed that the four-factor model, which included age, miR-146a rs2910164, miR-608 rs4919510, and miR-196a2 rs11614913, was the most accurate model with a testing accuracy of 54.91% and a perfect CVC of 10. However, the two-factor and three-factor models had decreased CVCs, suggesting the models were not very accurate.

Discussion

In this case–control study, we examined associations between five common SNPs in miRNAs (miR-149 rs2292832, miR-146a rs2910164, miR-605 rs2043556, miR-608 rs4919510, and miR-196a2 rs11614913) and HNSCC risk. The results revealed that rs2043556 in miR-605 and rs11614913 in miR-196a2 were significantly associated with OSCC risk in a Chinese population. However, no notable association was detected between other selected SNPs and HNSCC risk.

Once activated, the tumor suppressor p53 selectively modulates the expression of target genes involved in cell cycle arrest, apoptosis, and DNA repair [22]. A recent study indicated that miR-605 was a new component in the p53 gene network [23]. This network is transcriptionally activated by p53 and post-transcriptionally repressed by murine double minute 2 (Mdm2), which inhibits the function of p53. Thus, a positive feedback loop is created that aids in the rapid accumulation of p53 to facilitate its function in response to stress [23]. Id Said et al. [24] reported that high expression of miR-605 could result in a significant reduction in cell viability, clonogenicity, and cell migration in TP53-mutant cell types and that rs2043556-variant G allele could significantly result in a decreased expression of miR-605. Several studies have investigated the associations between miR-605 rs2043556 and cancer risk, and a recent meta-analysis concluded that miR-605 rs2043556 was associated with a significant overall risk of human cancer [25]. In this study, we first examined the effect of miR-605 rs2043556 on the risk of HNSCC and identified a significant linkage between this SNP and the decreased risk of OSCC in a Chinese population. Thus, we hypothesize that miR-605 rs2043556 may affect the expression of miR-605 and the
risk of OSCC, which may provide a visual cue regarding the role of this SNP in the development of OSCC.

Rs11614913, which is located at miR-196a2, impacts the expression of miR-196a2 and is involved in the carcinogenesis of different types of cancer [17, 26, 27]. For example, Tian et al. [28] reported that miR-196a2 rs11614913 was associated with the increased risk of non-small cell lung cancer and poor patient survival, and Hu et al. [29] reported its association with the increased risk of breast cancer. It was also reported that miR-196a2 rs11614913 influenced mature miR-196a expression (but not the pre-miR-196a2 level) and affected the

| SNP       | Genotype | Controls [number (%)] | Oral cancer patients [number (%)] | Adjusted OR (95% CI)p | pb | pc | Non-oral cancer patients [number (%)] | Adjusted OR (95% CI)p | pb |
|-----------|----------|-----------------------|-----------------------------------|-----------------------|----|----|--------------------------------------|-----------------------|----|
| miR-605   | AA       | 798 (51.6)            | 278 (60.3)                        | 1.00                  | 0.009 | 0.045 | 55 (48.2)                          | 1.00                  |    |
| rs2043556 | AG       | 631 (40.8)            | 160 (34.7)                        | 0.74 (0.59–0.93)      | 0.001 | 0.045 | 52 (45.6)                          | 1.19 (0.80–1.78)      | 0.396 |
|           | GG       | 119 (7.7)             | 23 (5.0)                          | 0.56 (0.35–0.88)      | 0.001 | 0.038 | 7 (6.1)                            | 0.85 (0.38–1.94)      | 0.708 |
|           |          |                       |                                   | 0.71 (0.58–0.88)      | 0.002 | 0.010 | NA                                   | 1.14 (0.77–1.67)      | 0.518 |
|           |          |                       |                                   |                       |      |      |                                      |                       |    |
| miR-196a2 | AA       | 503 (32.5)            | 122 (26.4)                        | 1.00                  | 0.001 | 0.005 | 40 (35.1)                          | 1.00                  |    |
| rs11614913 | AG      | 755 (48.7)            | 228 (49.4)                        | 1.25 (1.08–1.61)      | 0.001 | 0.005 | 56 (49.1)                          | 0.93 (0.61–1.43)      | 0.736 |
|           | GG       | 292 (18.8)            | 112 (24.2)                        | 1.64 (1.22–2.21)      | 0.001 | 0.005 | 18 (15.8)                          | 0.76 (0.43–1.37)      | 0.366 |
|           |          |                       |                                   |                       |      |      |                                      |                       |    |
| miR-149   | AA       | 726                   | 226                               | 1.00                  | 0.001 | 0.003 | 57                                  | 0.88 (0.59–1.33)      | 0.547 |
| rs2292832 | AG       | 647                   | 193                               | 0.96 (0.77–1.19)      | 0.001 | 0.003 | 38                                  | 0.80 (0.47–1.35)      | 0.402 |
|           | GG       | 175                   | 42                                | 0.76 (0.52–1.10)      | 0.001 | 0.003 | 19                                  | 0.88 (0.67–1.17)      | 0.386 |
|           |          |                       |                                   |                       |      |      |                                      |                       |    |
| miR-146a  | AA       | 497                   | 154                               | 1.00                  | 0.001 | 0.003 | 40                                  | 0.84 (0.56–1.27)      | 0.407 |
| rs2910164 | AG       | 773                   | 228                               | 0.95 (0.75–1.21)      | 0.001 | 0.003 | 53                                  | 1.05 (0.79–1.39)      | 0.735 |
|           | GG       | 278                   | 80                                | 0.93 (0.68–1.27)      | 0.001 | 0.003 | 21                                  | 0.90 (0.60–1.31)      | 0.556 |
|           |          |                       |                                   |                       |      |      |                                      |                       |    |
| miR-608   | AA       | 509                   | 137                               | 1.00                  | 0.001 | 0.003 | 40                                  | 0.87 (0.56–1.32)      | 0.546 |
| rs4919510 | AG       | 762                   | 232                               | 1.14 (0.90–1.45)      | 0.001 | 0.003 | 53                                  | 0.85 (0.55–1.31)      | 0.464 |
|           | GG       | 278                   | 80                                | 1.23 (0.91–1.67)      | 0.001 | 0.003 | 21                                  | 0.97 (0.56–1.70)      | 0.927 |
|           |          |                       |                                   |                       |      |      |                                      |                       |    |

Italic value indicate significance of p value (p < 0.05)
NA not available

a miR-605 rs2043556 was genotyped in 575 cases and 1548 controls; miR-196a2 was genotyped in 576 cases and 1550 controls; miR-149 rs2292832 was genotyped in 575 cases and 1548 controls; miR-146a rs2910164 was genotyped in 575 cases and 1548 controls; and miR-608 rs4919510 was genotyped in 576 cases and 1549 controls

b Adjusted by age, sex, smoking status, and drinking status
c P values of multiple comparisons for false discovery rate using the FDR method (n = 5, refer to the number of SNPs)
binding ability of miR-196a-3p to its targets [27]. Additionally, Hoffman et al. [30] demonstrated that mature miR-196a2 level was increased 9.3-fold in breast cancer cells transfected with pre-miR-196a2-C (rs11614913), but the levels were only increased 4.4-fold in cells transfected with pre-miR-196a2-T. Such associations were then further supported by studies on other types of cancers. A recent meta-analysis revealed that miR-196a2 rs11614913 was associated with cancer risk, especially risks of lung, colorectal, and breast cancers among Asian populations [31]. Specially, a few studies have investigated the association of rs11614913 in miR-196a2 with HNSCC risk in Caucasian populations, but the results were inconclusive. Liu et al. [32] found no association

| Number of risk alleles | Patients [number (%)] | Controls [number (%)] | Adjust OR (95% CI) | \( p^b \) |
|------------------------|-----------------------|-----------------------|-------------------|-------|
| 0–1                    | 66 (14.3)             | 303 (19.6)            | 1.00              |       |
| 2                      | 153 (33.2)            | 575 (37.2)            | 1.20 (0.87–1.66)  | 0.262 |
| 3                      | 168 (36.4)            | 517 (33.4)            | 1.51 (1.10–2.09)  | 0.011 |
| 4                      | 74 (16.1)             | 151 (9.8)             | 2.23 (1.51–3.29)  | <0.001|
| Trend                  | NA                    | NA                    | 1.21 (1.10–1.32)  | <0.001|

Table 4 Combined effects of miR-605 rs2043556 and miR-196a2 rs11614913 on oral squamous cell carcinoma (OSCC) risk

| Variable | miR-605 rs2043556 genotype (GG/AG/AA) | Adjusted OR (95% CI) | \( p^b \) | miR-196a2 rs11614913 genotype (GG/AG/AA) | Adjusted OR (95% CI) | \( p^b \) | Combined effect (0–2/3–4 risk alleles) | Adjusted OR (95% CI) | \( p^b \) |
|----------|---------------------------------------|----------------------|-------|----------------------------------------|----------------------|-------|----------------------------------------|----------------------|-------|
| Age (years) | | | | | | | | | |
| <60      | 10/7/5/125                            | 0.76 (0.59–1.00)     | 0.042 | 56/98/57                               | 1.33 (1.07–1.66)     | 0.011 | 102/105                               | 1.32 (0.97–1.81)     | 0.081 |
| ≥60      | 13/8/5/153                            | 0.73 (0.58–0.93)     | 0.011 | 56/130/65                              | 1.24 (1.01–1.52)     | 0.038 | 117/137                               | 1.62 (1.22–2.16)     | 0.001 |
| Sex      | | | | | | | | | |
| Female   | 12/6/8/124                            | 0.78 (0.60–1.02)     | 0.068 | 59/99/46                               | 1.64 (1.30–2.07)     | <0.001| 97/107                                | 1.54 (1.11–2.12)     | 0.010 |
| Male     | 11/9/2/154                            | 0.72 (0.57–0.91)     | 0.005 | 53/129/76                              | 1.08 (0.89–1.32)     | 0.434 | 122/135                               | 1.47 (1.11–1.94)     | 0.008 |
| Smoking  | | | | | | | | | |
| Never    | 15/9/9/160                            | 0.79 (0.63–0.99)     | 0.037 | 74/129/72                              | 1.32 (1.09–1.60)     | 0.004 | 135/139                               | 1.36 (1.03–1.79)     | 0.028 |
| Ever     | 8/6/1/118                             | 0.66 (0.50–0.89)     | 0.006 | 38/99/50                               | 1.25 (0.97–1.59)     | 0.081 | 84/103                                | 1.72 (1.22–2.42)     | 0.002 |
| Drinking | | | | | | | | | |
| Never    | 14/9/7/161                            | 0.76 (0.60–0.95)     | 0.016 | 72/134/67                              | 1.38 (1.14–1.67)     | 0.001 | 130/142                               | 1.46 (1.11–1.92)     | 0.006 |
| Ever     | 9/6/3/117                             | 0.70 (0.53–0.93)     | 0.014 | 40/94/55                               | 1.18 (0.93–1.51)     | 0.175 | 89/100                                | 1.58 (1.12–2.22)     | 0.009 |

Italic value indicate significance of \( p \) value (\( p < 0.05 \))

a These data are presented as the numbers of cases with genotypes GG, AG, or AA

b Adjusted by age, sex, smoking status, and drinking status

c These data are presented as the numbers of cases with 0–2 or 3–4 risk alleles
between \( \text{miR-196a2} \) rs11614913 and risk of HNSCC, whereas Christensen et al. [33] reported that the \( \text{miR-196a2} \) rs11614913 CC genotype was related with an increased HNSCC risk. Another study identified a significant association between rs11614913 and \( \text{miR-196a2} \) expression levels in tumor tissues from OSCC patients, but no association of \( \text{miR-196a2} \) rs11614913 with OSCC risk was noted [17]. In this study, we demonstrated that the \( \text{miR-196a2} \) rs11614913 G allele was significantly associated with an increased OSCC risk, which is consistent with the study by Christensen et al. [33]. The difference between our study and the other two studies [32, 33] may due to different ethnic backgrounds and different composition of cases. The MAF in our controls was 0.432, whereas it was either 0.420 [32] or not obtained [33] in the literature. Furthermore, the proportion of oral cancer was much higher in our study (80.2%) than that in the other two studies (29.4% and 55.6%, respectively). Larger studies with different ethnic backgrounds and functional investigation are needed to validate these findings.

Studies on associations between the other three SNPs (rs2292832 in \( \text{miR-149} \), rs2910164 in \( \text{miR-146a} \), and rs4919510 in \( \text{miR-608} \)) and cancer risk were inconsistent [34–38]. A recent meta-analysis of 12 studies, including 5937 cases and 6081 controls, revealed that \( \text{miR-149} \) rs2292832 was not associated with cancer risk [39]. Additionally, only two studies investigated the effect of \( \text{miR-149} \) rs2292832 on HNSCC risk, and neither produced significant results [32, 40]. A meta-analysis of 66 case–control studies reported that \( \text{miR-146a} \) rs2910164 was a risk factor for HNSCC, which included four studies from a Caucasian population and one study from a Chinese population [41]. However, the results from the Chinese population indicated that \( \text{miR-146a} \) rs2910164 was not significantly associated with oral cancer risk [40]. To date, two studies have focused on the associations of \( \text{miR-608} \) rs4919510 and cancer risk: one on colorectal cancer [38] and another on breast cancer [37], and their results were inconsistent. In our study, the results demonstrated that none of these three SNPs (rs2292832 in \( \text{miR-149} \), rs2910164 in \( \text{miR-146a} \), and rs4919510 in \( \text{miR-608} \)) contributed to the risk of HNSCC in a Chinese population. Given heterogeneous genetic backgrounds in different populations, these findings must be validated in further larger studies.

Several potential limitations of the present study warrant consideration. First, a relatively small sample size may limit the statistical power of our study, especially in the stratification analysis. We made multiple testing adjustments using the FDR method, and the results indicate that the associations between SNPs and OSCC risk remained significant. However, the effect of \( \text{miR-605} \) rs2043556 on HNSCC risk was borderline significant after the FDR correction. Thus, our results must be confirmed in further studies. Second, our study is a hospital-based, case–control study, and inherent selection bias cannot be completely excluded. Third, the functional significance of rs2043556 in \( \text{miR-605} \) and rs11614913 in \( \text{miR-196a2} \) for the development of HNSCC remains largely unknown.

In summary, we identified that \( \text{miR-605} \) rs2043556 and \( \text{miR-196a2} \) rs11614913 were associated with OSCC risk in a Chinese population. Further replication studies with diverse ethnic groups and functional characterization are warranted to validate our findings.

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**Competing interests**

The authors declare that they have no competing interests.

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**Table 6** Multifactor dimensionality reduction (MDR) analysis for OSCC risk prediction

| Best model                      | Training bal. acc. | Testing bal. acc. | \( P^a \) | CVC |
|--------------------------------|--------------------|-------------------|-----------|-----|
| One-factor (age)               | 0.6063             | 0.5570            | 0.1602    | 10/10 |
| Two-factor (age and \( \text{miR-605} \) rs2043556) | 0.6575             | 0.5590            | 0.1511    | 5/10 |
| Three-factor (age, \( \text{miR-146a} \) rs2910164, and \( \text{miR-196a2} \) rs11614913) | 0.7276             | 0.5314            | 0.4663    | 6/10 |
| Four-factor (age, \( \text{miR-146a} \) rs2910164, \( \text{miR-608} \) rs4919510, and \( \text{miR-196a2} \) rs11614913) | 0.8221             | 0.5491            | 0.2411    | 10/10 |

Training bal. acc. training balanced accuracy, Testing bal. acc. testing balanced accuracy, \( \text{CVC} \) cross-validation consistency

\( a \) \( P \) values for testing balanced accuracy
