

The association, clinicopathological significance, and diagnostic value of *CDHI* promoter methylation in head and neck squamous cell carcinoma: a meta-analysis of 23 studies

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Abstract: Epithelial cadherin (encoded by the *CDHI* gene) is a tumor suppressor glycoprotein that plays a role in the invasion and metastasis of human cancers. As previous studies regarding the association between *CDHI* promoter methylation and head and neck squamous cell carcinoma (HNSCC) have yielded inconsistent conclusions, a meta-analysis was performed. A systematic literature review was undertaken from four databases: PubMed, Embase, Google Scholar, and Web of Science. Finally, a total of 23 studies (including 1,727 cases of HNSCC and 555 normal controls) were included in the present study. Our results showed that the frequency of *CDHI* promoter methylation in HNSCC was statistically greater than in controls (odds ratio [OR] = 5.94, 95% confidence interval [CI]: 3.36–10.51, $P < 0.001$). In reported cases of HNSCC, *CDHI* promoter methylation was statistically associated with tumor stage (OR = 0.46, 95% CI: 0.27–0.78, $P = 0.004$) and a history of alcohol consumption (OR = 6.04, 95% CI: 2.41–15.14, $P < 0.001$). Moreover, the sensitivity, specificity, and area under the curve of the summary receiver operator characteristic for the included studies were 0.50 (95% CI: 0.4–0.61), 0.89 (95% CI: 0.79–0.95), and 0.74 (95% CI: 0.70–0.78), respectively. In conclusion, our meta-analyses indicated that *CDHI* promoter methylation was associated with HNSCC risk, and may be utilized as a valuable diagnostic biomarker for HNSCC.

Keywords: *CDHI*, methylation, diagnosis, head and neck squamous cell carcinoma, HNSCC

Introduction

Head and neck cancer is the sixth most common cancer worldwide and the main histological type is head and neck squamous cell carcinoma (HNSCC).^{1,2} In the US alone, 48,330 new cases of HNSCC and 9,570 deaths from HNSCC are projected to occur in 2016.³ Although there have been some developments in the diagnosis and treatment of HNSCC during the recent decades,^{4–6} there has been limited improvement in patient survival and mortality rates,^{7–9} especially for advanced-stage disease and elderly patients.¹⁰ Therefore, the development of biomarkers that allow for early detection of HNSCC would be of value at this time.

There are many unknown mechanisms in the etiology and pathogenesis of HNSCC, but the role of alcohol consumption and smoking, as well as infection with high-risk subtypes of human papillomavirus are now known risk factors for HNSCC.^{11,12} Genetic and epigenetic factors are also involved in the initiation and progression of HNSCC.^{13,14} More recently, there has been increasing evidence in the published literature that

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aberrant methylation of cytosine-guanosine dinucleotide (CpG) islands of tumor suppressor gene (TSG) promoter regions is one of the most common epigenetic alterations that has a role in the pathogenesis of HNSCC.^{15,16} There are now more accurate and easily performed detection methods for DNA methylation, which have provided increasing evidence that abnormal DNA methylation patterns may be potential diagnostic biomarkers for the early detection of HNSCC.¹⁷

The *CDHI* gene is located on chromosome 16 (16q22.1), encodes a transmembrane 120 kDa glycoprotein, epithelial cadherin (E-cadherin), and is a TSG that plays a role in the invasion and metastasis of human cancers.^{18,19} Cadherins belong to the family of cell-cell adhesion molecules, which are involved in maintaining intercellular connections and establishing the normal architecture of epithelial tissues.^{18,19} There has been increasing evidence showing that loss of *CDHI* expression is involved in tumor cell invasion and metastasis in cancer, including HNSCC.^{20–22} Several studies have found that promoter methylation of *CDHI* may lead to transcriptional inactivation of *CDHI* and that this mechanism is involved in several types of malignancy, including breast,²³ gastric,²⁴ and colorectal cancers,²⁵ and HNSCC.^{26,27}

However, among the increasing number of studies on the role of *CDHI* promoter methylation and HNSCC, some of the findings of these studies have been contradictory. Some studies have concluded that *CDHI* methylation was related to the development of HNSCC.^{15,28} However, there have been other studies that the association between *CDHI* methylation and HNSCC did not reach statistical significance.^{16,29}

Therefore, in the current study, we performed a meta-analysis to quantitatively evaluate the association between *CDHI* promoter methylation and HNSCC. Furthermore, we estimated the relationship between *CDHI* promoter methylation and clinicopathological parameters in HNSCC. We also assessed the diagnostic value of *CDHI* methylation for HNSCC, in order to provide evidence for the future application of *CDHI* in the diagnosis of HNSCC.

Materials and methods

Study search strategy

A comprehensive literature search was performed from the following electronic databases: PubMed, Embase, Google Scholar, and Web of Science, without language restrictions. The last search was updated on March 3, 2016. The following key words were used in the database literature search: “methylation” or “DNA methylation” or “promoter methylation” or “demethylation” or “hypermethylation”; “squamous cell carcinoma” or “cancer”; “oral” or “oropharyngeal” or

“oropharynx” or “head and neck” or “tonsil”; “*CDHI*” or “E-cadherin” or “epithelial cadherin” or “cadherin-1” or “uvomorulin”. Additionally, a manual search was conducted to find potentially relevant articles.

Literature selection criteria

The following criteria were used to evaluate the eligibility of included studies: 1) the study focused on the association between *CDHI* promoter methylation and HNSCC; 2) all patients had a histologically confirmed diagnosis of HNSCC; and 3) the study provided sufficient information about the frequency of *CDHI* promoter methylation. The study was excluded if it could not meet the required inclusion criteria. If the authors had published several studies using the same study population, only the most recent or the study with the largest sample size was included in the meta-analysis.

Data quality assessment

The quality of the studies was assessed according to the Newcastle–Ottawa Scale (NOS) criteria.³⁰ The NOS study quality evaluation system includes three considerations: 1) the subject selection: 0–4 points; 2) comparability of the subject: 0–2 points; and 3) clinical outcome: 0–3 points. The NOS scores range from 0 to 9; a score ≥ 7 indicates a good quality study.

Data extraction

The data were independently extracted from the eligible studies by two authors using a standard data extraction form, including the first author’s name, country, year of publication, patient ethnicity, sample size, sample type in the case and the control group, clinicopathological characteristics, detection method of methylation and methylation frequency of *CDHI* promoter, both in HNSCC cases and controls. Clinicopathological characteristics of the subjects – including age, gender (male vs female), smoking behaviors (cigarette smoking history vs no cigarette smoking history), alcohol consumption (alcohol consumption history vs no alcohol consumption history), differentiation grade (well vs moderate or poor), tumor stage (T_{1+2} vs T_{3+4}), clinical stage (I + II vs III + IV), lymph node metastasis (yes vs no) – were noted. If there were any disagreements, a third reviewer and consensus were used.

Statistical analysis

In the current study, STATA-12.0 software (Stata Corporation, College Station, TX, USA) was used to analyze the data. The summary odds ratios (ORs) with its corresponding 95% confidence intervals (CIs) were calculated to determine

the correlation between *CDHI* promoter methylation and HNSCC, as well as the clinicopathological characteristics. Between-study heterogeneity was assessed and visually represented using χ^2 -based Cochran Q statistic test and I^2 test.^{31,32} If the Q-test showed a $P < 0.05$ or $I^2 > 50\%$, indicating significant heterogeneity, the random effect model (DerSimonian–Laird method)³³ was conducted; otherwise, the fixed effect model (Mantel–Haenszel method)³⁴ was used. The sources of heterogeneity were analyzed by meta-regression and subgroup analyses. Subgroup analysis was performed by control types (autogenous vs heterogeneous), ethnicity (African vs Caucasian vs Asian), sample size (≥ 60 vs < 60), methylation detection method (with methylation-specific polymerase chain reaction [MSP] vs without MSP) and publication year (before 2010 vs during or after 2010). To evaluate the effect of single study on the pooled ORs, a sensitivity analysis was performed. The publication bias was exhibited by the funnel plot and assessed by Begg’s linear regression test.³⁵ The Fail safe number (N_{fs}) was calculated to estimate the influence of publication bias to our conclusion by the Meta package in R (version 3.22, <http://www.r-project.org/>). The pooled sensitivity, specificity, and area under curve (AUC) of the summary receiver operator characteristic (ROC) with their 95% CIs were analyzed to determine the diagnostic value of *CDHI* promoter methylation for HNSCC.³⁶ All the tests were two-sided and a P -value of < 0.05 was of statistical significance. All data were computed separately by two investigators and a final consensus was reached.

Results

Baseline characteristics of included studies

An initial total of 319 publications were selected with 318 publications from database searches and one publication from manual searching. Of these initial 319 publications, 172 studies were excluded due to study duplication and 130 studies due to lack of relevance. In final, there were 23 studies included in our systematic quantitative analysis, including 1,727 cases of HNSCC and 555 control cases. Among these 23 studies, 17 case–control studies assessed the association between *CDHI* promoter methylation and HNSCC.^{15,16,26–29,37–47} Four of these 17 studies also evaluated the relationship of *CDHI* promoter methylation and the clinicopathological characteristics of HNSCC.^{27,41–43} Eventually, a further six studies^{21,48–52} combined with these four studies were used to quantitatively assess the association between methylated *CDHI* and the clinicopathological characteristics of the HNSCC cases. Figure 1 shows the selection procedure

of our analysis. The NOS criteria³⁰ scores of all included studies were more than 6. The individual characteristics of the included studies are summarized in Table 1.

Association between *CDHI* promoter methylation and HNSCC risk

The results of the meta-analysis indicated that the frequency of *CDHI* methylation in patients diagnosed with HNSCC was significantly elevated when compared with normal controls (OR = 5.94, 95% CI: 3.36–10.51, $P < 0.001$, Figure 2). There was significant heterogeneity across the included studies ($I^2 = 64.6\%$, $P < 0.001$). The potential sources of heterogeneity were investigated by applying meta-regression analysis and subgroup analysis. However, the source of heterogeneity was not identified by meta-regression analysis (Table 2). The subgroup analysis was performed based on ethnicity, control types, sample size, methods for detecting methylation, and the study publication year. In the ethnicity-based stratified analyses, the pooled OR for *CDHI* methylation in HNSCC compared with normal controls in the Asian group was 13.39 (95% CI: 5.35–33.48, $P < 0.001$), and was greater than that in the African (OR = 8.52, 95% CI: 3.18–22.83, $P < 0.001$) and Caucasian groups (OR = 2.80, 95% CI: 1.95–4.02, $P < 0.001$). Furthermore, the degree of heterogeneity was reduced in all the three ethnic subgroups (Figure 3). The heterogeneity did not change remarkably in the other subgroup analysis. The detailed subgroup analysis results are shown in Table 3. Therefore, sensitivity analysis was performed by omitting each study in turn, under the random effects model, which demonstrated that no single study could essentially influence the overall pooled ORs, supporting the robust nature of the meta-analysis (Figure 4).

Association between *CDHI* promoter methylation and the clinicopathological features of HNSCC

A total of 10 studies, which included 991 patients, were performed to analyze the associations between *CDHI* promoter methylation and the HNSCC clinicopathological features, including age, gender, smoking behavior, alcohol consumption, tumor stage, clinical stage, histological tumor grade, and the presence of lymph node metastasis (Table 4). The result showed that the *CDHI* promoter methylation was significantly associated with tumor stage (pooled OR = 0.46, 95% CI: 0.27–0.78, $P = 0.004$, Figure 5) and alcohol consumption (pooled OR = 6.04, 95% CI: 2.41–15.14, $P < 0.001$, Figure 6). However, there was no association between other clinicopathological characteristics and *CDHI* promoter methylation in HNSCC.

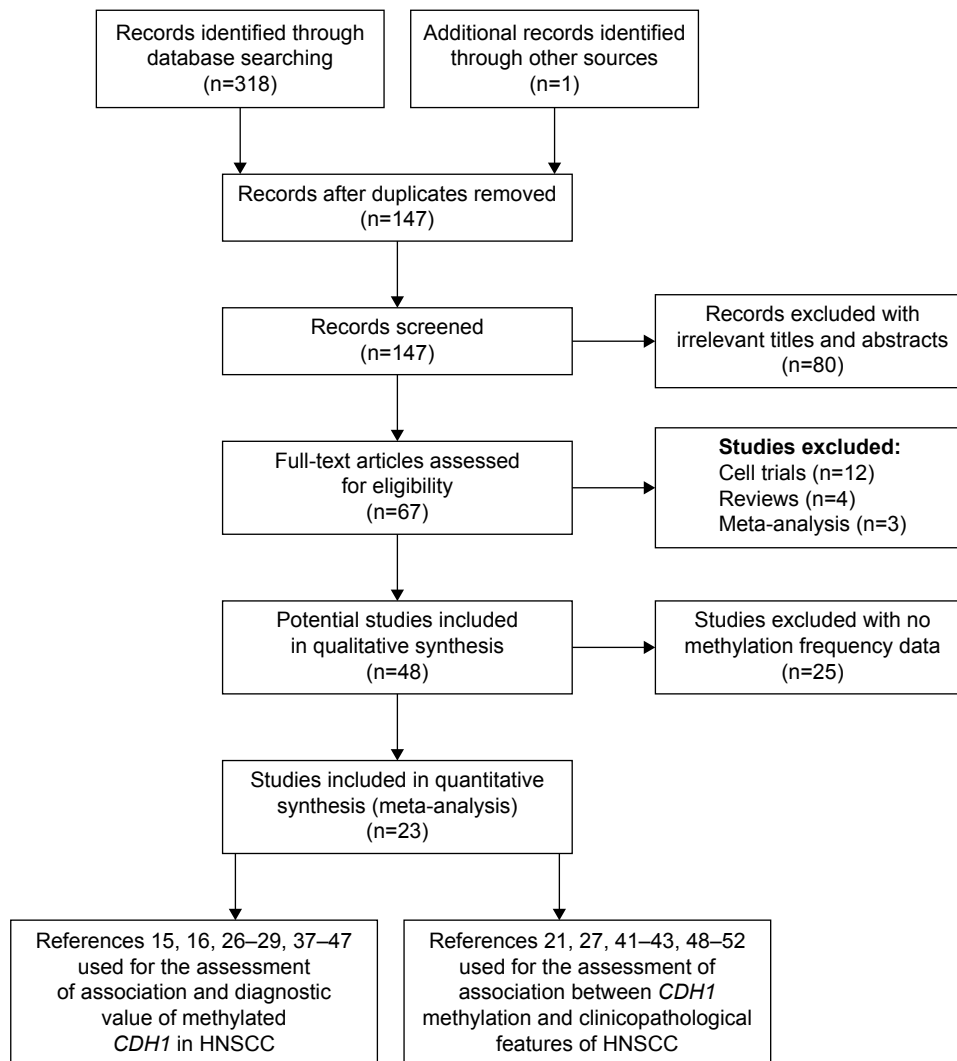


Figure 1 Flow diagram of the study selection process in this meta-analysis.

Abbreviation: HNSCC, head and neck squamous cell carcinoma.

Diagnostic value of *CDH1* promoter methylation for HNSCC

In the current analysis, 17 eligible case–control studies were included to assess the diagnostic value of *CDH1* promoter methylation for HNSCC. Figure 7 shows the pooled sensitivity and specificity for all included studies, which were 0.50 (95% CI: 0.40–0.61) and 0.89 (95% CI: 0.79–0.95). The AUC of the summary ROC was 0.74 (95% CI: 0.70–0.78) (Figure 8), indicating that the detection of *CDH1* methylation was associated with a diagnosis of HNSCC, representing a potential diagnostic biomarker.

Publication bias

A Begg's funnel plot was performed to assess the publication bias of literatures. Figure 9 shows that the shape of the funnel plot showed no evidence of publication bias ($P=0.077$). Furthermore, we conducted an N_{fs} to assess the efficacy of

the meta-analysis ($N_{fs0.05}=611$, $N_{fs0.01}=181$), which indicated that our results were robust.

Discussion

Previous studies have shown that hypermethylation of TSG promoters in many cancers can contribute to tumor progression.^{53,54} Specifically, *CDH1*, which encodes the cell adhesion protein E-cadherin, is an important TSG.⁵⁵ Studies have shown that loss of *CDH1* expression by promoter hypermethylation is involved in several types of cancer, including colorectal,⁵⁶ lung,⁵⁷ breast,²³ and gastric cancers.⁵⁸ This meta-analysis was done to resolve some of the inconsistent reports of the association between *CDH1* promoter methylation and HNSCC.^{15,16}

In this meta-analysis, a total of 23 studies included 1,727 cases of HNSCC (and 555 control cases). The results showed that the frequency of *CDH1* promoter methylation in

Table 1 The main characteristics of included studies in this meta-analysis

| References | Year | Country | Ethnicity | Method | Sample type | Case | | Control | | Control source |
|--------------------------------------|------|----------------------------|-----------|----------------|-------------|------|-------|---------|-------|----------------|
| | | | | | | M | Total | M | Total | |
| Saito et al ²⁷ | 1998 | Japan | Asian | MSRE | Tissue | 9 | 52 | 0 | 52 | Autologous |
| Yeh et al ³⁸ | 2002 | People's Republic of China | Asian | MSP | Tissue | 41 | 48 | 16 | 48 | Autologous |
| Chang et al ²⁶ | 2002 | People's Republic of China | Asian | MSRE | Tissue | 45 | 70 | 0 | 11 | Heterogeneous |
| Viswanathan et al ³⁷ | 2003 | India | African | MSRE | Tissue | 35 | 99 | 0 | 25 | Autologous |
| Shaw et al ²⁹ | 2006 | UK | Caucasian | Pyrosequencing | Tissue | 30 | 71 | 6 | 18 | Autologous |
| de Moraes et al ³⁹ | 2008 | Brazil | Caucasian | MSP | Tissue | 27 | 46 | 0 | 5 | Heterogeneous |
| Righini et al ⁴⁰ | 2007 | France | Caucasian | MSP | Tissue | 32 | 90 | 0 | 30 | Autologous |
| Steinmann et al ⁴¹ | 2009 | Germany | Caucasian | MSP | Tissue | 23 | 54 | 2 | 23 | Autologous |
| Su et al ⁴² | 2010 | People's Republic of China | Asian | MSP | Tissue | 21 | 31 | 12 | 31 | Autologous |
| Kordi-Tamandani et al ⁴³ | 2010 | Iran | Caucasian | MSP | Tissue | 47 | 76 | 31 | 57 | Heterogeneous |
| Weiss et al ¹⁶ | 2011 | Germany | Caucasian | MSP | Tissue | 13 | 37 | 7 | 31 | Heterogeneous |
| Supic et al ²⁸ | 2011 | Serbia | Caucasian | MSP | Tissue | 20 | 47 | 6 | 47 | Autologous |
| Nagata et al ⁴⁴ | 2012 | Japan | Asian | MSP | Rinse | 32 | 34 | 5 | 24 | Heterogeneous |
| Xu et al ⁴⁷ | 2012 | People's Republic of China | Asian | MSP | Tissue | 22 | 60 | 2 | 50 | Heterogeneous |
| Asokan et al ⁴⁵ | 2014 | India | African | MSP | Tissue | 6 | 10 | 0 | 5 | Heterogeneous |
| Mielcarek-Kuchta et al ⁴⁶ | 2014 | Poland | Caucasian | MSP | Tissue | 24 | 53 | 14 | 53 | Autologous |
| Choudhury and Ghosh ¹⁵ | 2015 | India | African | MSP | Tissue | 23 | 71 | 4 | 45 | Autologous |
| Hasegawa et al ⁵⁰ | 2002 | USA | Caucasian | MSP | Tissue | 29 | 80 | na | na | na |
| Calmon et al ⁴⁸ | 2007 | Brazil | Caucasian | MSP | Tissue | 38 | 43 | na | na | na |
| Dikshit et al ⁴⁹ | 2007 | France | Caucasian | MSP | Tissue | 82 | 190 | na | na | na |
| Marsit et al ⁵¹ | 2008 | USA | Caucasian | MSP | Tissue | 113 | 340 | na | na | na |
| Supic et al ⁵² | 2009 | Serbia | Caucasian | nMSP | Tissue | 33 | 77 | na | na | na |
| Pannone et al ²¹ | 2014 | Italy | Caucasian | MSP | Tissue | 14 | 48 | na | na | na |

Notes: Autologous: control from the HNSCC group; Heterogeneous: control from other individuals.

Abbreviations: M, methylation; MSRE, methylation-sensitive restriction endonuclease; MSP, methylation-specific polymerase chain reaction; nMSP, nested methylation-specific polymerase chain reaction; na, not available; HNSCC, head and neck squamous cell carcinoma.

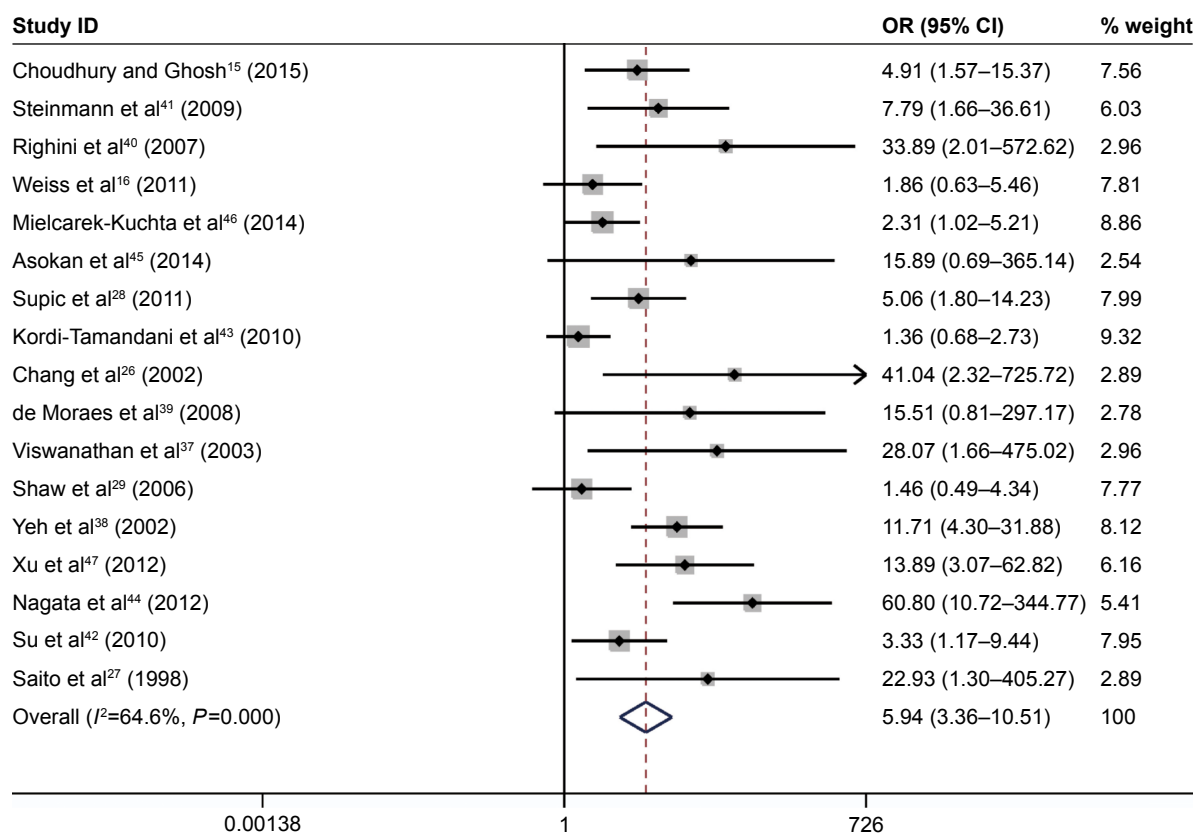


Figure 2 Forest plot for evaluating the association between *CDHI* promoter methylation and head and neck squamous cell carcinoma (HNSCC) by application of the random-effect model.

Note: Weights are from random effects analysis.

Abbreviations: CI, confidence interval; OR, odds ratio; HNSCC, head and neck squamous cell carcinoma.

Table 2 Meta-regression analysis based on publication year, ethnicity, detection method, control type, case size

| Heterogeneity sources | Coefficient | 95% CI | | P-value |
|-----------------------|-------------|--------|-------|---------|
| | | Lower | Upper | |
| Publication year | -0.058 | -0.307 | 0.191 | 0.613 |
| Ethnicity | | | | |
| African | -0.102 | -2.89 | 2.686 | 0.936 |
| Caucasian | -1.046 | -2.797 | 0.706 | 0.21 |
| Detection method | | | | |
| MSP | -0.578 | -4.056 | 2.89 | 0.716 |
| Pyrosequence | -1.645 | -5.465 | 2.176 | 0.356 |
| Control type | 0.209 | -1.24 | 1.658 | 0.752 |
| Case size | <0.001 | -0.043 | 0.043 | 0.996 |

Abbreviations: CI, confidence interval; MSP, methylation-specific polymerase chain reaction.

HNSCC was statistically greater than in controls (OR =5.94, 95% CI: 3.36–10.51, $P < 0.001$). In reported cases of HNSCC, *CDHI* promoter methylation was associated with tumor stage (OR =0.46, 95% CI: 0.27–0.78, $P = 0.004$) and a history of alcohol consumption (OR =6.04, 95% CI: 2.41–15.14,

$P < 0.001$). The findings demonstrated almost sixfold greater level of *CDHI* methylation in the HNSCC patient group compared with normal controls, indicating that hypermethylation of *CDHI* was strongly associated with HNSCC, which would support its role as a diagnostic biomarker. The reduced value of I^2 found in the stratified analysis by ethnicity indicated that ethnicity might account for some of the study heterogeneity. The OR of the Asian subgroup with HNSCC was greater than that of the Caucasian and African subgroups with HNSCC, indicating that the Asian population may be more susceptible to *CDHI* promoter methylation, which is supported by a previous study.⁵⁹

This study showed that there was an increased frequency of *CDHI* promoter methylation with more advanced tumor stage HNSCC compared with early tumor stage disease, which may also support a role for *CDHI* promoter methylation in the invasion progression of HNSCC. Alcohol consumption is a known predisposing factor for HNSCC⁶⁰ and has been shown to induce DNA methylation

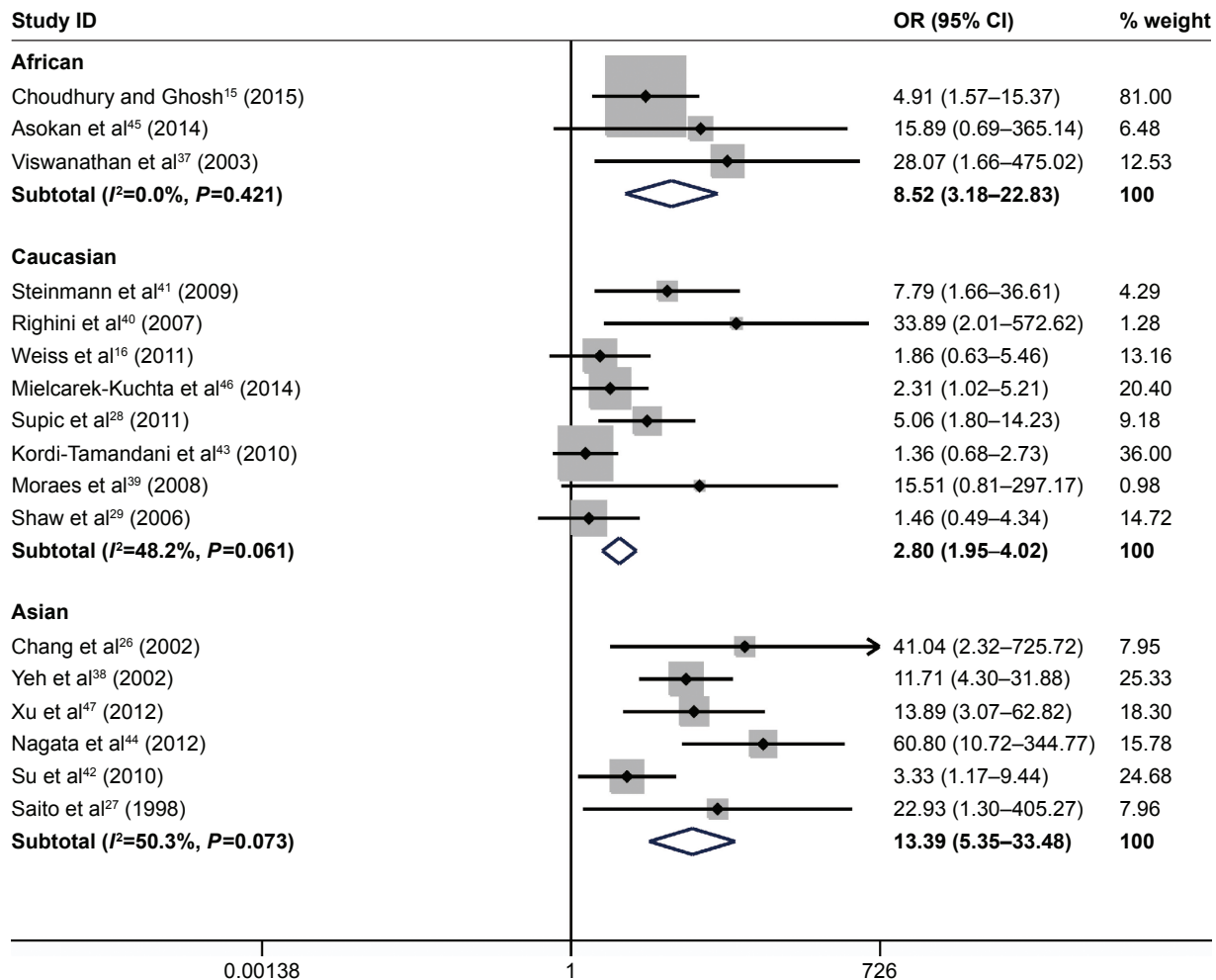


Figure 3 Forest plot for the subgroup analyses by ethnicity. **Abbreviations:** CI, confidence interval; OR, odds ratio; HNSCC, head and neck squamous cell carcinoma.

Table 3 Subgroup analyses of *CDHI* promoter methylation in HNSCC

| Subgroup | Case | | Control | | M-H pooled OR | D-L pooled OR | Heterogeneity | |
|----------------|------|-----|---------|-----|--------------------------|-----------------------------|--------------------|---------|
| | M | U | M | U | OR (95% CI) | OR (95% CI) | I ² (%) | P-value |
| Total | 450 | 499 | 105 | 450 | 4.82 (3.64–6.39) | 5.94 (3.36–10.51) | 64.6 | <0.001 |
| Race | | | | | | | | |
| African | 64 | 116 | 4 | 71 | 8.52 (3.18–22.83) | 6.893 (2.53–18.78) | 0 | 0.42 |
| Caucasian | 216 | 258 | 66 | 198 | 2.80 (1.95–4.02) | 2.803 (1.56–5.03) | 48.2 | 0.06 |
| Asian | 170 | 125 | 35 | 181 | 11.84 (6.74–20.79) | 13.39 (5.35–33.48) | 50.3 | 0.07 |
| Control types | | | | | | | | |
| Autologous | 258 | 358 | 60 | 312 | 5.22 (3.61–7.56) | 4.975 (2.83–8.75) | 46 | 0.05 |
| Heterogeneous | 192 | 141 | 45 | 138 | 4.27 (2.76–6.61) | 8.69 (2.36–31.96) | 78.4 | <0.001 |
| Methods | | | | | | | | |
| MSP | 267 | 271 | 79 | 277 | 4.55 (3.36–6.15) | 5.56 (3.05–10.14) | 65.8 | <0.001 |
| No MSP | 119 | 173 | 6 | 100 | 6.58 (2.98–14.56) | 29.763 (5.72–154.95) | 71.8 | 0.01 |
| Sample size | | | | | | | | |
| <60 | 216 | 196 | 62 | 257 | 5.44 (3.73–7.94) | 5.64 (3.08–10.33) | 56.6 | 0.01 |
| ≥60 | 234 | 303 | 43 | 193 | 4.20 (2.75–6.41) | 10.95 (1.25–96.12) | 73.2 | 0 |
| Published year | | | | | | | | |
| <2010 | 242 | 288 | 24 | 188 | 9.21 (5.24–16.18) | 9.947 (3.77–26.23) | 50.8 | 0.05 |
| ≥2010 | 208 | 211 | 81 | 262 | 3.56 (2.52–4.91) | 4.36 (2.23–8.55) | 67.7 | 0 |

Note: The pooled OR with 95% CI was calculated by appropriate effect model based on heterogeneity and highlighted in boldface.

Abbreviations: CI, confidence interval; HNSCC, head and neck squamous cell carcinoma; M, methylation; MSP, methylation-specific polymerase chain reaction; OR, odds ratio; U, unmethylation.

in oncogenesis.⁶¹ In the current study, *CDHI* promoter methylation was significantly increased in patients with high alcohol consumption, indicating that it may contribute to HNSCC via the induction of hypermethylation of *CDHI*. These findings support the need for further controlled studies with large patient sample sizes to evaluate these ethnic,

social, and etiological factors involved in the etiology and pathogenesis of HNSCC.

The findings of this meta-analysis support a possible diagnostic role for *CDHI* promoter methylation evaluation in HNSCC, with pooled sensitivity and specificity of 0.5 and 0.89, respectively. Previous studies have shown

Meta-analysis estimates, given named study is omitted

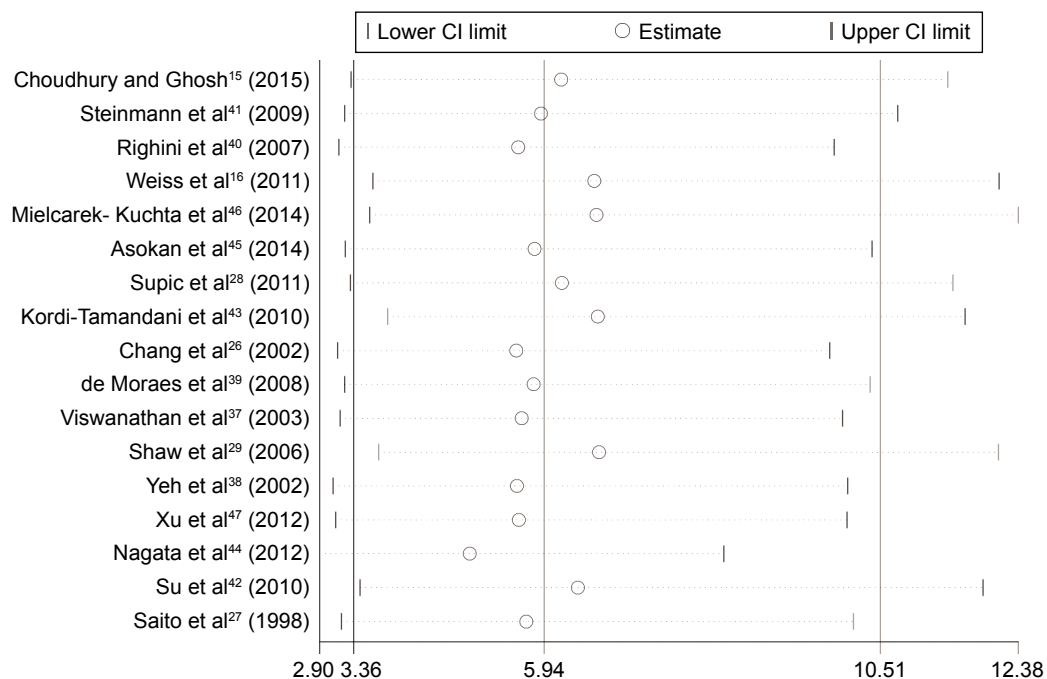


Figure 4 Sensitivity analysis of pooled OR for *CDHI* promoter methylation and HNSCC under the random effects model.

Abbreviations: CI, confidence interval; OR, odds ratio; HNSCC, head and neck squamous cell carcinoma.

Table 4 The association between and *CDH1* promoter methylation and the clinicopathological features in HNSCC

| Characteristics | No ^a | Case/control types | Cases/controls | OR (95% CI) | P-value | I ² % |
|-----------------------|-----------------|------------------------------------|----------------|-------------------|---------|------------------|
| Age | 4 | Older/younger | 354/329 | 0.84 (0.61–1.15) | 0.277 | 0 |
| Gender | 5 | Male/female | 560/156 | 0.82 (0.55–1.20) | 0.306 | 0 |
| Smoking behavior | 5 | Yes/no | 523/128 | 1.14 (0.76–1.70) | 0.539 | 29.8 |
| Alcohol consumption | 2 | Yes/no | 40/62 | 6.04 (2.41–15.14) | <0.001 | 0 |
| Differentiation grade | 4 | Well/moderate or poor | 61/115 | 0.42 (0.08–2.24) | 0.312 | 56.7 |
| Tumor stage | 6 | T ₁₊₂ /T ₃₊₄ | 139/234 | 0.46 (0.27–0.78) | 0.004 | 26.8 |
| Clinical stage | 4 | I + II/III + IV | 65/170 | 0.63 (0.33–1.18) | 0.149 | 25 |
| Lymph node metastasis | 6 | Yes/no | 191/182 | 0.93 (0.56–1.56) | 0.794 | 41.4 |

Note: ^aThe number of included studies.

Abbreviations: CI, confidence interval; OR, odds ratio; HNSCC, head and neck squamous cell carcinoma.

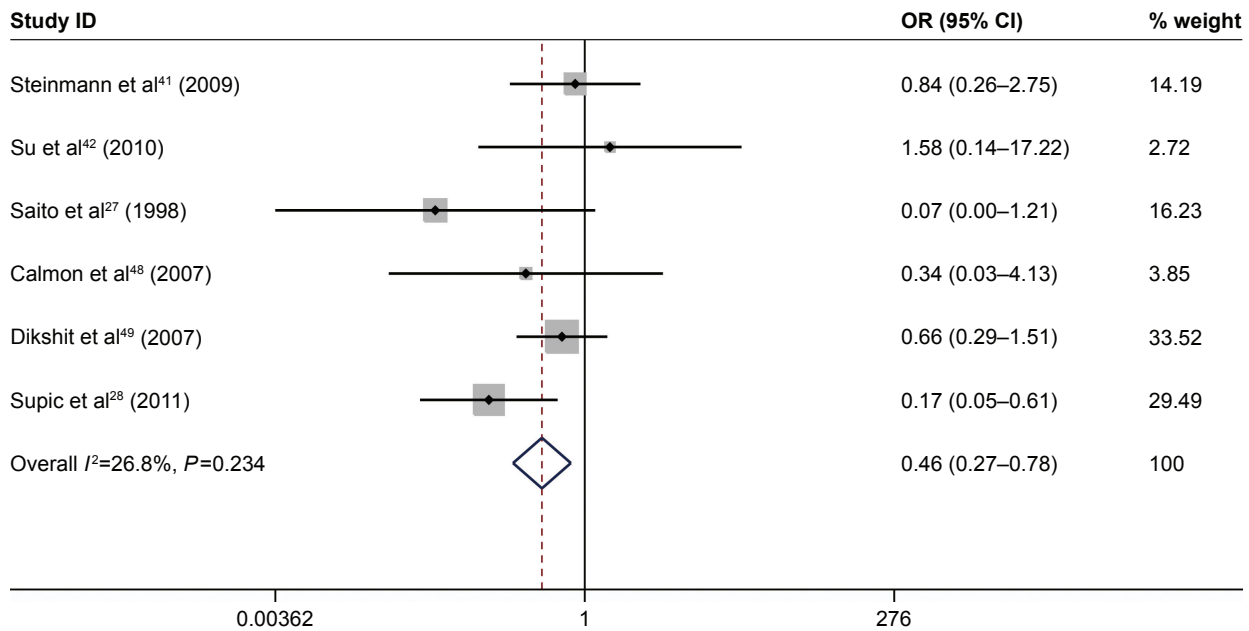


Figure 5 Forest plot for the associations between *CDH1* promoter methylation and tumor stage in HNSCC.

Abbreviations: CI, confidence interval; OR, odds ratio; HNSCC, head and neck squamous cell carcinoma.

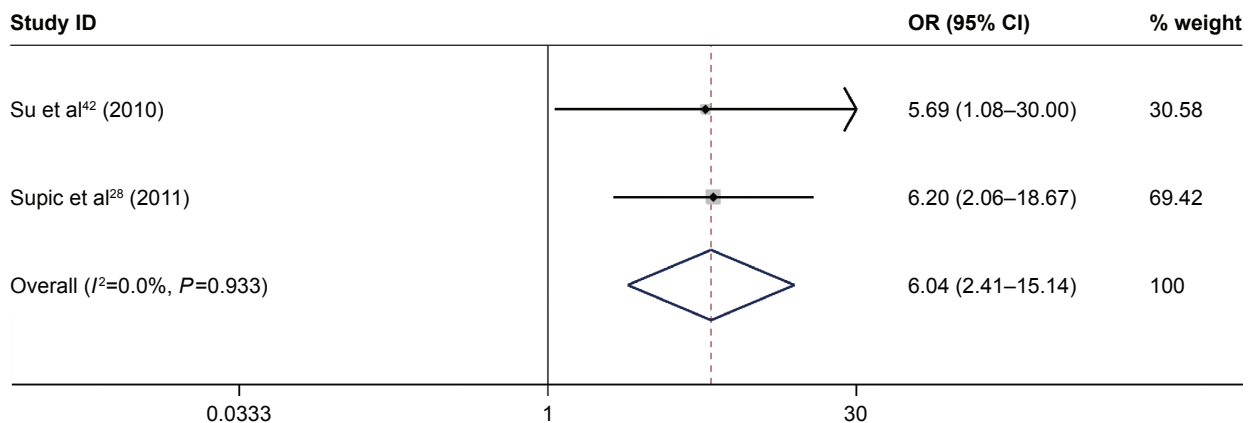


Figure 6 Forest plot for the associations between *CDH1* promoter methylation and alcohol consumption in HNSCC.

Abbreviations: CI, confidence interval; OR, odds ratio; HNSCC, head and neck squamous cell carcinoma.

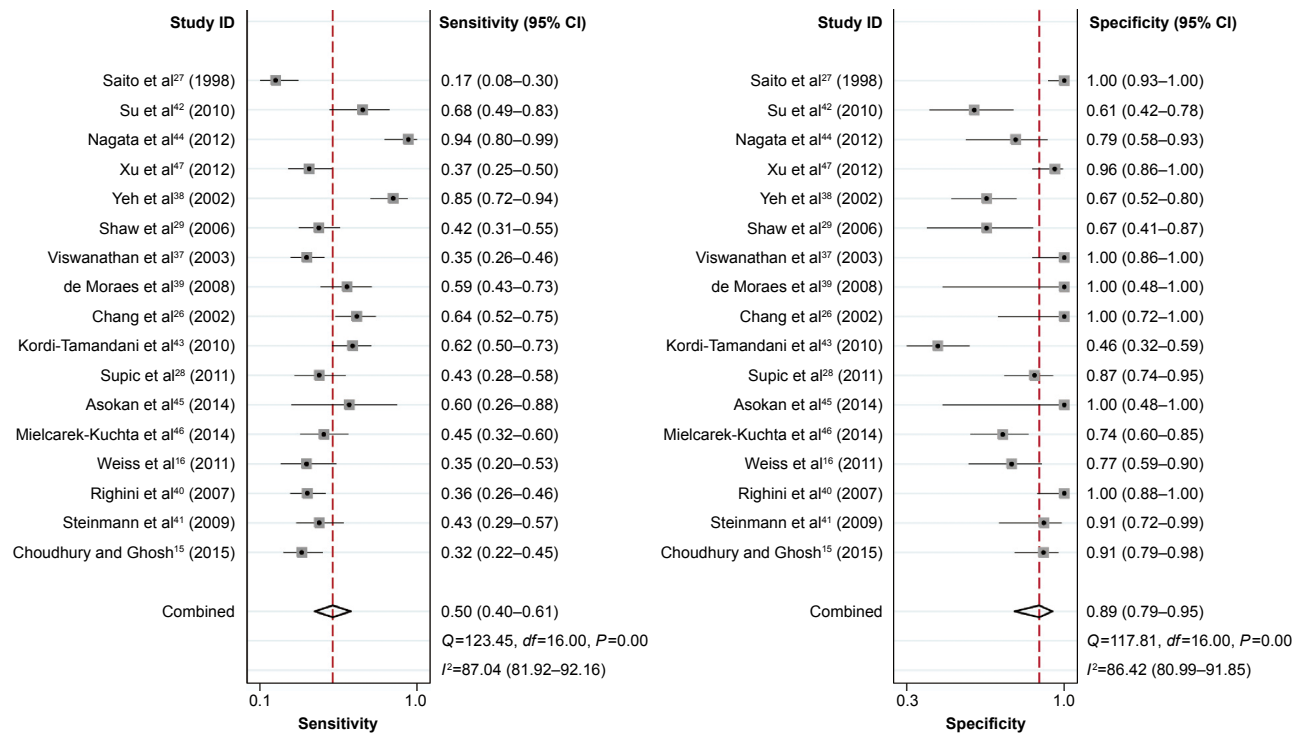


Figure 7 Forest sensitivity and specificity of *CDHI* promoter methylation for head and neck squamous cell carcinoma (HNSCC).
Abbreviations: CI, confidence interval; HNSCC, head and neck squamous cell carcinoma.

that the combination of several methylation biomarkers can improve the sensitivity and specificity of diagnosis testing for cancers, including HNSCC.^{62–64} Therefore, it would be logical to combine *CDHI* methylation testing with other epigenetic biomarkers. This combined diagnostic approach

requires further studies to determine the diagnostic power in HNSCC. When the AUC of the ROC is close to 1.0, this signifies a good risk predictor,^{65,66} and in this study, the AUC for detection of *CDHI* promoter methylation in HNSCC was 0.74, indicating a qualified diagnostic accuracy for *CDHI* promoter methylation in HNSCC.

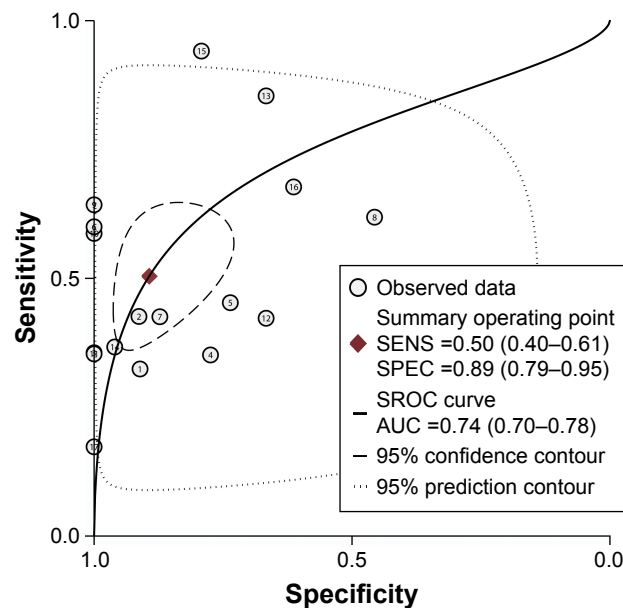


Figure 8 SROC plot with best-fitting asymmetric curve of methylated *CDHI* for the diagnosis of HNSCC.
Abbreviations: AUC, area under curve; HNSCC, head and neck squamous cell carcinoma; SENS, sensitivity; SPEC, specificity; SROC, summary of receiver operating characteristic.

The present meta-analysis had several limitations. First, it must be acknowledged that studies with positive findings on *CDHI* promoter methylation in HNSCC are more likely to be those that are published, resulting in possible publication bias. Second, a significant heterogeneity was observed in the data analysis, which means that the findings should be interpreted

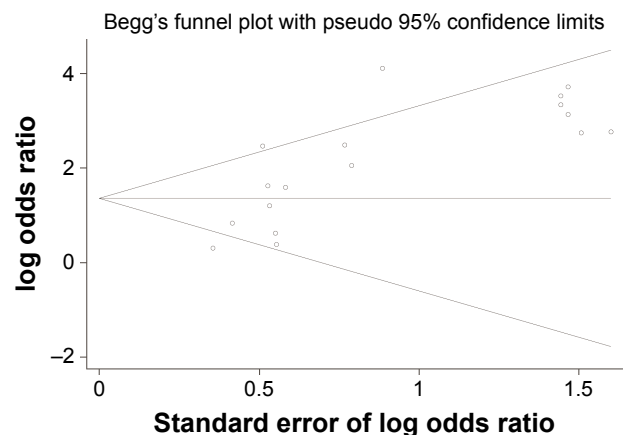


Figure 9 Begg's funnel plot of publication bias.

with caution. Third, although studies in all languages were included, it is possible that relevant studies published in other languages may have been missed. Therefore, in future, we recommend that an updated meta-analysis, including more high quality studies with larger sample sizes, should be done to support or add to the findings of this present study.

Conclusion

In summary, our meta-analysis results have supported the role of promoter methylation of *CDH1* in the diagnosis of HNSCC. These findings may have implications for a future role of this biomarker in the diagnosis of HNSCC.

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Author contributions

ZSS and CCZ conceived and designed the experiments. CCZ and JYL performed the experiments. CCZ and JW analyzed the data. CCZ, HXD, and QL contributed analysis tools. CCZ and JYL wrote the manuscript. All authors contributed toward data analysis, drafting and critically revising the paper, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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