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ORIGINAL RESEARCH

The clinicopathological significance of CDH1 in gastric cancer: a meta-analysis and systematic review

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Background: CDH1 is a protein encoded by the *CDH1* gene in humans. Loss of CDH1 function contributes to cancer progression by increasing proliferation, invasion, and/or metastasis. However, the association and clinicopathological significance between *CDH1* hypermethylation and gastric cancer (GC) remains unclear. In this study, we systematically reviewed the studies of *CDH1* hypermethylation and GC, and evaluated the association between *CDH1* hypermethylation and GC using meta-analysis methods.

Methods: A comprehensive search of the PubMed and Embase databases was performed for publications up to July 2014. Methodological quality of the studies was also evaluated. The data were extracted and assessed by two reviewers independently. Analyses of pooled data were performed. Odds ratios (ORs) were calculated and summarized.

Results: A final analysis of 1,079 GC patients from 14 eligible studies was performed. *CDH1* hypermethylation level in the cancer group was significantly higher compared to the normal gastric mucosa (OR =8.55, 95% confidence interval [CI]: 2.39–33.51, Z=5.47, P<0.00001). *CDH1* hypermethylation was not significantly higher in GC than in adjacent gastric mucosa (OR =3.68, 95% CI: 0.96–14.18, Z=1.90, P=0.06). However, *CDH1* hypermethylation was higher in adjacent gastric mucosa compared to that in normal gastric mucosa (OR =2.55, 95% CI: 1.22–5.32, Z=2.49, P<0.01). In addition, *CDH1* hypermethylation was correlated with *Helicobacter pylori* (HP) status in GC. The pooled OR from six studies including 280 HP-positive GCs and 193 HP-negative GCs is 1.72 (95% CI: 1.13–2.61, Z=2.55, P=0.01).

Conclusion: The results of this meta-analysis reveal that *CDH1* hypermethylation levels in cancer and adjacent gastric mucosa are significantly higher compared to normal gastric mucosa. Thus, *CDH1* hypermethylation is significantly correlated with GC risk. *CDH1* hypermethylation is correlated with HP status, indicating that it plays a more important role in the pathogenesis of HP-positive GC and might be an interesting potential drug target for GC patients.

Keywords: methylation, tumor suppressor gene, odds ratio

Background

Stomach cancer, also known as gastric cancer (GC), is the second most common cause of cancer-related death according to the World Health Organization, and 800,000 cancer-related deaths are caused by GC each year globally.[1] Although diagnostic methods, surgical techniques, targeted therapy, and perioperative care have undergone considerable advancements, GC remains difficult to cure and prognosis remains poor with a median overall survival of 12 months for advanced disease in Western countries.[2,3] Thus, in order to improve the clinical outcome of GC patients, investigation on the mechanism of incidence and progression of GC, as well as identification

of new biomarkers and drug targets, are still needed and will help to select patients with high chances of GC recurrence and provide better prognosis and individualized treatments. Aberrant methylation of CpG dinucleotides is a commonly observed epigenetic modification and plays an important role in the initiation and progression in human cancer.[4–6] Thus, the analysis of specific gene promoter methylation as a diagnostic and/or prognostic marker has been widely used for many different cancers including GC.[7,8]

Cadherin-1 (CDH1), also known as epithelial cadherin (E-cadherin), CAM 120/80, or uvomorulin, a member of the transmembrane glycoprotein family, is encoded by the *CDH1* gene (16q22.1).[9,10] CDH1, a calcium-dependent cell–cell adhesion glycoprotein which contains three domains, ie, five extracellular cadherin repeats, a transmembrane region, and a highly conserved cytoplasmic tail, plays an essential role in maintaining cell adhesion and adherent junction in normal tissues.[11] CDH1 expression is frequently inactivated or absent in a variety of epithelial tumors, and loss of normal intercellular junctions results in promoted cancer invasion and metastasis and is correlated with several types of cancers including GC.[12–16] Although previous studies indicated that inactivation of *CDH1* is mainly induced by hypermethylation of the *CDH1* gene, the reported rates of *CDH1* hypermethylation in GC were remarkably diverse. In addition, the correlation and incidence between *CDH1* promoter hypermethylation and GC remains unclear. In this study, we systematically investigated studies of *CDH1* promoter hypermethylation and GC, and validated the correlation between *CDH1* promoter hypermethylation and GC using meta-analysis methods. We will summarize these findings and discuss the tumor suppressor function, as well as the clinicopathological significance of *CDH1* in GC.

Methods

Publication selection

A systematic literature search was performed using Pubmed, Embase, and Web of Science for publications up to July 15, 2014 without any language restrictions. The following keywords and terms were used: [methylation or DNA methylation or hypermethylation or de-methylation] and [CDH1 or Cadherin-1 or CAM 120/80 or epithelial cadherin (E-cadherin) or uvomorulin] and [gastric cancer or gastric carcinoma or gastric tumor]. Also, references from these publications were manually searched to acquire additional studies. Titles, abstracts, and keywords of the articles were initially evaluated for appropriate purpose. Then, details and additional information were identified and collected from full texts of these articles.

Inclusion and exclusion criteria

A study included for the meta-analysis needed to meet the following criteria: 1) studies which evaluated the correlation between *CDH1* methylation and GC; 2) the subjects in every study included clinical cohort and case control; 3) when the same groups of patients were reported in multiple papers, only the most recent and complete paper was selected to avoid overlap; 4) numbers of patients and controls needed to be larger than three; 5) only the tissue data were selected and the blood data was excluded from the study. If a study did not meet the inclusion criteria, it was excluded.

Data extraction and quality assessment

Two researchers independently collected the information and extracted the data regarding the authors, year, source of publication, inclusion criteria, *CDH1* methylation frequencies, sexual status, smoking history, pathological types, clinical staging, differentiation degree, lymph node metastasis, epidermal growth factor receptor (EGFR) status, and prognostic conditions in patients and control groups. Any discrepancy was adjusted by discussion until they reached an agreement. The data are summarized in Table 1 based on the criteria mentioned above. Methodological evaluation was assessed by two independent researchers according to REMARK guidelines and the European Lung Cancer Working Party quality scale.[17,18]

Data analysis

Meta-analysis was performed using Reviewer Manager 5 (Cochrane Collaboration, Oxford, UK). The pooled odds ratios (ORs) and confidence intervals (CIs) were calculated to assess the correlation between *CDH1* methylation and GC. Cochran's Q-test and I^2 were adopted to assess heterogeneity among studies.[19] If the Q-test showed $P<0.05$ or I^2 test was $>50\%$, it indicated significant heterogeneity and a fixed effects model was used to calculate the parameters. Otherwise, a random effects model was used to pool data and attempt to identify potential sources of heterogeneity based on subgroup analyses.[20,21] "Events" means number of hypermethylation cases. An overall effect is calculated as a weighted average of the individual summary statistics. Greater weights are given to the results from studies that provide more information. The weights are often the inverse of the variance (the square of the standard error) of the methylation rates, which relates closely to sample size. The typical graph for displaying the results of a meta-analysis is called a "forest plot".

Table 1 Basic characteristics of the included studies

Study	Country	Patients	Methods	Primary aim	Methylation site	CDH1 expression
Oh et al[36]	South Korea	102	MSP	To identify cancer-risk epigenotypes in GC patients	Promoter, CpG islands	–
Lee et al[37]	South Korea	72	MSP/IHC	To analyze the epigenetic alterations of *CDH1* gene in GC patients	Promoter, CpG islands	+
Li et al[38]	People's Republic of China	19	MSP/IHC	To evaluate the role of *CDH1* gene in the occurrence of sporadic or hereditary GC	Promoter, CpG islands	+
Yu et al[39]	People's Republic of China	92	MSP	To investigate the clinical value of *CDH1* methylation in GC patients	Promoter, CpG islands	–
Ben Ayed-Guerfali et al[40]	Tunisia	83	MSP	To determine the methylation status of 5 tumor suppressors in GC patients	Promoter, CpG islands	–
Borges et al[41]	Brazil	33	MSP	To determine genetic and epigenetic alterations of CDH1 in GC patients	Promoter, CpG islands	–
Tahara et al[42]	Japan	139	MSP	To investigate the association between cyclin D1 gene G870A polymorphism and the methylation status of 4 genes in GC patients	Promoter, CpG islands	–
Ferrasi et al[43]	Brazil	89	MSP	To determine the methylation status of 5 tumor suppressors in GC patients	Promoter, CpG islands	–
Kim et al[44]	South Korea	148	MSP	To determine the methylation status of 11 tumor suppressors in GC patients	Promoter, CpG islands	–
Zhang et al[45]	People's Republic of China	47	MSP	To determine the methylation status of 6 tumor suppressors in GC patients	Promoter, CpG islands	–
Leal et al[46]	Brazil	65	MSP	To determine DNA methylation changes of 4 genes in GC patients	Promoter, CpG islands	–
Oue et al[47]	Japan	75	MSP, RT-PCR	Detection of methylation status of 12 gene promoters and mRNA expression in GC patients	Promoter, CpG islands	+
Liu et al[48]	People's Republic of China	45	MSP/IHC	To analyze DNA polymorphism and methylation status of *CDH1* in GC patients	Promoter, CpG islands	+
Graziano et al[49]	Italy	70	MSP/IHC	To evaluate *CDH1* methylation status and correlation with CDH1 protein in GC patients	Promoter, CpG islands	+

Abbreviations: GC, gastric cancer; IHC, immunohistochemistry; MSP, methylation-specific polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction.

Publication bias was detected by the Begg's test and funnel plots.[22] The analysis of meta-regression and publication bias were performed using STATA version 10.0.

Results

The selection process of articles used in this report is shown in Figure 1. Ninety-three articles were searched by electronic database and additional information was sorted manually. Seventy-nine articles were excluded due to duplicated articles, irrelevant title and abstract, laboratory studies, non-original articles (review), or studies irrelevant to the current analysis. Finally, 14 reliable studies published from 2004 to 2014 were screened out based on the inclusion and exclusion criteria in the pooled analysis. A total of 1,079 GC patients from the People's Republic of China, South Korea, Japan, Tunisia, Brazil, and Italy were included. Their basic characteristics are summarized in Table 1.

By analyzing 587 cancer tissues and 389 normal mucosa tissues, the frequency of *CDH1* hypermethylation ranged from 28.6% to 82.2% (average 61%) in cancer tissues and from 0.00% to 54.5% (average 16%) in normal mucosa, respectively. This result indicates that the occurrence of *CDH1* hypermethylation in cancer tissues is higher than in normal mucosa. Under the random model, the meta-analysis result shows that nine studies were pooled OR as shown in Figure 2 (OR =8.55, 95% CI: 2.39–33.51, test for overall effect, Z=5.47, $P<0.00001$). These results indicate that *CDH1* hypermethylation is the key molecular event in cancer tissue rather than normal mucosa, and the results show the heterogeneity across the included studies (I^2 is 64% which is larger than 50%).

Then, we determined whether or not the *CDH1* hypermethylation rate in GC was significantly higher than that in adjacent gastric mucosa. The pooled OR from six studies including 467 GC tissues and 298 adjacent gastric mucosa tissues is shown in Figure 3 (OR =3.68, 95% CI: 0.96–14.18, Z=1.90, P=0.06), which indicates that *CDH1* hypermethylation is not significantly higher in GC than in adjacent gastric mucosa.

We determined whether or not *CDH1* hypermethylation was higher in adjacent gastric mucosa compared to that in normal gastric mucosa. There was no evidence of heterogeneity across the studies (P for heterogeneity =0.41; I^2=0%). The pooled OR from four studies including 205 adjacent gastric mucosa tissues

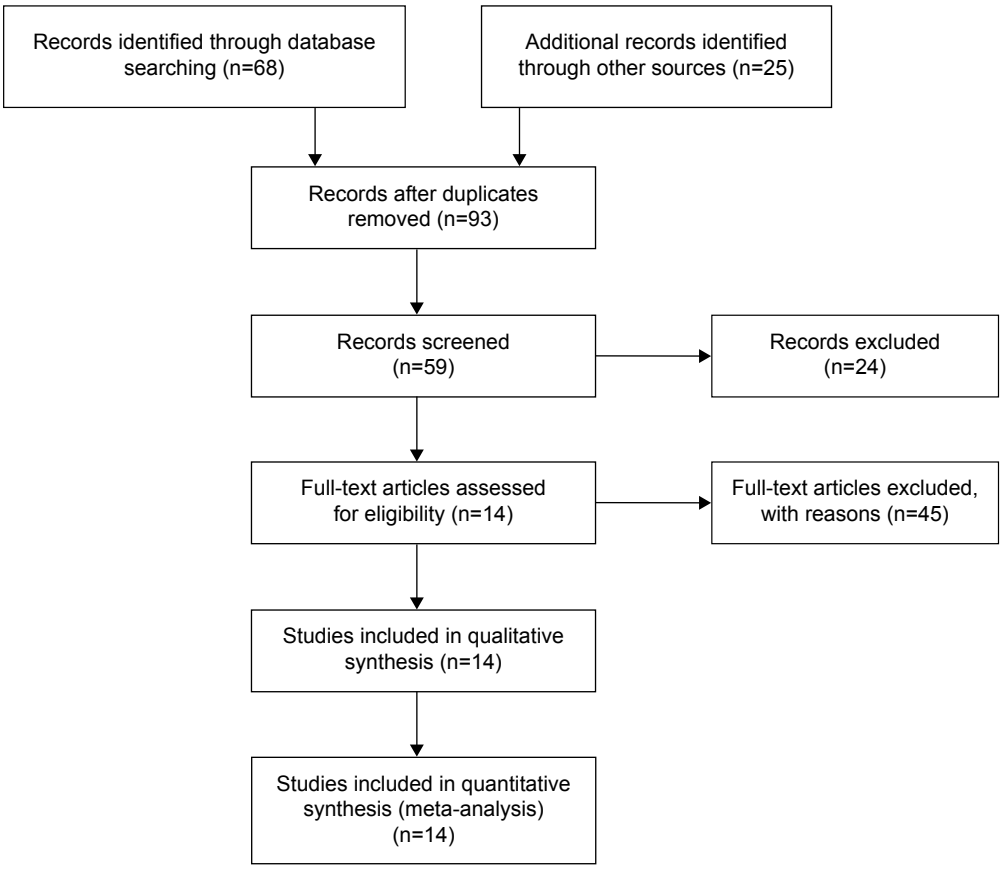

Figure 1 Flow diagram of the literature search strategy and assessment of studies identified for meta-analysis.

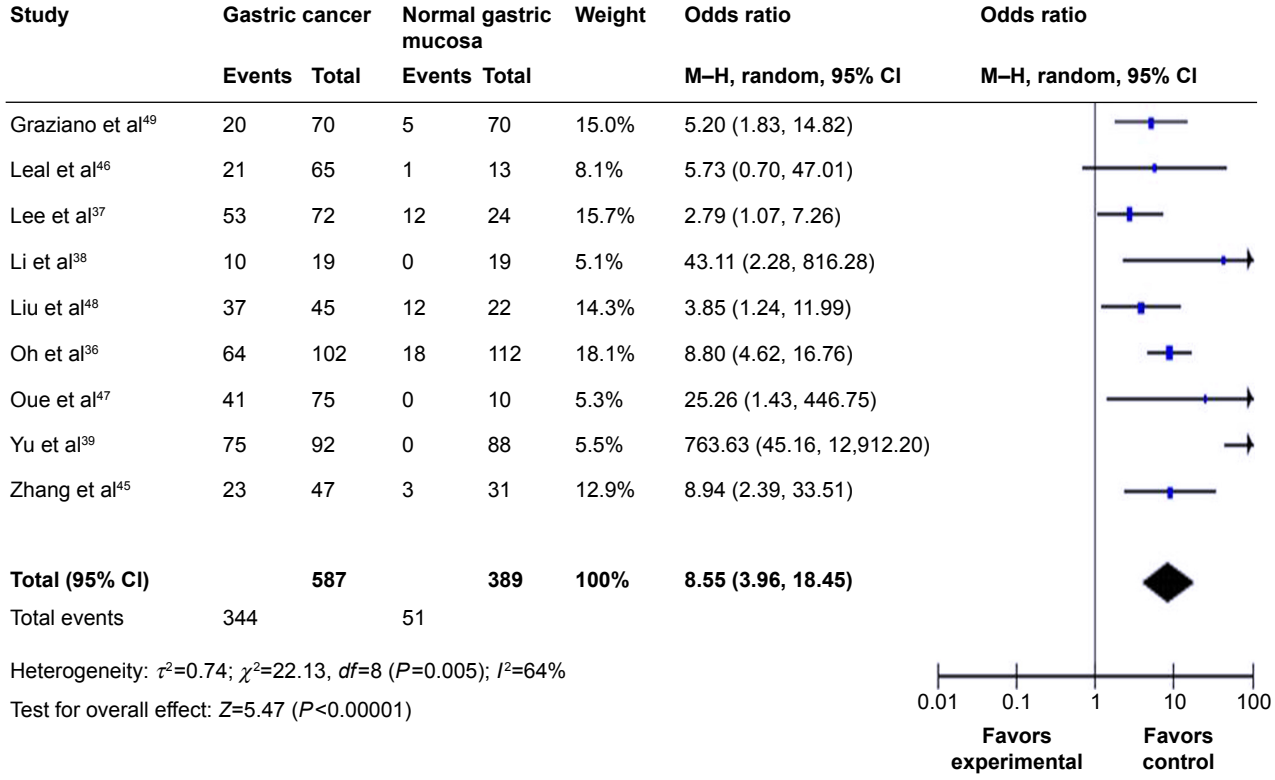

Figure 2 The pooled OR from nine studies including 587 gastric cancer tissues and 389 normal mucosa tissues.
Notes: OR =8.55, 95% CI: 2.39–33.51, test for overall effect, *Z*=5.47, *P*<0.00001.
Abbreviations: CI, confidence interval; *df*, degrees of freedom; OR, odds ratio; M–H, Mantel–Haenszel.

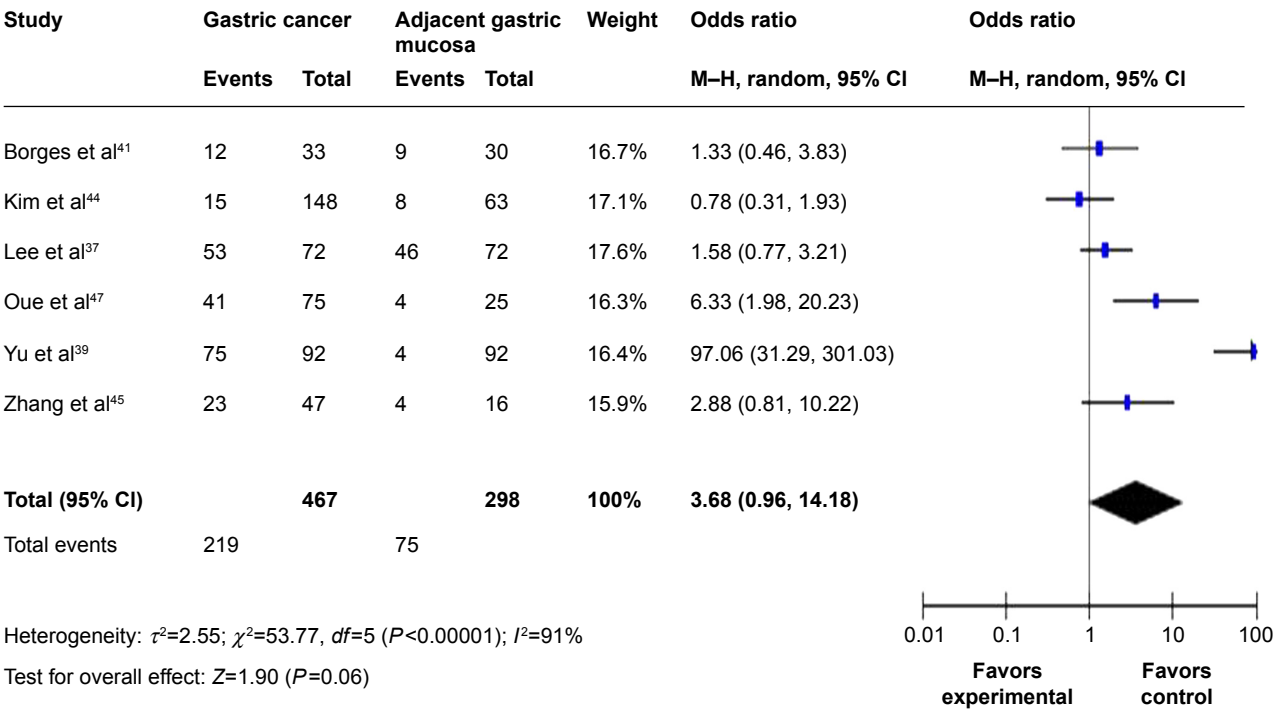

Figure 3 The pooled OR from six studies including 467 gastric cancer tissues and 298 adjacent gastric mucosa tissues.
Notes: OR =3.68, 95% CI: 0.96–14.18, *Z*=1.90, *P*=0.06.
Abbreviations: CI, confidence interval; *df*, degrees of freedom; OR, odds ratio; M–H, Mantel–Haenszel.

and 153 normal gastric mucosa tissues is shown in Figure 4 (OR =2.55, 95% CI: 1.22–5.32, Z=2.49, P<0.01), which indicates that *CDH1* hypermethylation plays a more important role in the pathogenesis of adjacent gastric mucosa.

Since it was described that DNA hypermethylation was associated with *Helicobacter pylori* (HP) infection but the mechanisms are not yet identified,[23,24] we determined whether or not *CDH1* hypermethylation was correlated with HP status in GC. The pooled OR from six studies including 280 HP-positive GCs and 193 HP-negative GCs is shown in Figure 5 (OR =1.72, 95% CI: 1.13–2.61, Z=2.55, P=0.01), which indicates that *CDH1* hypermethylation plays a more important role in the pathogenesis of HP-positive GC.

A sensitivity analysis, in which one study was removed at a time, was conducted to assess the result stability. The pooled ORs were not significantly changed, indicating the stability of our analyses. The funnel plots were largely symmetric (Figure 6), suggesting there were no publication biases in the meta-analysis.

Discussion

DNA methylation is an important epigenetic mechanism for gene expression regulation. The imbalance of gene methylation can induce a variety of human diseases. The hypermethylation of tumor suppressor genes and hypomethylation of oncogenes are two essential components of the molecular mechanism in the gene epigenomic regulation for cancer initiation and progression. *CDH1* is genetically or epigenetically altered in many different kinds of primary and advanced carcinomas. Inactivation of *CDH1* by promoter hypermethylation plays an important role in tumorigenesis in several types of tumors including GC.[25–27] To date, there have been some studies describing the methylation status of *CDH1* in GC; however, the roles of methylation of *CDH1* in GC and clinical significance have not been thoroughly investigated. In this meta-analysis, we mainly focused on the correlation between *CDH1* hypermethylation and GC. We analyzed the data from 14 previous scientific articles. The results show that the *CDH1* hypermethylation level of the cancer group was significantly higher than in normal gastric mucosa. The total OR is 8.55 (95% CI: 2.39–33.51, test for overall effect, Z=5.47, P<0.00001). *CDH1* hypermethylation plays a key role in the induction of GC due to silencing the tumor suppressor gene *CDH1*. Analysis of the pooled data also shows that *CDH1* hypermethylation was not significantly higher in GC than in adjacent gastric mucosa (OR =3.68, 95% CI: 0.96–14.18, Z=1.90, P=0.06). However, *CDH1* hypermethylation was higher in adjacent gastric mucosa compared to that in normal gastric mucosa (OR =2.55, 95% CI: 1.22–5.32, Z=2.49, P<0.01). In addition, *CDH1* hypermethylation was correlated with HP status in GC. The pooled OR from six studies including 280 HP-positive GC and 193 HP-negative GC is 1.72 (95% CI: 1.13–2.61, Z=2.55, P=0.01). The results from the current study demonstrate that the hypermethylation rate of *CDH1* gene promoter in GC is strongly associated with GC incidence. Since changes

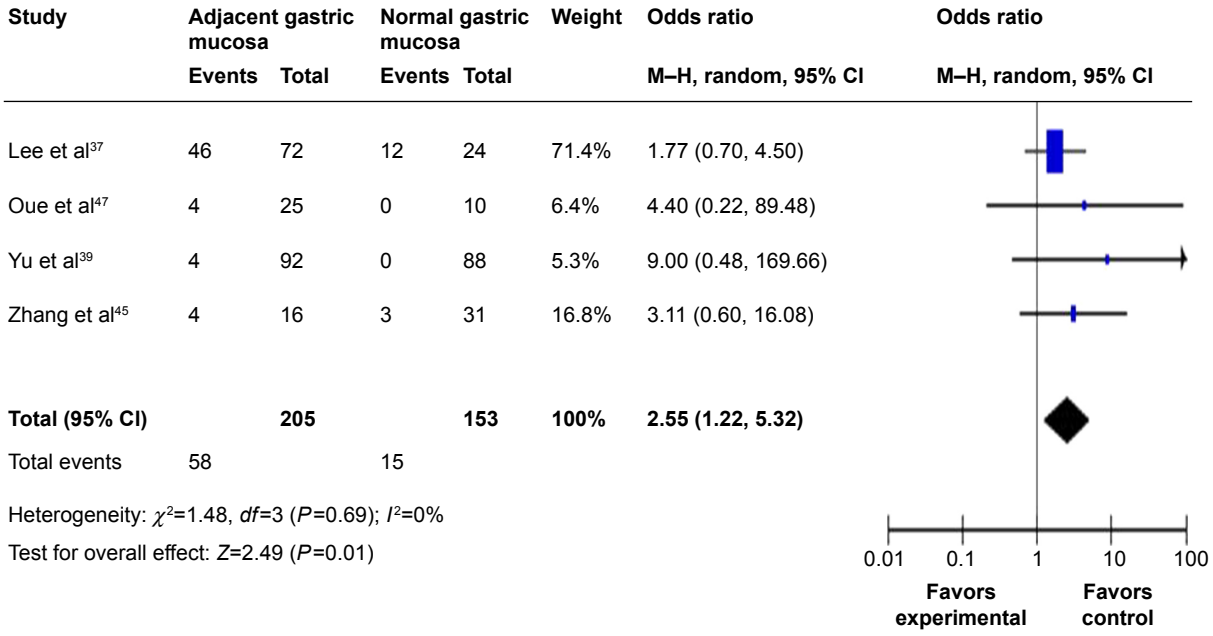

Figure 4 The pooled OR from four studies including 205 adjacent gastric mucosa and 153 normal gastric mucosa tissues.
Notes: OR =2.55, 95% CI: 1.22–5.32, Z=2.49, P<0.01.
Abbreviations: CI, confidence interval; *df*, degrees of freedom; OR, odds ratio; M–H, Mantel–Haenszel.

Study	HP-positive Events	Total	HP-negative Events	Total	Weight	Odds ratio M–H, fixed, 95% CI
Ben Ayed-Guerfali et al[40]	16	26	23	47	18.7%	1.67 (0.63, 4.43)
Ferrasi et al[43]	31	55	15	34	24.0%	1.64 (0.69, 3.87)
Liu et al[48]	26	38	19	29	20.2%	1.14 (0.41, 3.18)
Tahara et al[42]	82	111	15	28	18.5%	2.45 (1.04, 5.76)
Yu et al[39]	38	42	37	50	9.5%	3.34 (1.00, 11.18)
Zhang et al[45]	3	8	4	5	9.1%	0.15 (0.61, 2.05)
Total (95% CI)		**280**		**193**	**100%**	**1.72 (1.13, 2.61)**
Total events	196		113			

Heterogeneity: χ^2=5.78, *df*=5 (*P*=0.33); I^2=14%

Test for overall effect: *Z*=2.55 (*P*=0.01)

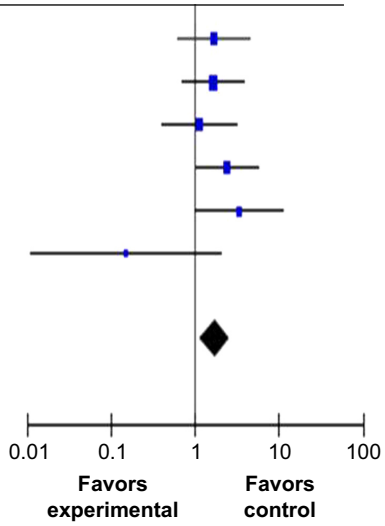

Figure 5 The pooled OR from six studies including 280 HP-positive and 193 HP-negative gastric cancers.
Notes: OR =1.72, 95% CI: 1.13–2.61, *Z*=2.55, *P*=0.01.
Abbreviations: CI, confidence interval; *df*, degrees of freedom; HP, *Helicobacter pylori*; OR, odds ratio; M–H, Mantel–Haenszel.

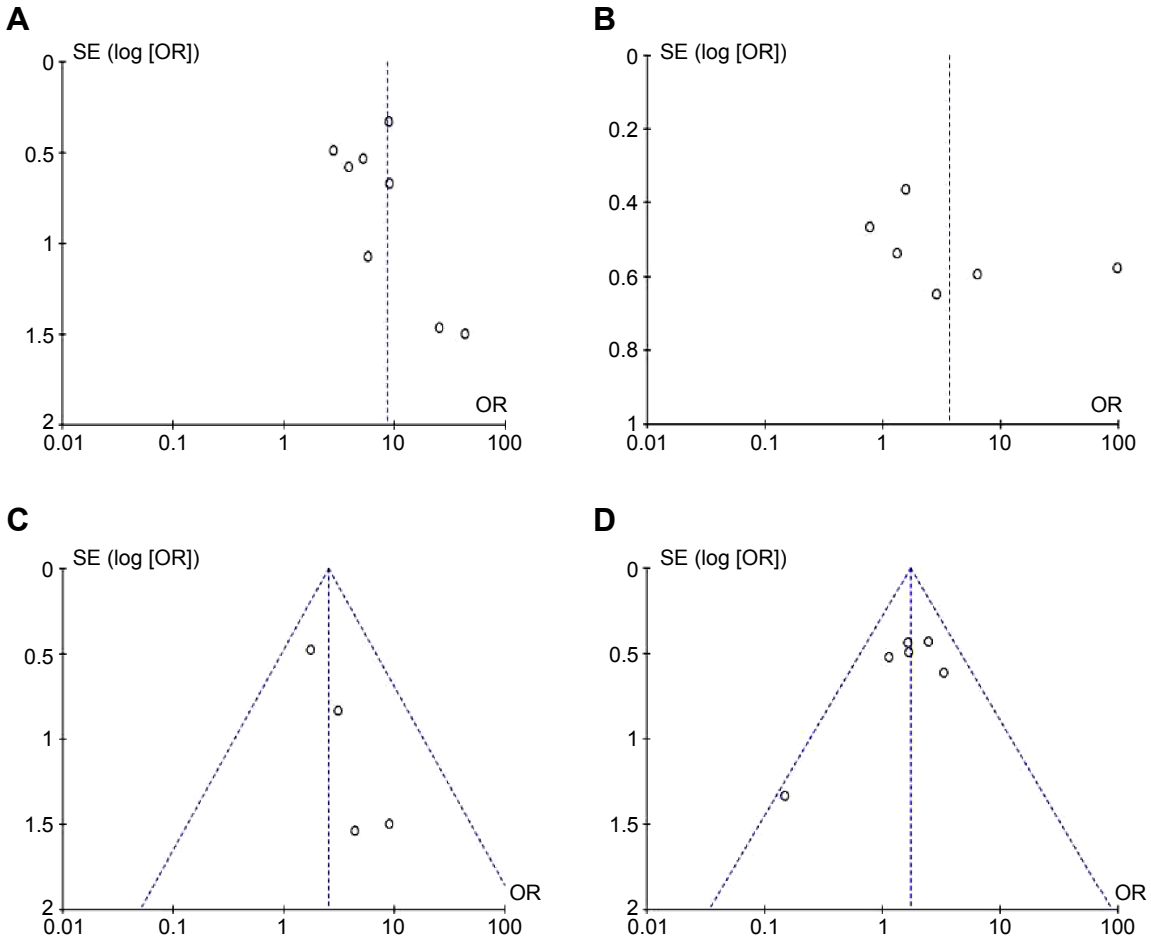

Figure 6 Funnel plot for assessment of publication bias in the meta-analysis.
Notes: (**A**) Gastric cancer tissues versus normal gastric mucosa. (**B**) Gastric cancer tissues versus adjacent gastric mucosa. (**C**) Adjacent gastric mucosa versus normal gastric mucosa. (**D**) *Helicobacter pylori*-positive versus -negative.
Abbreviations: OR, odds ratio; SE, standard error.

in *CDH1* promoter hypermethylation are reversible, drug treatment through demethylation may be useful to delay carcinogenesis and progression. In fact, treatment of CDH1-negative tumor cells with the demethylating agent 5-aza-2′-deoxycytidine induced re-expression of *CDH1* mRNA and/or protein in several types of tumor cells including colorectal cancer,[28] esophageal cancer,[29] lung cancer,[30] as well as prostate cancer.[31] A combination of histone deacetylase inhibitors and DNA methyltransferase inhibitors suppresses the growth of endometrial cancer, which is likely mediated by upregulation of CDH1 and downregulation of Bcl-2.[32] Transfection of CDH1 cDNA into R-HepG2 cells, in which *CDH1* promoter was hypermethylated in drug resistance of a doxorubicin-induced multidrug-resistant hepatocellular carcinoma cell line, led to increased amount of doxorubicin uptake, decreased cell viability, decreased P-glycoprotein expression, and increased apoptotic population of cells exposed to doxorubicin.[33] In addition, 1α,25(OH)(2)D(3) promoted differentiation of breast cancer MDA-MB-231 cells by inducing de novo E-cadherin expression, an effect that was time- and dose-dependent.[34] Therefore, this kind of approach targeting CDH1 may bring new directions and hope for cancer treatment through gene-targeted therapy.

CDH1, as a tumor suppressor gene, functionally keeps cell–cell adhesion and controls epithelial cell arrangement in normal order and layer. An in vitro study demonstrated that loss of the expression or function of *CDH1* can initiate the activation of transcription factors which are associated with epithelial–mesenchymal transition, finally leading to cancer cell metastasis.[35] To better understand the correlation between *CDH1* methylation and GC, comprehensive evaluation on the methylation makers in GC should be further addressed. Although a large number of studies have demonstrated the potential relationship between *CDH1* methylation and GC, a meta-analysis can summarize the studies and compare different subgroup characters.

Consistent results were shown in sensitivity analyses, and no evidence of publication bias was found. This study has several potential limitations. First, the possibility of information and selection biases and unidentified confounders could not be completely excluded because all of the included studies were observational. Second, the search strategy was restricted to articles published in English. Articles with potentially high-quality data that were published in other languages were not included because of anticipated difficulties in obtaining accurate medical translation. Most selected publications were from Asia, hence caution should be taken when our findings are interpreted among general populations. In addition, there are high heterogeneities in the data of Figures 2 and 3, I^2 test was >50%, thus we used a random effects model to pool data. Data heterogeneity may come from tissue sample preparation, DNA isolation condition, polymerase chain reaction condition, etc.

Conclusion

In summary, *CDH1* promoter hypermethylation is associated with GC risk based on the meta-analysis, which indicates that *CDH1* hypermethylation might be a biomarker of GC, with potential value as a drug candidate for the therapy of GC patients. In addition, further large-scale studies, especially multicenter and well-matched cohort research, will provide more insight into the role of *CDH1* in the carcinogenesis and clinical implementation of GC patients.

Disclosure

The authors report no conflicts of interest in this work.

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