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ZNF582 methylation as a potential biomarker to predict cervical intraepithelial neoplasia type III/worse

A meta-analysis of related studies in Chinese population

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Abstract

Objective: DNA methylation markers have been assessed as potential biomarkers for early cervical cancer detection. Herein, we evaluated the diagnostic performance of zinc finger protein 582 (ZNF582) methylation for cervical cancer detection.

Methods: Eligible studies were systematically searched from the electronic databases. The quality of enrolled studies was evaluated using the second version of the check list for Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2). The bivariate meta-analysis model was employed to plot the summary receiver operator characteristic (SROC) curve using Stata 14.0 software. Cochran's *Q* test and I^2 statistics were applied to assess heterogeneity among studies. Publication bias was evaluated by the Deeks' funnel plot asymmetry test.

Results: Seven studies composed of 1749 patients were eventually included. The pooled sensitivity of ZNF582 methylation was estimated to be 0.71 [95% confidence interval (CI): 0.67–0.75] in differentiating patients with cervical intraepithelial neoplasia type III/worse (CIN3+), corresponding to a specificity of 0.81 (95% CI: 0.79–0.83) and area under the curve (AUC) of 0.85. Our stratified analysis suggested that sequential combined of HPV DNA and ZNF582 methylation test (AUC, sensitivity, and specificity of 0.876, 0.75, and 0.87, respectively) achieved higher diagnostic accuracy than single HPV DNA testing test (AUC, sensitivity and specificity of 0.669, 0.96, and 0.41, respectively).

Conclusions: ZNF582 methylation has a prospect to be an auxiliary biomarker for cervical cancer screening. A new strategy of co-testing HPV DNA and ZNF582 methylation test in cervical scrapings confers an improved diagnostic accuracy than single HPV DNA testing.

Abbreviations: AUC = area under the curve, CIN = cervical intraepithelial neoplasia, DOR = diagnostic odds ratio, hrHPV = high-risk human papillomavirus, NLR = negative likelihood ratio, PLR = positive likelihood ratio, SROC = summary receiver operator characteristic.

Keywords: cervical cancer, diagnosis, DNA methylation, zinc finger protein 582

1. Introduction

Cervical cancer is one of the main causes of death of women worldwide.[1–3] The most widely used screening methods for cervical cancer are the cytology-based Pap smear and high-risk human papillomavirus (hrHPV) test. However, cytomorphological examination of cervical smears is not ideal because of its relatively low sensitivity.[4] Although hrHPV testing improves the sensitivity of cervical screening,[5] the specificity of hrHPV testing, especially in a young screening population, is relatively low.[6] Therefore in hrHPV primary screening programs, the less specific screening test may lead to substantially heavy burden on health care resources, such as unnecessary referral to colposcopy makes triage testing compulsory. In this respect, discovering and developing new biomarkers which confer high sensitivity and specificity for cervical cancer detection is a matter of great urgency in the clinic.

Gene silencing by promoter hypermethylation has been shown to contribute to cervical carcinogenesis and methylation analysis of cervical-cancer-specific genes has been suggested as a valuable, alternative or additive triage tool.[7–10] Among these altered and methylated genes, the ZNF582 was highlighted.[11–16] As reported, ZNF582 is frequently silenced by methylation in cervical cancers, and literature have documented the promise of ZNF582 methylation in the detection of cervical precancerous lesions.[17] In order to make a comparison of the accuracy of DNA methylation and HPV DNA testing, we performed a comprehensive meta-analysis and evaluated the diagnostic performance of ZNF582 methylation for the detection of cervical intraepithelial neoplasia (CIN) type III/worse.

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2. Methods

2.1. Search strategy

This meta-analysis was conducted in compliance with the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) Statement issued in 2015.[18] Three electronic databases, Pubmed/Medline, Embase, and Cochrane, were searched for relevant studies until March 1, 2018, using the following Keywords: (cervical cancer or cervical intraepithelial neoplasia or CIN or uterine cervical neoplasm or uterine cervical dysplasia) and (methylation marker or methylation or DNA methylation) and (zinc finger protein 582 OR ZNF582 or HPV or human papillomavirus) and (screening or detection or diagnostic or diagnos*).

2.2. Study selection

The references of all publications were hand-searched in order to identify missing relevant publications. The following criteria were used for the literature selection in this meta-analysis:

(1) studies evaluated the diagnostic performance of ZNF582 methylation or HPV DNA testing in the diagnosis of high-grade squamous intraepithelial lesion (HSIL) or cervical neoplasms;
(2) studies explicitly mentioned the sample size, sensitivity, specificity and their 95% confidence intervals (CIs) or other more detailed information;
(3) Matched controls were included.

Literature was excluded according to the following criteria:

(1) the control group and sample sizes were unclear;
(2) studies without complete data including missing information of sensitivity, specificity or area under the curve (AUC) value, and so on;
(3) studies did not use histology as gold standard and
(4) basic research, animal studies, meta-analysis, review articles, letters, commentaries, abstracts presented at conferences, and so on.

2.3. Data extraction and quality assessment

All the included studies were carefully reviewed independently by 2 investigators (Li and He). All analyses were based on previously published studies, thus no ethical approval and patient consent are required. Data from these articles were extracted according to a predefined registration form. The following information was extracted: the first author, country, year of publication, patient size, study design, CIN degrees, test method, and the diagnostic results, methylation methods, cut-off value, HPV status. In studies contained both a training and a validation group, data from each group was treated as a single study in the meta-analysis. The quality of each included study was evaluated using the second version of the check list for Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) 19. A score was given to 4 domains (participant selection, triage test, reference standard, and flow & timing), based on a set of signaling questions assigned to each domain. Any disagreement was resolved by group consensus.

2.4. Statistical analysis

Statistical analysis was undertaken using Stata 14.0 (Stata Corporation, College Station, TX), and Meta-disc 1.4 (XI Cochrane Colloquium, Barcelona, Spain) software. The bivariate meta-analysis model was employed to summarize the sensitivity, specificity, diagnostic odds ratio (DOR), positive likelihood ratio (PLR) and negative likelihood ratio (NLR) and to generate the bivariate summary receiver operator characteristic (SROC) curves with their corresponding 95% CI. The pooled diagnostic indices were calculated by using a random-effects model.[20] Heterogeneity from threshold and non-threshold effects were reflected by the Spearman correlation coefficient, Cochran's Q and I^2 tests,[21] respectively. Meta-regression and subgroup analysis were performed to trace potential sources of study heterogeneity. The covariates included the following: age (average age ≤ 45 or >45), publication year (≤ 2014 or >2014), sample size (≤ 200 or >200), study location (China or Chinese Taipei). Deeks' funnel plot asymmetry test was conducted to evaluate the potential publication bias, and significant level was set at $P < .05$.

3. Results

3.1. Study characteristics and quality

A total of 422 studies were retrieved from a primary literature search in electronic databases, and 406 studies were excluded due to the status that unrelated to ZNF582 methylation or cervical cancer diagnosis. Nine studies were left for full-text evaluation. In 1 study, the clinical accuracy calculated only in cervical adenocarcinoma was further excluded.[22] Another study didn't use histology as gold standard was discarded as well.[23] Seven studies for ZNF582 methylation and 4 studies for HPV DNA test were included in this meta-analysis. The selection process for relevant studies is shown in Figure 1.

All of the 7 studies were conducted in Asia, including 4 studies in Chinese Taiwan and 3 in China mainland. The final diagnoses of all studies were determined by tissue-proven histopathology, and the evaluation method for DNA methylation was quantitative methylation-specific polymerase chain reaction (QMSP). The main features of each included study were described in Table 1. We evaluated the study quality of each included publications according to the QUADAS2 assessment tool.[19] As shown in Figure 2, all of the 7 studies revealed lower risks of bias, suggesting a relatively high quality of the included studies.

3.2. Heterogeneity

Heterogeneity from threshold and non-threshold effects was assessed using Meta-disc 1.4 software. The P-values of spearman correlation coefficient in ZNF582 methylation test, HPV DNA test, and ZNF582/HPV DNA test were more than 0.05, indicating that there was no heterogeneity from threshold effect. For the individual ZNF582 methylation test, the Cochran's-Q test yielded a Q value of 7.97 ($P > .01$), with $I^2 < 50\%$, suggesting that non-threshold effect is not likely to be a source of heterogeneity. However, heterogeneity generated by non-threshold effects appeared in the other pooled analyzed with P values of Cochran's Q test less than .01, accompanied by $I^2 > 50\%$ (Supplement Digital Content 1, which demonstrates the threshold effect analysis, http://links.lww.com/MD/C804).

3.3. Diagnostic performance

As indicated in Table 2, the pooled accuracies for ZNF582 methylation was determined to assess their usefulness as a biomarker for screening of patients with CIN3+. The pooled sensitivity, specificity, DOR, and AUC for ZNF582 methylation

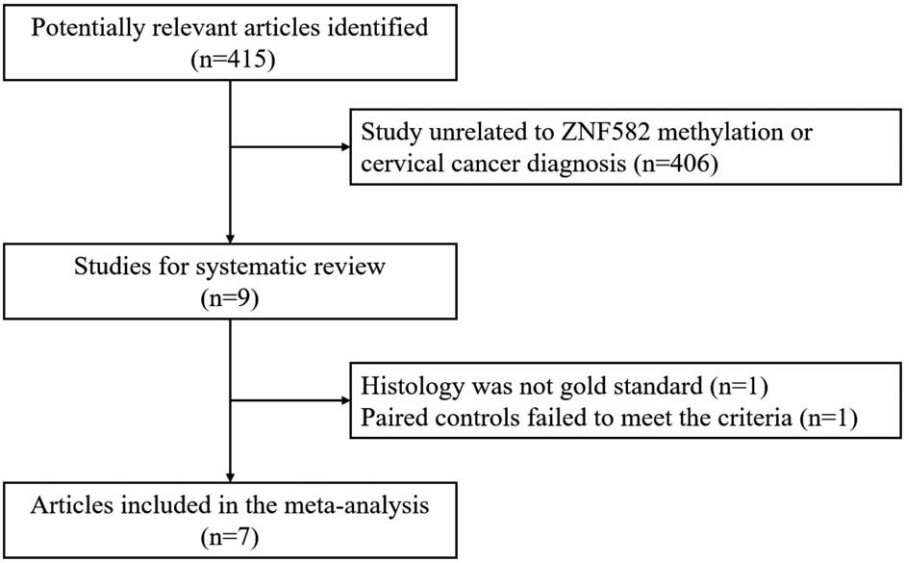

Figure 1. Flowchart of the literature search and study selection procedure.

test were 0.71 (95% CI: 0.67–0.75), 0.81 (95% CI: 0.79–0.83), 12.72 (95% CI: 9.93–12.68), and 0.85, respectively. The forest plots of pooled sensitivity, specificity, and SROC curves for ZNF582 methylation are displayed in Figures 3 and Supplement Digital Content 2, http://links.lww.com/MD/C804. For the HPV DNA testing, it yielded an AUC value of 0.669, with pooled sensitivity of 0.96 (95% CI: 0.93–0.98) and specificity of 0.41 (95% CI: 0.37–0.45).

A random effect model was applied in the stratified meta-analyses due to the existence of significant heterogeneities among studies. We further validated the diagnostic accuracy of the parallel and sequential combinations of ZNF582/HPV DNA test. The results for the stratified analyses were listed in Table 2. The paralleled and sequential combinations of ZNF582/HPV tests achieved AUC values of 0.793 and 0.876, under which, the pooled sensitivity were 0.97 (95% CI: 0.94–0.99) and 0.75 (95% CI: 0.69–0.80), the pooled specificity were 0.48 (95% CI: 0.44–0.52) and 0.87 (95% CI: 0.84–0.89) respectively.

3.4. Influence assay and meta-regression

We performed influence analysis based on the platform of Stata 14.0 software. No outlier studies were identified in ZNF582 methylation test (Supplement Digital Content 3, http://links.lww.com/MD/C804). Furthermore, meta-regression and subgroup analyses were conducted by assessing the impacts of 4 pre-specified covariates (average age, publication year, sample size, study location) on pooled sensitivity and specificity. Our data revealed that these covariates introduce heterogeneity in specificity with a *P* value less than .05. However, these covariates showed a low likelihood of sources of inter-study heterogeneity in sensitivity (Table 3, Supplement Digital Content 4, http://links.lww.com/MD/C804).

3.5. Publication bias

The funnel plots for publication bias showed no asymmetry for the pooled ZNF582 methylation analysis. The slope of coefficient was associated with a *P* value of .36, implying that no publication bias existed in the studies (Supplement Digital Content 5A, http://links.lww.com/MD/C804). For single HPV DNA test (Supplement Digital Content 5B, http://links.lww.com/MD/C804) and paneled ZNF582 tests (data not shown) also showed a low likelihood of publication bias.

4. Discussion

Because CIN is a dynamic process, the approximate regression rates for CIN I, CIN II, and CIN III are 60%, 40%, and 33%,

Table 1

The main features of included studies for ZNF582 methylation in diagnosing cervical cancer.

Author	Year	Study location	Patient size			HPV type	Method	Cut-off value
			Total	CIN3+/cancer	Control size			
Liou et al[11]	2016	China	449	158	291	—	QMSP	based on ROC
Chang et al[12]	2015	China Taiwan	53	7	46	—	QMSP	based on ROC
Chang et al[13]	2015	China Taiwan	136	66	70	—	QMSP	based on ROC
Liou et al[16]	2015	China	242	74	168	—	QMSP	based on ROC
Lin et al[14]	2014	China Taiwan	230	15	215	—	QMSP	specificity 70%
Huang et al[15]	2012	China Taiwan	327	85	242	—	QMSP	M-index 0.62
Tian et al[17]	2017	China	312	155	157	High risk	QMSP	$\Delta Cp \leqq 11.0$

HPV = human papillomavirus; QMSP = quantitative methylation-specific polymerase chain reaction.

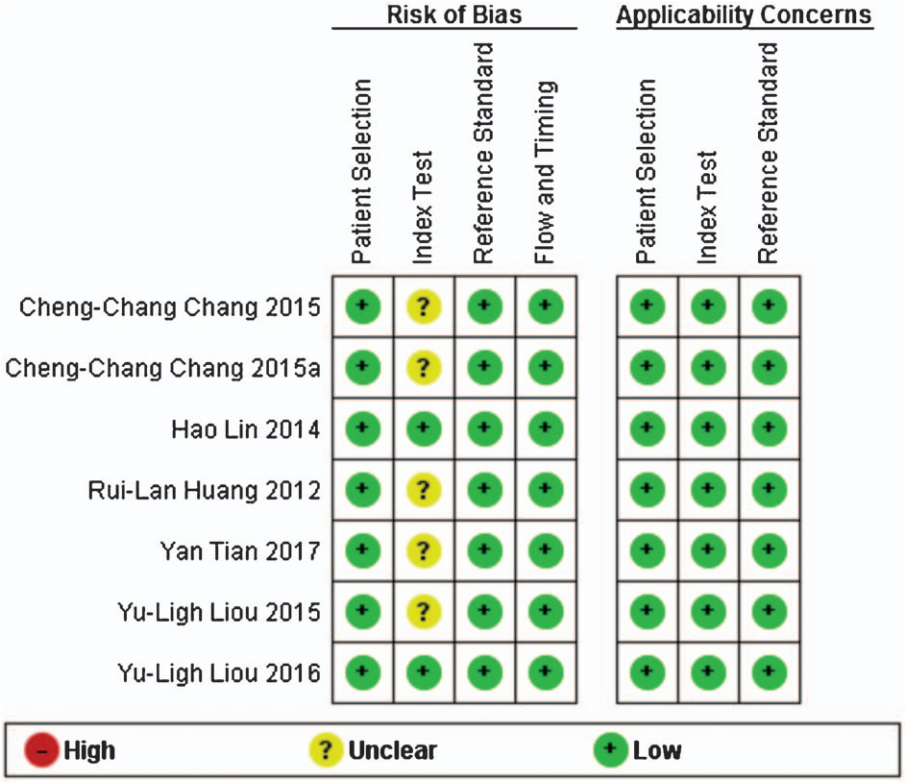

Figure 2. Summary of assessment of the included studies analyzed using the QUADAS2 tool: studies with low, mediate (unclear), and high risk of bias. QUADAS = quality assessment for studies of diagnostic accuracy.

respectively, and their corresponding rates of progression to invasive cervical cancer are 1%, 5%, and 12%, respectively.[24] Therefore, early diagnosis and treatment of CIN can reduce cancer mortality rate through effective screening programs drastically.

Papanicolaou cytology screening programs detect most CIN with a potential to transform into malignancy and for which treatment may prevent the cancer. Unfortunately, the cytology test is difficult to implement and retain at high quality, especially in underdeveloped countries.[25] The sensitivity of HPV DNA testing is satisfactory, whereas the high prevalence of transient HPV infections had limited the specificity of this approach.[26,27] Of greater importance are accurate molecular prognostic classifiers which could be done on the screening specimen and would reflexively indicate the future risk of progression. The ability to accurately tell whether the HPV infection will become a CIN3 or disappear would radically trans-form screening programs. The results would be reduced testing, lower costs, fewer overtreatments, and less anxiety.[28]

ZNF582, located at chromosome 19q13.43, encodes the Krüppel-type zinc finger protein 582 (HGNC: 26421), which contains 1 KRAB-A-B domain and 9 zinc-finger motifs.[29] However, the biological function of ZNF582 is not yet well characterized. Most KRAB-ZNF proteins contain the KRAB (AB) domain and bind KRAB-associated protein 1 (KAP1) to co-repress gene transcription.[30,31] Members of the KRAB-ZNF family are probably involved in a variety of biological processes related to the DNA damage response, proliferation, cell cycle control, and neoplastic transformation.[30] Recent studies revealed that methylation of its promotor CPG island is an

Table 2

Diagnostic indices of ZNF582 methylation for cervical cancer screening.

Analysis	Pooled sensitivity (95% CI)	Pooled specificity (95% CI)	Pooled PLR (95% CI)	Pooled NLR (95% CI)	Pooled DOR (95% CI)	AUC
ZNF582	0.71 (0.67–0.75)	0.81 (0.79–0.83)	4.19 (3.62–4.85)	0.34 (0.30–0.39)	12.72 (9.93–12.68)	0.85
hrHPV	0.96 (0.93–0.98)	0.41 (0.37–0.45)	1.69 (1.56–1.83)	0.1 (0.06–0.18)	18.44 (9.33–36.47)	0.6693
hrHPV/ZNF582	0.97 (0.94–0.99)	0.48 (0.44–0.52)	2.45 (2.14–2.80)	0.06 (0.04–0.12)	31.26 (15.03–65.03)	0.7928
hrHPVand ZNF582	0.75 (0.69–0.80)	0.87 (0.84–0.89)	5.91 (2.94–11.91)	0.34 (0.22–0.53)	19.23 (8.09–45.7)	0.8762

No outlier studies identified in ZNF582 methylation test. AUC = area under the curve; CI = confidence interval; DOR = diagnostic odds ratio; NLR = negative likelihood ratio; PLR = positive likelihood ratio.

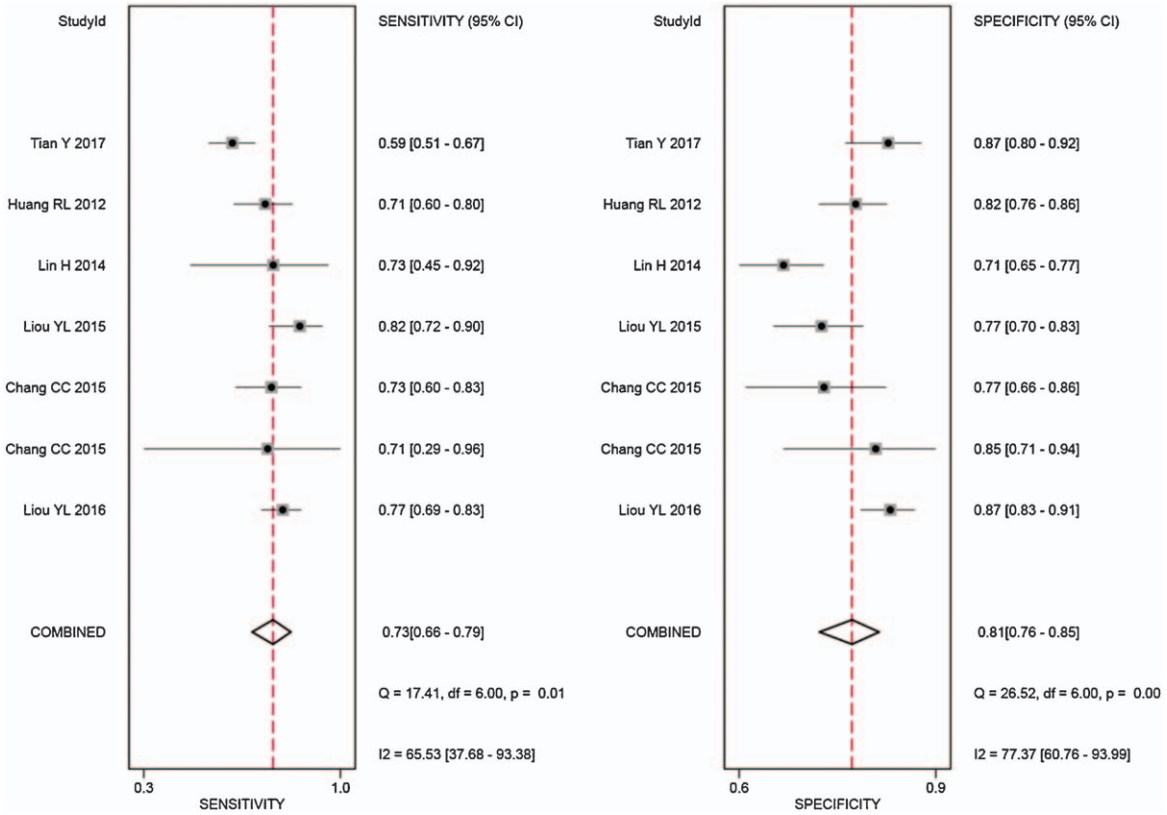

Figure 3. Forest plots of the pooled sensitivity and specificity for ZNF582 methylation. Only first author of each study was given. Sensitivity and specificity were given with CI. CIs = confidence intervals.

important regulating manner in epigenetics, which is closed related to the development of malignant tumor, such as oral cancer,[32,33] esophageal squamous cell carcinoma,[34] colorectal cancer,[35] and leukemia.[36]

In the development of cervical cancer, ZNF582 is silenced by hypermethylation, hence the methylation of ZNF582 has been proposed as a potential biomarker for the detection of cervical cancer.[23] As the potential diagnostic value of DNA methylation for cervical cancer screening has not yet been well elucidated thus far, we performed a comprehensive meta-analysis and evaluated the diagnostic performance of ZNF582 methylation for the detection CIN3+. And we also evaluated the pooled diagnostic accuracy of HPV DNA test from the published studies. As shown in our data, the pooled sensitivity and specificity of ZNF582 methylation was 0.71 and 0.81, respectively. Although the pooled sensitivity appeared not very high, the ROC AUC was 0.85, suggesting an overall high accuracy of this diagnostic test. DOR is one of the key indicators in assessing the accuracy of 1 diagnostic test, and that a DOR smaller than 1.0 often suggests a low discriminating value for a diagnostic test.[37,38] Importantly, the pooled DOR for ZNF582 methylation was 12.72, indicating a better discriminatory test performance of ZNF582 methylation for CIN3+ detection. Moreover, the pooled PLR of 4.19, also suggested that patients with CIN3+ had nearly 4 fold higher

Table 3

Meta-regression (inverse variance weights) for the potential source of heterogeneity.

Stratified analysis	No. studies	Sensitivity (95% CI)	*P*1	Specificity (95% CI)	*P*2
Average age, y					
≤45	4	0.74 (0.66–0.81)	.27	0.81 (0.76–0.86)	.02
>45	2	0.72 (0.56–0.87)		0.81 (0.71–0.91)	
Sample size					
≤200	3	0.77 (0.68–0.86)	.19	0.79 (0.72–0.87)	.00
>200	3	0.70 (0.62–0.79)		0.82 (0.77–0.87)	
Publication year					
2012-2014	2	0.74 (0.60–0.87)	.15	0.77 (0.69–0.84)	.00
2015-2016	4	0.73 (0.66–0.81)		0.83 (0.79–0.88)	
Study location					
China	2	0.73 (0.64–0.82)	.07	0.84 (0.79–0.89)	.00
Chinese Taipei	4	0.73 (0.63–0.83)		0.78 (0.72–0.84)	

CI = confidence interval.

chance of being ZNF582 methylation test positive than individuals without CIN3+. A pooled NLR of 0.34 means that that the probability of the individuals having CIN3+ is 34% when the ZNF582 test is negative. HPV DNA test harbored much high pooled sensitivity for the detection of CIN3+, but with much lower specificity with AUC of 0.669. Our data provide evidence that ZNF582 methylation confers better diagnostic accuracy in detecting CIN3+.

We further conducted the stratified analyses to compare the diagnostic accuracy of ZNF582 methylation and HPV DNA test. Combined sequential testing of HPV DNA and ZNF582 methylation achieved an improved diagnostic accuracy compared to HPV DNA test alone with AUC and DOR of 0.876 and 19.23.

In this study, heterogeneity from non-threshold effects existed in the pooled studies. It is speculated that sample size, age, and study location may contribute to the heterogeneity sources. We further conducted influence and meta-regression analyses and our results revealed that the study location and sample size were likely to be a source of heterogeneity.

Although we did our best to conduct a comprehensive analysis, some limitations still exist. Only 7 studies were included in this meta-analysis, and all the studies included in this meta-analysis were conducted in Chinese Taipei and China. The results of this analysis in Chinese populations should be applicable to other developing countries with high incidence of CIN.

In conclusion, our meta-analysis revealed that ZNF582 achieves a promising diagnostic performance for CIN3+. And combined sequential HPV DNA and ZNF582 methylation test achieves an improved diagnostic accuracy compared to HPV DNA test alone. Therefore, we suggest that ZNF582 methylation assay can be used as an auxiliary biomarker for cervical cancer screening. Further high-quality studies from other geographies are still warranted to confirm our analyses.

Author contributions

Data curation: Na Li, Ya He.
Formal analysis: Na Li, Peng Mi.
Supervision: Yuanjing Hu.
Writing – original draft: Na Li.
Writing – review & editing: Yuanjing Hu.

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