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**Original Paper**

# The Association and Clinical Significance of CDKN2A Promoter Methylation in Head and Neck Squamous Cell Carcinoma: a Meta-Analysis

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**Key Words**
CDKN2A • Methylation • Head and neck squamous cell carcinoma • Diagnosis • Prognosis • Carcinogenesis

**Abstract**
***Background/Aims:*** The association between cyclin-dependent kinase inhibitor 2A (*CDKN2A*) hypermethylation and head and neck squamous cell carcinoma (HNSCC) risk has been investigated by a number of studies. However, these studies have not demonstrated consistent results. Moreover, the role of *CDKN2A* methylation in HNSCC carcinogenesis and its clinical significance remain unclear. ***Methods:*** We performed a systematic meta-analysis based on 72 articles (including 3399 HNSCCs, 668 premalignant lesions, and 2393 normal controls) from the PubMed, Google Scholar, Web of Science, Embase, China National Knowledge Infrastructure and Wanfang databases. ***Results:*** Our study showed a significant increase in the frequency of *CDKN2A* methylation during HNSCC carcinogenesis (HNSCC vs. normal controls, odds ratio (OR) = 6.72, $P < 0.01$; HNSCC vs. precancerous lesions, OR = 1.89, $P < 0.05$; precancerous lesions vs. normal controls, OR = 14.70, $P < 0.01$). Moreover, *CDKN2A* methylation was significantly associated with gender (OR = 1.34; $P < 0.05$) and lymph node metastasis (OR = 2.32; $P < 0.01$). The area under summary receiver operating characteristic curve (AUC) for diagnosis of HNSCC based on all samples and saliva sample subgroup were 0.77 and 0.96, respectively. Additionally, *CDKN2A* hypermethylation was significantly associated with shorter overall survival (OS) (hazard ratio (HR) = 1.01, $P < 0.05$) and recurrence-free survival (RFS)

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(HR = 1.77, $P < 0.05$). ***Conclusion:*** Our findings indicate *CDKN2A* methylation is involved in the carcinogenesis, progression, and metastasis of HNSCC. Furthermore, methylated *CDKN2A* could be a potential diagnostic and prognostic biomarker for HNSCC.



## Introduction

Cancer of the head and neck region is the sixth most common cancer and fifth leading cause of cancer-related death worldwide. More than 90% of these tumors are squamous cell carcinomas (HNSCC) occurring in the epithelial mucosal membranes of the upper-aerodigestive tract (oral and nasal cavity, oropharynx, hypopharynx, and larynx) [1]. The worldwide incidence of HNSCC has been increasing for several decades for reasons that are not entirely clear, but may be related to the increasing prevalence of risk factors such as smoking, alcohol consumption, and high-risk human papilloma virus (HPV) infection [2]. In the United States alone, there were an estimated 63, 030 new cases and 13, 360 deaths for head and neck cancer in 2017 [3]. Despite the combination of adequate treatment modalities, including surgical resection, chemotherapy, and radiotherapy, loco-regional or distant recurrence rates in HNSCC patients remain high [4]. Due to the lack of symptoms in the early stage and effective screening techniques, the majority of HNSCC patients are diagnosed at an advanced stage [5]. Recurrence, distant failure, and advanced stage cancer represent a highly aggressive disease with dismal survival rates [6]. Indeed the 5-year survival rate of HNSCC is less than 50% [7]. Therefore, more effective and reliable biomarkers are urgently in demand for the early screening, diagnosis, and identification of risk of recurrence and subsequent death, which are of tremendous importance in achieving a better prognosis.

HNSCC develops in a multistep process that involves undergoing different molecular alterations including the accumulation of multiple genetic and epigenetic changes with tumor progression [8]. As one of the most important epigenetic alterations, hypermethylation in the promoter region frequently associated with transcriptional silencing, may serve as a crucial mechanism to inactivate tumor suppressor genes (TSGs) in several cancers, including breast [9], liver [10], esophageal [11], and thyroid [12] cancer. Aberrant methylations are believed to be early events in cancer [13], which may serve as biomarkers for cancer diagnosis and prognosis [14-16]. Cyclin-dependent kinase inhibitor 2A (*CDKN2A*) located on chromosome 9p21, encodes two functionally distinct tumor suppressor genes, $p14^{ARF}$ and $p16^{INK4a}$, which play active roles in p53 and retinoblastoma (RB) tumor suppressive pathways [17]. *CDKN2A* is involved in tumorigenesis by the regulation of cell division and apoptosis, and maintenance of cellular homeostasis by decelerating cell cycle progression at G1/S phase [18, 19]. Hypermethylation of *CDKN2A* promoter was shown to be responsible for the loss of its expression in numerous cancers, including hepatocellular carcinoma [20], cervical cancer [21], oral squamous cell carcinoma [22], and non-small cell lung cancer [23].

Recently, many researchers have studied the association between *CDKN2A* promoter methylation and HNSCC or clinicopathological features of patients. However, due to small sample sizes or errors among different studies, the results have been inconsistent. Additionally, the role of *CDKN2A* promoter methylation in HNSCC carcinogenesis, and its clinical application for HNSCC diagnosis and prognosis remain less intensely investigated. Therefore, to obtain more reliable and systematic results, we performed a meta-analysis to assess the association between *CDKN2A* promoter methylation and HNSCC risk and carcinogenesis, as well as clinicopathological characteristics. In addition, we also evaluated the diagnostic and prognostic value of *CDKN2A* methylation for HNSCC.

## Materials and Methods

*Literature search*

PubMed, Google Scholar, Web of Science, Embase, China National Knowledge Infrastructure and Wanfang databases were systematically searched to find eligible studies without language restrictions published prior to August 5, 2017. We used the following key words and search terms individually as well as in various combinations: "*p16*," "*p16INK4a*," "*p14ARF*", "*p14*", "*CDKN2A* (*p16* and *p14*)," "cyclin-dependent kinase inhibitor 2A," "methylation," "DNA methylation," "promoter methylation," "squamous cell carcinoma," "cancer;" "oral," "oropharyngeal," "oropharynx," "head and neck," and "tonsil." Furthermore, we manually reviewed the reference lists of the initially identified articles to find more potentially relevant studies.

*Inclusion criteria*

For studies to be eligible for inclusion in the meta-analysis, they had to meet the following criteria: (1) study samples were confirmed by pathology, including HNSCCs, precancerous lesions, and normal controls; (2) studies related to *CDKN2A* gene promoter methylation and HNSCC progression or clinicopathological features or prognosis; (3) studies were case-control or cohort designs; and (4) studies provided sufficient data regarding methylation frequency of *CDKN2A* promoter to enable the calculation of odds ratios (ORs) and 95% confidence intervals (CIs), or have reported hazard ratios (HRs) and corresponding 95% CIs. If authors published several articles using the same (or overlapping) data, only the study with the most complete or up-to-date information was included in the meta-analysis.

*Data quality assessment*

The quality of studies was assessed according to the Newcastle–Ottawa Scale (NOS) criteria [24, 25]. The NOS evaluation system includes three aspects: (1) subject selection, 0–4 points; (2) comparability of subjects, 0–2 points; and (3) clinical outcome, 0–3 points. NOS scores range from 0 to 9, and a score ≥ 7 indicates good quality. Only studies with scores ≥ 7 were included in the analysis.

*Data extraction*

Three reviewers (CZ, HL, and JL) extracted relevant data from qualified articles using a standardized form. The following information was extracted: name of first author, publication year, countries, ethnicity of subjects, number of samples, control type, control source, methylation detection methods, the number of *CDKN2A* promoter methylation and unmethylation in the case and control group, HR and corresponding 95% CI for HNSCC patients with methylated *CDKN2A*, and clinicopathological characteristics including age, gender, smoking behavior, alcohol consumption, differentiation grade, tumor stage, lymph node metastasis, and clinical stage. The three reviewers discussed any inconsistencies and eventually reached consensus.

*Statistical analyses*

All analyses were conducted using Stata 12.0 statistical software (Stata Corporation, College Station, TX, USA). The pooled ORs and corresponding 95% CIs were used to quantify the strengths of the associations between *CDKN2A* methylation and carcinogenesis of HNSCC, along with clinicopathological features. The assessment of potential heterogeneity was quantified by *I* squared ($I^2$) tests based on Q tests [26]. A $P < 0.05$ or $I^2 > 50\%$ was defined as significant heterogeneity. A random effects model (DerSimonian–Laird method) [27] was used to calculate pooled OR when significant heterogeneity was observed; otherwise, a fixed effects model (Mantel–Haenszel method) [28] was applied. Subgroup analyses stratified by ethnicity, sample type, control source, detection method, sample size, and publication year were performed to detect potential sources of heterogeneity and lower the between-study heterogeneity. A sensitivity analysis was performed to assess the robustness of the results and determine the influence of individual studies on the pooled results [29]. Begg's linear regression tests were used to evaluate publication bias of included studies [30]. HRs with 95% CIs were calculated to evaluate the association between *CDKN2A* methylation and prognosis of HNSCC patients. The area under the summary receiver operator characteristic (SROC) curve (AUC), pooled sensitivity, and specificity were calculated to assess the overall diagnostic power of *CDKN2A* methylation test for HNSCC [31]. All *P* values were two-sided, and a *P* value of less than 0.05 was considered statistically significant.

## Results

*Qualified study characteristics*

According to the retrieval strategy, we initially obtained 1203 articles from a search of six databases and five articles from manual searching. The flow chart of the selection process is presented in Fig. 1. Ultimately, 76 articles (including 72 case-control and 19 cohort studies) met the inclusion criteria and were used in the meta-analysis. The basic characteristics of all eligible studies [22, 32-92] are presented in Supplemental Table 1 (/ For all supplemental material see www.karger.com/10.1159/000494473/).

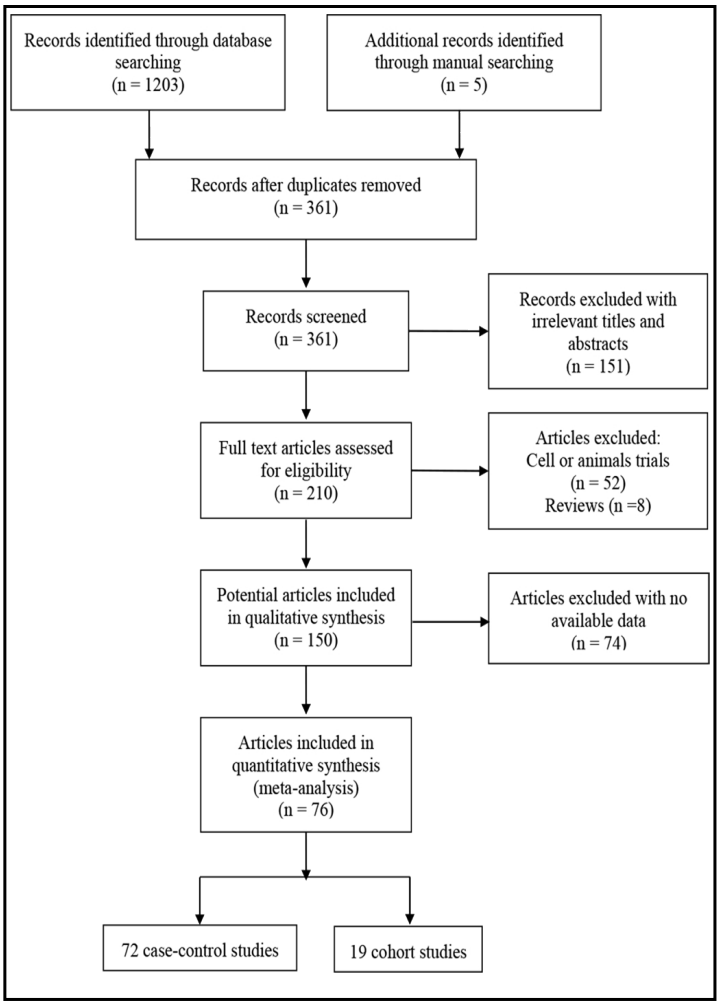



**Fig. 1.** Flow diagram of the selection process for this meta-analysis.

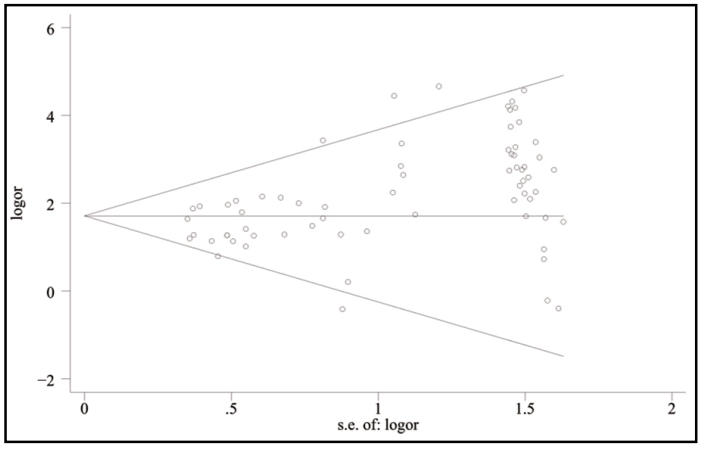
*The association between CDKN2A promoter methylation and HNSCC*

A total of 67 case-control studies, including 3399 HNSCCs and 2393 normal controls were used to assess the association between *CDKN2A* promoter methylation and HNSCC. Our results indicated that the frequency of *CDKN2A* promoter methylation was significantly higher in HNSCCs than normal controls (OR = 6.72, 95% CI: 5.67–7.98, $P < 0.01$; Supplemental Fig. 1). A subgroup analysis was conducted by ethnicity, sample type, control source, detection method, sample size, and publication year. The results of this subgroup analysis showed that *CDKN2A* hypermethylation was significantly associated with HNSCC in all subgroups (Table 1). Subgroup analysis by ethnicity revealed an OR of 5.17 (95% CI: 4.05–6.60, $P < 0.01$) for Caucasian populations,

**Table 1.** Subgroup analyses of CDKN2A promoter methylation in HNSCC. M+: positive for CDKN2A methylation test. MSP, methylation-specific polymerase chain reaction

| Subgroup | Case | | Control | | Pooled OR (95% CI) | P | Heterogeneity | |
|---|---|---|---|---|---|---|---|---|
| | M+ | total | M+ | total | | | $I^2$ (%) | P |
| Ethnicity | | | | | | | | |
| Caucasian | 517 | 1520 | 142 | 1160 | 5.165 (4.045 -6.596) | <0.001 | 8.8 | 0.332 |
| Asian | 477 | 1219 | 60 | 825 | 8.704 (6.467-11.715) | <0.001 | 20.4 | 0.165 |
| African | 286 | 660 | 43 | 411 | 9.886 (4.647-21.027) | <0.001 | 49.2 | 0.039 |
| Sample type | | | | | | | | |
| Tissue | 1162 | 3108 | 238 | 2235 | 6.404 (5.37-7.637) | <0.001 | 21.8 | 0.074 |
| Blood | 72 | 113 | 4 | 44 | 16.029 (5.221-49.21) | <0.001 | 14.8 | 0.309 |
| Saliva | 46 | 178 | 3 | 117 | 12.449 (4.414-35.11) | <0.001 | 23 | 0.268 |
| Control source | | | | | | | | |
| Autologous | 799 | 2197 | 171 | 1492 | 5.506 (4.088-7.417) | <0.001 | 28.8 | 0.048 |
| Heterogeneous | 624 | 1499 | 74 | 904 | 8.09 (5.167-12.668) | <0.001 | 44.5 | 0.004 |
| Methods | | | | | | | | |
| MSP | 1067 | 2617 | 229 | 1801 | 5.642 (4.406-7.226) | <0.001 | 26.4 | 0.045 |
| No MSP | 213 | 782 | 16 | 595 | 11.378 (6.958-18.606) | <0.001 | 0 | 0.701 |
| Sample size | | | | | | | | |
| < 60 | 618 | 1639 | 118 | 1449 | 6.426 (4.653-8.874) | <0.001 | 30.1 | 0.033 |
| ≥ 60 | 662 | 1760 | 127 | 947 | 6.832 (5.419-8.612) | <0.001 | 9.1 | 0.336 |
| Publish year | | | | | | | | |
| < 2010 | 628 | 2038 | 105 | 1378 | 7.015 (5.468-9) | <0.001 | 23.2 | 0.096 |
| ≥ 2010 | 652 | 1361 | 140 | 1018 | 6.445 (5.096-8.151) | <0.001 | 26.1 | 0.112 |

**Fig. 2.** Begg's funnel plot with 95% confidence limits of publication bias.



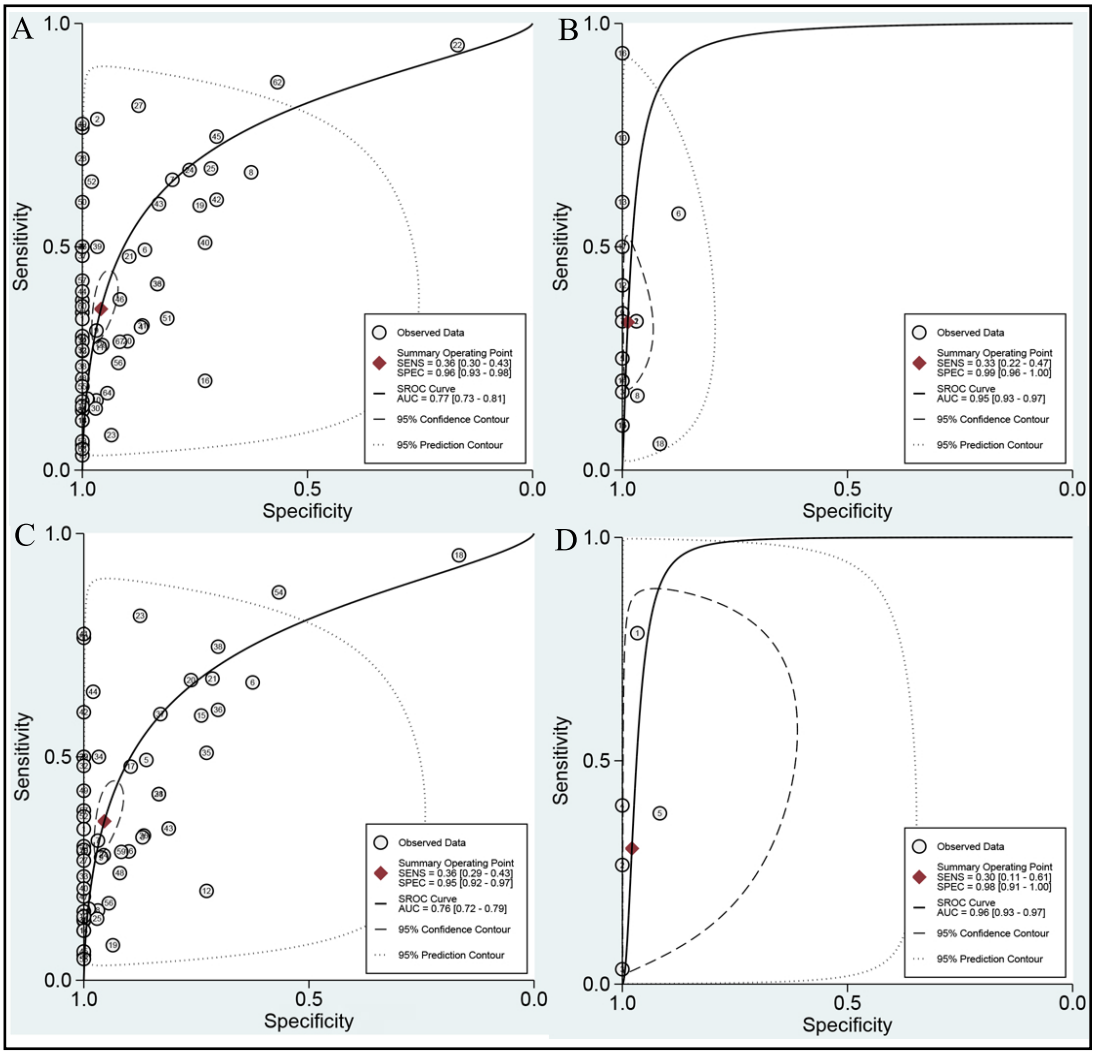




**Fig. 3.** Summary receiver operating characteristic (SROC) plots of methylated CDKN2A. A: SROC plot of methylated CDKN2A in distinguish of HNSCC from normal control by tissue and saliva samples; B: SROC plot of methylated CDKN2A in distinguish of precancerous patients from normal control by tissue and saliva samples; C: SROC plot of methylated CDKN2A in distinguish of HNSCC from normal control using tissue samples; D: SROC plot of methylated CDKN2A in distinguish of HNSCC from normal control using on saliva samples.

8.70 (95% CI: 6.47–11.72, $P < 0.01$) for Asian populations, and 9.89 (95% CI: 4.65–21.03, $P < 0.01$) for African populations. To test the robustness of our results, a sensitivity analysis was performed to determine the influence of an individual study on overall pooled ORs. The omission of individual studies did not significantly change the pooled OR, suggesting that the results were stable and credible (Supplemental Table 2). The potential publication bias was assessed by Begg's funnel plot analysis. The results indicated there might be some publication bias among the studies under analysis (Fig. 2).

*The association between CDKN2A promoter methylation and HNSCC carcinogenesis*

Furthermore, a total of 15 studies involving 621 HNSCCs and 460 precancerous lesions were included to evaluate the association between *CDKN2A* methylation in HNSCCs and precancerous lesions. We observed that the methylation frequency of *CDKN2A* was markedly elevated in HNSCCs compared to precancerous lesion samples (OR = 1.89; 95% CI: 1.05–3.40; $P < 0.05$; Supplemental Fig. 2). The analysis of the association between *CDKN2A* methylation and HNSCC precancerous lesions included 639 precancerous samples and 419 normal controls from 18 studies. As demonstrated in Supplemental Fig. 3, the methylation frequency of *CDKN2A* was significantly higher in precancerous lesions than controls (OR = 14.70; 95% CI: 8.26–26.17; $P < 0.01$).

*CDKN2A promoter methylation and clinicopathological features of HNSCC*

We also evaluated the association between *CDKN2A* methylation and clinicopathological features of HNSCC patients, including age, gender, smoking behavior, alcohol consumption, differentiation grade, T stage, lymph node metastasis, and clinical stage. Our analyses

**Table 2.** The association between CDKN2A promoter methylation and clinicopathological features of HNSCC patients. No.: number of studies

| Characteristics | No. | Case /control | Pooled OR (95% CI) | P | Heterogeneity | |
|---|---|---|---|---|---|---|
| | | | | | $I^2$% | P |
| Age | 15 | older/younger | 1.05 (0.81-1.38) | 0.7 | 28 | 0.148 |
| Gender | 19 | Male/female | 1.34 (1.00-1.80) | 0.047 | 0 | 0.566 |
| Smoking behavior | 12 | Yes/No | 0.96 (0.66-1.39) | 0.821 | 32.1 | 0.134 |
| Alcohol consumption | 11 | Yes/no | 0.93(0.93-1.32) | 0.681 | 0 | 0.471 |
| Differentiation grade | 11 | Poor/Well and moderate | 1.09 (0.66-1.79) | 0.734 | 0 | 0.566 |
| T stage | 12 | $T_{3+4}/T_{1+2}$ | 1.24 (0.88-1.74) | 0.223 | 7.4 | 0.373 |
| Lymph node metastasis | 17 | Yes/No | 2.32 (1.44-3.75) | 0.001 | 62 | <0.001 |
| Clinical stage | 20 | Ⅲ+Ⅳ/ Ⅰ+Ⅱ | 1.14 (0.85-1.51) | 0.381 | 17 | 0.242 |

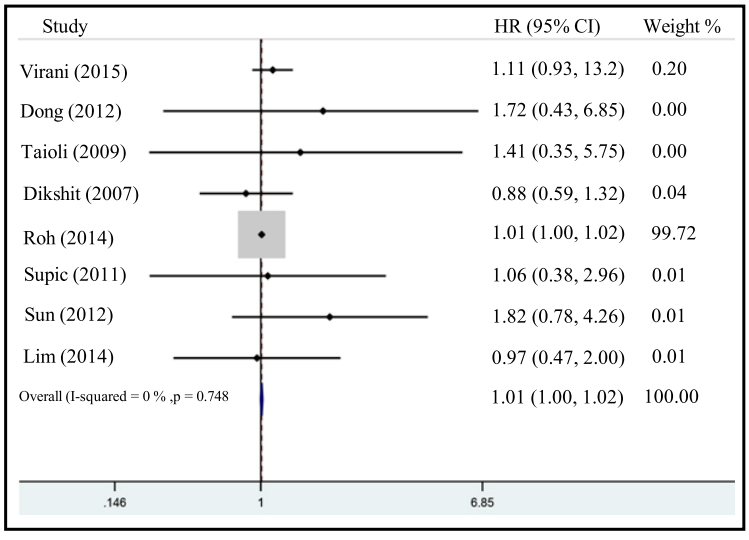



**Fig. 4.** Forest plot for pooled hazard ratio (HR) and the corresponding 95% confidence interval (CI) of CDKN2A methylation for overall survival (OS) of HNSCC patients.

demonstrated that *CDKN2A* methylation was significantly associated with gender (OR = 1.34; 95% CI: 1.00–1.80; *P* = 0.047) and lymph node metastasis (OR = 2.32; 95% CI: 1.44–3.75; *P* < 0.01). However, there was no correlation between *CDKN2A* promoter methylation and other clinicopathological characteristics of HNSCC patients (Table 2).

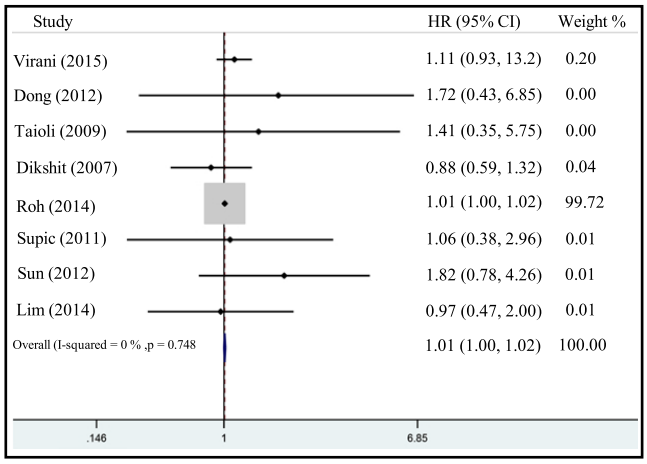



**Fig. 5.** Forest plot for pooled hazard ratio (HR) and the corresponding 95% confidence interval (CI) of CDKN2A methylation for recurrence-free survival (RFS) of HNSCC patients.

*Diagnostic value of CDKN2A promoter methylation for HNSCC*

We further calculated the pooled sensitivity, specificity, and AUC to evaluate the diagnostic value of *CDKN2A* promoter methylation for HNSCC and precancerous patients. The pooled sensitivity, specificity, and AUC for HNSCC patients were 0.36, 0.96, and 0.77, respectively (Fig. 3A). The pooled sensitivity, specificity, and AUC for precancerous patients were 0.33, 0.99, and 0.95, respectively (Fig. 3B). Furthermore, we performed a subgroup analysis based on the retrieved sample materials. The subsequent detailed results showed that the AUC for tissue and saliva samples were 0.76 (Fig. 3C) and 0.96 (Fig. 3D), respectively.

*Prognostic value of CDKN2A promoter methylation for* HNSCC *patients*

Eight and six studies were used to assess the association of *CDKN2A* promoter methylation with overall survival (OS) and recurrence-free survival (RFS) of HNSCC patients, respectively. The results revealed that *CDKN2A* methylation was significantly associated with both shortened OS (HR = 1.01, 95% CI: 1.00–1.02, *P* < 0.05, Fig. 4) and RFS (HR = 1.77, 95% CI: 1.17–2.69, *P* < 0.05, Fig. 5) of HNSCC patients.

## Discussion

The multistep carcinogenesis of HNSCC involves various genetic and/or epigenetic alterations that lead to the functional loss of TSGs [93]. *CDKN2A* as a classical TSG has been reported that hypermethylation and inactivation of *CDKN2A* promoter region in several types of malignancy [67] [94, 95]. Due to the conclusion of the role of *CDKN2A* promoter methylation in HNSCC is still inconsistent and controversial since the use of different sample types, ethnicities, and detection methods, we conducted a comprehensive meta-analysis to achieve further insight into the association between *CDKN2A* promoter methylation and HNSCC carcinogenesis.

The results of this study showed higher *CDKN2A* promoter methylation frequency in HNSCC samples than normal controls, which is consistent with the results found in other types of cancers [11, 94, 95]. The sensitivity analysis and the absence of heterogeneity indicated that our results were stable and credible. In subgroup analysis grouped by ethnicity, a strong positive association was observed in Caucasian, Asian, and African populations. African populations had a higher OR (OR = 9.89) than Caucasian (OR = 5.17) and Asian (OR = 8.70) populations in cancer versus control patients, suggesting that African populations might be more susceptible to *CDKN2A* promoter methylation. Moreover, the subgroup analysis based on sample type showed that the OR in blood (OR = 16.03) and saliva (OR = 12.45) samples

were higher than in tissue samples (OR = 6.40), indicating *CDKN2A* promoter methylation in body fluid samples was associated with a higher risk of developing HNSCC.

HNSCC is a progressive disease that originates predominantly from dysplasia [96].

Patients with precancerous, such as erythroplakia and leukoplakia, may have a higher risk of malignant [97]. There are the molecular basis for the difference between precancerous lesion and HNSCCs, such as loss of heterozygosity (LOH), microsatellite instability or allelic imbalance (AI), and epigenetic events, including DNA methylation [97]. Notably, we found a significantly higher methylation level of *CDKN2A* promoter in HNSCC than premalignant lesions. Meanwhile, the frequency of *CDKN2A* methylation was markedly higher in premalignant lesions than healthy controls. Interestingly, the OR of premalignant lesions vs. normal controls was higher than that of HNSCC vs. normal controls, which suggests abnormal methylation might be an early event in this cancer [98]. Taken together, these results indicate *CDKN2A* promoter hypermethylation might correlate with carcinogenesis of HNSCC.

In addition, we also determined the correlations between *CDKN2A* promoter methylation and clinicopathological characteristics of HNSCC patients. Our findings revealed a preponderance of *CDKN2A* promoter methylation in male patients, which may account for the higher incidence and mortality of HNSCC in males [3]. Lymphatic metastasis and clinical stage are vital factors that affect outcomes of cancer patients [99, 100]. Interestingly, our analysis demonstrated a significantly elevated frequency of *CDKN2A* promoter methylation in patients with lymph node metastasis, suggesting that *CDKN2A* promoter methylation may play a critical role in HNSCC metastasis.

The screening for HNSCC depends on clinical symptoms and imaging examinations such as computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) [101]. However, because of nonspecific symptoms in the early stage and lack of effective diagnostic biomarkers, a low early diagnostic rate brings challenges for effective treatment. Abnormal methylation biomarkers have proven to be useful in diagnosing numerous cancers [102, 103]. In the current study, the results demonstrated that the combined sensitivity, specificity, and AUC values of *CDKN2A* methylation were 0.36, 0.96, and 0.77, respectively, indicating that detection of *CDKN2A* promoter methylation has a moderate diagnostic accuracy for HNSCC. We further assessed the diagnostic effect of *CDKN2A* promoter methylation in HNSCC precancerous patients vs. healthy subjects. Interestingly, the AUC was 0.95 and revealed efficient diagnostic power of *CDKN2A* promoter methylation in diagnosis of HNSCCs from precancerous samples, which can reveal that methylation is a relatively early molecular change during carcinogenesis as previous study concluded [13, 104]. Some studies have suggested that DNA methylation can be detected in body fluid samples (blood, bronchial aspirates, brushing, saliva, and urine) as a non-invasive molecular biomarker for cancer screening and diagnosis [105-107]. We conducted a subgroup analysis based on sample type and the results showed that the AUC of saliva was 0.96, which was greater than that of tissue. Compared to tissue, saliva has a significant diagnostic advantage because its collection is non-invasive and simple [39]. Because of only three studies reported the association between *CDKN2A* methylation and HNSCC using blood samples, the data were insufficient and unavailable to calculate the AUC. More studies with sufficient data are needed in the future. Taken together, these results suggested that methylated *CDKN2A* promoter has a potential value as a non-invasive biomarker for HNSCC screening and diagnosis.

Tumor node metastasis (TNM) staging is still the vital tool in predicting cancer prognosis [108, 109]. However, the latest edition of the TNM classification is unable to absolutely satisfy clinical application due to the heterogeneous molecular mechanisms and clinical behaviors of HNSCC. Effective prognostic biomarkers can be of assistance in identifying patients that are at risk of recurrence or poor outcomes, and may therefore benefit from treatment stratification and intensified surveillance [110]. Previous studies have shown that

methylated *CDKN2A* were precise prognostic markers for cancers [111, 112]. In the present study, the results indicated that compared to HNSCC patients with *CDKN2A* hypomethylation, those with *CDKN2A* hypermethylation had a 1.01-fold higher risk of poor OS and 1.77-fold higher risk of RFS, indicating hypermethylation of *CDKN2A* promoter is a potential prognostic biomarker for HNSCC patients, especially for predicting recurrence. However, the HR for *CDKN2A* hypermethylation influencing the OS was very small since limited studies were analyzed, rigorous clinical research studies with larger sample sizes will be essential to corroborate our findings.

Several limitations of our meta-analysis should be considered. First, only articles published in English and Chinese were included in the study, which may have contributed to selection bias. Second, because only three studies used blood samples, the data were insufficient and not available to evaluate the diagnostic power of methylated *CDKN2A* for HNSCC based on blood samples. Further studies with larger sample populations are needed. Third, Egger's test and funnel plot exhibited publication bias for the analysis of association between *CDKN2A* methylation and HNSCC.

## Conclusion

In conclusion, this integrated analysis provides strong evidence that *CDKN2A* methylation is significantly associated with the carcinogenesis and metastasis of HNSCC. Additionally, methylated *CDKN2A* is a promising biomarker for the diagnosis and prognosis of HNSCC. Future large-scale studies are necessary to confirm our results.

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Conceived and designed the experiments: CZ, ZS and JL. Performed the experiments: CZ, HG and JL. Analyzed the data: CZ, DY, and HD. Contributed analysis tools: JL and CZ. Wrote the manuscript: JL, CZ and QL.

## Disclosure Statement

None of the authors has any commercial or other associations that might pose a conflict of interests.

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