

# Meta-analysis of possible role of cadherin gene methylation in evolution and prognosis of hepatocellular carcinoma with a PRISMA guideline

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## Abstract

**Background:** Cadherins (CDHs) have been reported to be associated with cancer. However, the clinical significance of *CDH* gene methylation in hepatocellular carcinoma (HCC) remains unclear.

**Methods:** Based on the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement criteria, available studies were identified from online electronic database. The overall odds ratio (OR) and the corresponding 95% confidence interval (95% CI) were calculated and analyzed.

**Results:** A total of 29 eligible studies with 2562 HCC samples and 1685 controls were included. E-cadherin (*CDH1*) hypermethylation was observed to be significantly higher in HCC than in benign, adjacent, or normal samples. Moreover, *CDH1* hypermethylation was not associated with gender, tumor grade, clinical stage, hepatitis B virus (HBV), or hepatitis C virus (HCV) infection in HCC patients. H-cadherin (*CDH13*), protocadherin-10 (*PCDH10*), P-cadherin (*CDH3*), and *CDH15* methylation may have an increased risk of HCC in fewer than 4 studies, and methylated *CDH8* and *CDH11* had a similar OR in HCC and adjacent samples. When HCC samples were compared with normal samples, the analysis of sample type revealed a significantly higher OR in normal blood samples than in normal tissues for hypermethylated *CDH1* (50.82 vs 4.44).

**Conclusion:** *CDH1* hypermethylation may play a key role in the carcinogenesis of HCC. However, *CDH1* hypermethylation was not correlated with clinicopathological features. Methylated *CDH13*, *PCDH10*, *CDH3*, and *CDH15*, but not methylated *CDH8* or *CDH11*, may lead to an increased risk of HCC. Hypermethylated *CDH1* may become a noninvasive blood biomarker. Further studies with more data are necessary.

**Abbreviations:** 95% CI = 95% confidence interval, CDH = cadherin, CDH1 = E-cadherin, CDH13 = H-cadherin, CDH3 = P-cadherin, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, MSP = methylation-specific polymerase chain reaction, OR = odds ratio, PCDH10 = protocadherin-10, TSG = tumor suppressor genes.

**Keywords:** cadherins, *CDH1*, clinical significance, HCC, methylation

## 1. Introduction

Worldwide, hepatocellular carcinoma (HCC) is the 5th most frequent malignant disease and the 2nd most common cause of

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cancer death, with China accounting for approximately half of HCC cases and deaths.<sup>[1]</sup> A low incidence of this disease has been recorded in Europe.<sup>[2]</sup> According to global cancer statistics, an estimated 782,500 new cases of HCC were diagnosed in 2012, with approximately 745,500 HCC-related deaths.<sup>[1]</sup> Several risk factors have been suggested to be associated with the majority of HCC cases, such as chronic liver diseases associated with hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, liver cirrhosis, aflatoxin exposure, alcohol consumption, obesity, type 2 diabetes, and tobacco smoking, etc.<sup>[1,3]</sup> The majority of HCC patients are diagnosed at a late stage, and symptomatic HCC has a 5-year survival rate of 3%.<sup>[1,4]</sup>

Epigenetic events involving DNA methylation, histone modifications, nucleosome positioning, and noncoding RNAs have been reported to play key roles in the carcinogenesis and progression of cancers.<sup>[5,6]</sup> DNA methylation, a major mechanism of epigenetic alterations, occurs more often in human tumor cells than gene mutations.<sup>[7]</sup> Epigenomic regulation displays 2 essential molecular mechanisms: hypermethylation of tumor suppressor genes (TSGs) and hypomethylation of oncogenes.<sup>[8,9]</sup> The classical cadherins (CDHs) are a superfamily of transmembrane glycoproteins involved in calcium-dependent cell-cell adhesion in embryonic development and epithelial tissues.<sup>[10]</sup> CDHs are also associated with signaling, mechanotransduction, cancer progression, and tissue morphogenesis.<sup>[11–14]</sup> Many

CDHs are related to cancer. For example, the expression of E-cadherin (*CDH1*), a TSG, is reduced through hypermethylation in several cancers, including in HCC.<sup>[15–17]</sup> In addition, aberrant methylation of the TSG H-cadherin (*CDH13*) associated with gene inactivation has been found in some primary tumors, including ovarian, renal cell, and breast cancers.<sup>[18]</sup> Reduction of protocadherin-10 (*PCDH10*) expression via methylation has also been reported in some cancers.<sup>[19,20]</sup> In contrast, increased expression of P-cadherin (*CDH3*) is clinically associated with several cancers, such as breast and colon tumors.<sup>[21,22]</sup>

In the present study, we first determined whether methylated *CDH* genes were significantly associated with the risk of HCC. In addition, we assessed the clinicopathological significance of *CDH1* hypermethylation in HCC patients.

## 2. Materials and methods

### 2.1. Search strategy

We systemically performed literature searches in electronic databases (PubMed, EMBASE, Web of Science, EBSCO, and Cochrane Library) for eligible studies published in English prior to October 10th, 2016. The following keywords and search terms were used: (liver OR hepatocellular OR hepatic) AND (cancer OR tumor OR neoplasm OR carcinoma) AND (*CDH*\* OR cadherin) AND (methylation OR epigene\*). Furthermore, the reference lists of the identified articles were manually searched to identify additional relevant studies.

### 2.2. Study criteria

The studies included in our analysis had to meet the following inclusion criteria: patients were limited to individuals with HCC based on histopathological examination, without restriction of sample type; studies reported that sufficient data were obtained to evaluate the association between *CDH* gene methylation and HCC in the cancer and control groups; and studies on *CDH1* methylation were performed to estimate the clinicopathological significance of *CDH1* methylation in HCC patients. The major exclusion criteria were as follows: studies using cell lines and animal studies; reviews, case reports, letters, and conference abstracts; or studies with insufficient data or duplicated data.

### 2.3. Ethical review from patients

The current study was a secondary analysis regarding human subject data published in the public domain.

### 2.4. Data extraction

For eligible studies, the following data were extracted: surname of the first author, year of publication, country, race, methylation detection methodology, sample type, number of HCC cases, number of HCC controls, methylation rate, number of *CDH* gene methylations, and clinicopathological parameters (including gender status, tumor grade, clinical stage, HBV status, and HCV status). Benign samples included liver disease associated with chronic hepatitis or cirrhosis, normal samples were from normal healthy subjects without chronic hepatitis, cirrhosis, or other disease. The present meta-analysis met the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).<sup>[23]</sup>

## 2.5. Data analysis

The pooled odds ratios (ORs) were calculated using STATA software (version 12.0, Stata Corporation, College Station, TX). The pooled ORs with the corresponding 95% confidence intervals (95% CIs) were calculated to assess the correlation between *CDH* gene methylation and HCC in the HCC and control groups. In addition, the correlation of the *CDH1* hypermethylation status with gender status, tumor grade, tumor stage, HBV status, and HCV status in HCC patients was determined to estimate the clinicopathological significance of *CDH1* hypermethylation in HCC patients. The heterogeneity among studies was examined based on the chi-square test and Q statistics.<sup>[24]</sup> The pooled OR was calculated and summarized under a random-effects model. If heterogeneity was significant ( $I^2 \geq 50\%$ ), meta-regression analyses were performed to assess the potential sources of heterogeneity. Moreover, a sensitivity analysis was conducted to evaluate the influence of an individual study on the results and the effect of omitting a single study on the stability of the results.<sup>[25,26]</sup> Possible publication bias was identified using Egger test.<sup>[27]</sup>

## 3. Results

### 3.1. General characteristics of the included studies

After being carefully retrieved from the above electronic databases according to our inclusion criteria (as shown in Fig. 1), 29 eligible studies<sup>[17,28–51]</sup> involving 2562 HCC specimens and 1685 controls were included in the final meta-analysis. Twenty-three of the studies analyzed the association between *CDH1* hypermethylation and HCC in cancer and control groups.<sup>[17,28,29,31,32,34,36–42,46–51]</sup> Among these 23 studies, 12 studies that included a total of 594 HCC samples and 581 benign samples analyzed the association between *CDH1* hypermethylation and HCC.<sup>[28,29,31,34,40,46,48–50,53–55]</sup> Twelve studies, involving 598 HCC tissue samples and 471 adjacent tissue samples, analyzed the association between *CDH1* hypermethylation and HCC.<sup>[17,29,36–39,41,42,47,49,51,52]</sup> Nine studies, involving 494 HCC samples and 166 normal samples, analyzed the association between *CDH1* hypermethylation and HCC.<sup>[28,29,32,38,40,47,48,50,51]</sup>

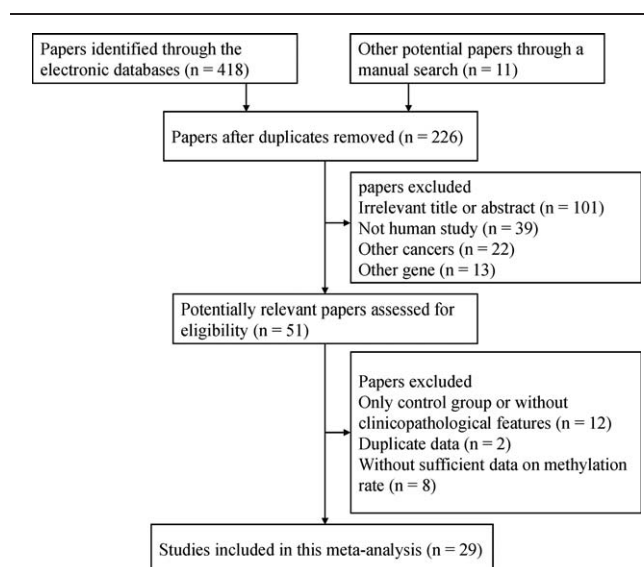
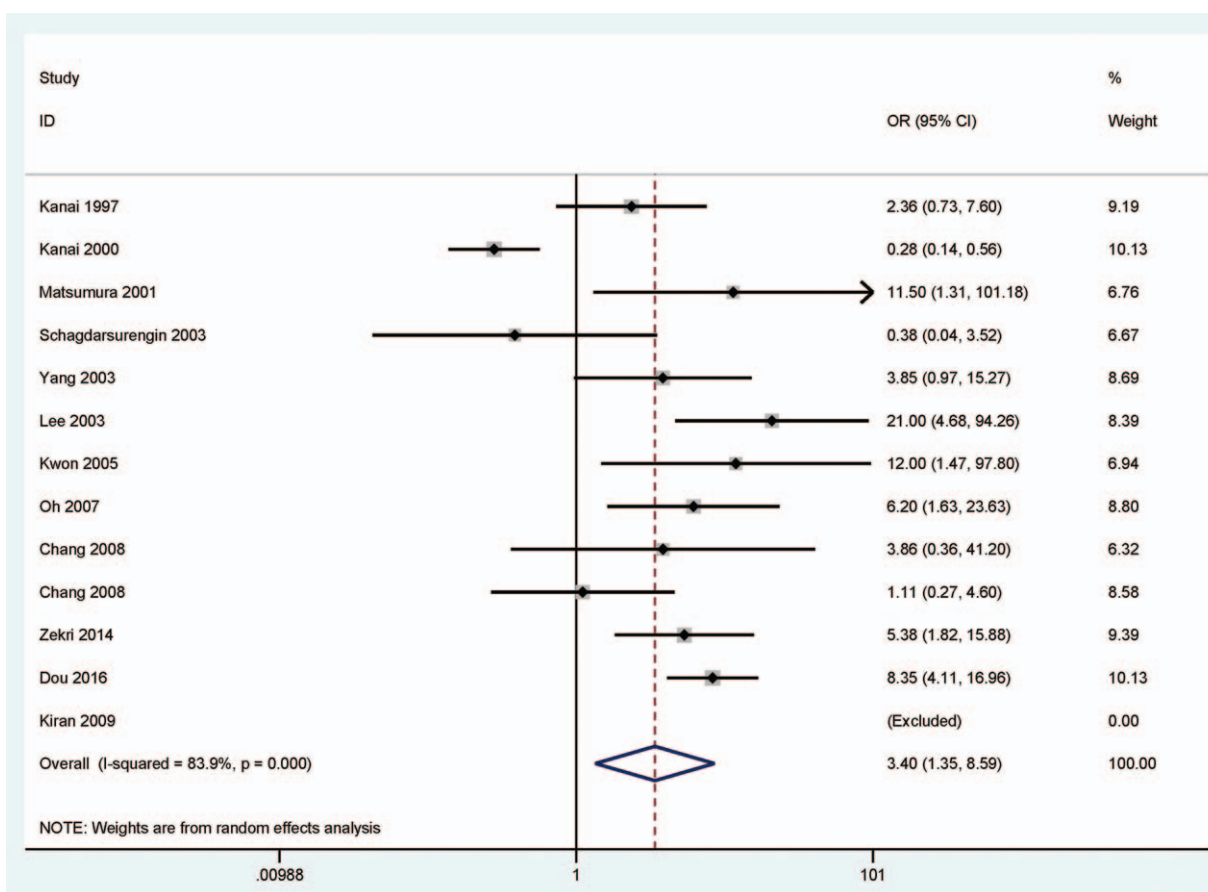


Figure 1. Flow chart of the literature search strategy.



**Figure 2.** Forest plot for the correlation of *CDH1* hypermethylation status from 12 publications showing the pooled OR under a random-effects model in 594 HCC versus 581 benign samples, OR=3.40, 95% CI= 1.35 to 8.59. *CDH1* = E-cadherin, CI=confidence interval, HCC=hepatocellular carcinoma, OR=odds ratio.

Sixteen studies, involving 1110 HCC patients, assessed the correlation of *CDH1* hypermethylation with clinicopathological parameters<sup>[17,28,32–34,36,38,41–44,46,49,50,52,53]</sup> in HCC. Three studies analyzed the association between *CDH13* methylation and HCC in cancer and control groups.<sup>[35,39,51]</sup> Two studies evaluated the relationship between *PCDH10* methylation and HCC in cancer and control groups.<sup>[30,45]</sup> One study evaluated the relationship of methylated *CDH3*, *CDH5*, *CDH8*, *CDH11*, and *CDH15* with HCC in cancer and control groups.<sup>[39]</sup> The basic characteristics of the included studies are summarized in supplemental Table S1, <http://links.lww.com/MD/B656>.

**3.2. Correlation between *CDH1* hypermethylation and HCC**

In a random-effects model (Figs. 2–4), the pooled OR of *CDH1* hypermethylation showed that HCC exhibited a significantly different OR than the benign samples, adjacent samples, and normal samples (benign samples: OR = 3.40, 95% CI= 1.35–8.59, *P* = .01; adjacent samples: OR = 2.42, 95% CI= 1.01–5.83, *P* = .049; and normal samples: OR = 7.17, 95% CI= 2.80–18.35, *P* < .001), which demonstrated that *CDH1* hypermethylation was significantly associated with an increased risk of HCC.

**3.3. Correlation between the methylation of other *CDH* genes and HCC**

As shown in Table 1, the overall OR from 3 studies involving 125 HCC samples and 125 adjacent tissue samples demonstrated that

the level of *CDH13* methylation was slightly higher in HCC than adjacent tissues (OR = 4.11, 95% CI = 1.04–16.23, *P* = .044).

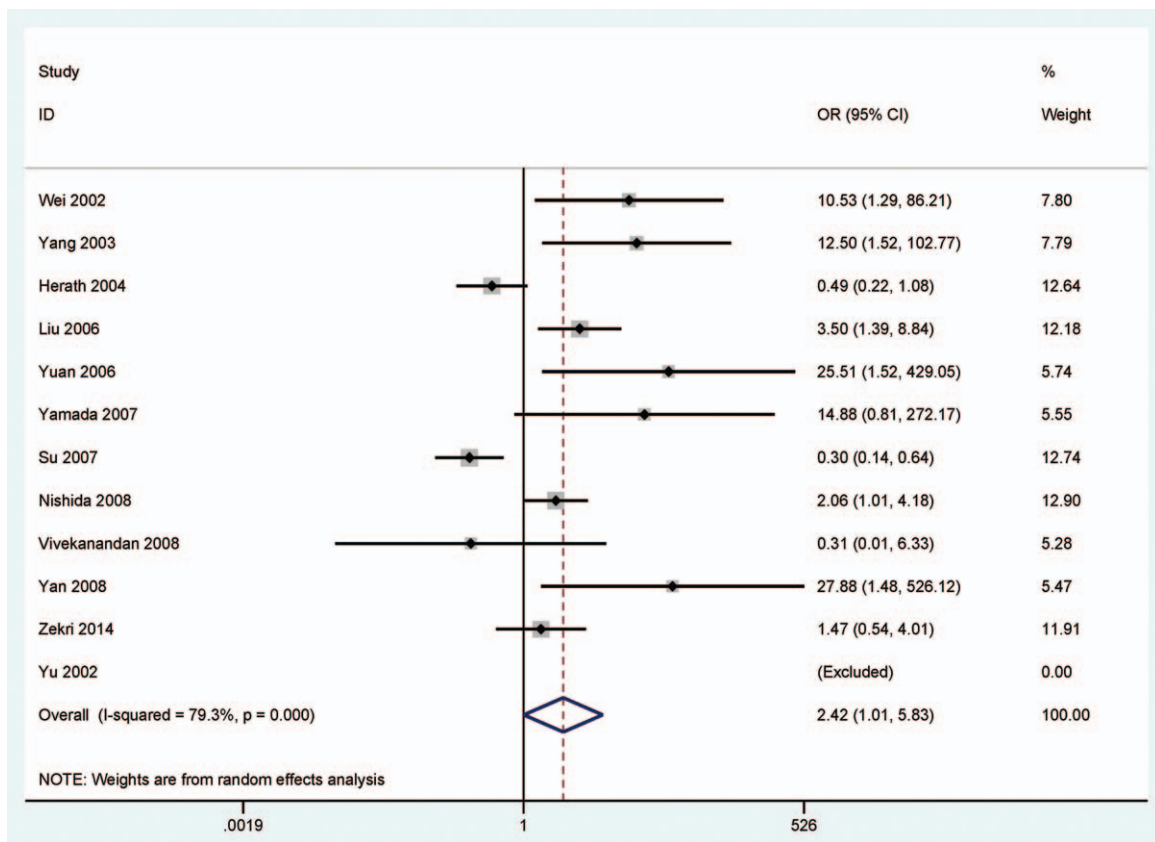
The pooled OR from 2 studies involving 62 HCC tissues and 56 adjacent and normal tissues showed that *PCDH10* methylation exhibited a significantly higher OR in HCC than in adjacent and normal tissue samples (OR = 5.00, 95% CI = 2.18–11.45, *P* < .001).

The pooled OR from 1 study involving 47 HCC tissues and 47 adjacent tissues showed that *CDH3* and *CDH15* methylation exhibited a higher OR in HCC than in adjacent tissue samples (*P* < .01), while methylated *CDH8* and *CDH11* showed a similar OR between HCC and adjacent tissues (*P* > .05). The *CDH5* gene exhibited no methylation in 47 HCC tissues and 47 adjacent tissues.

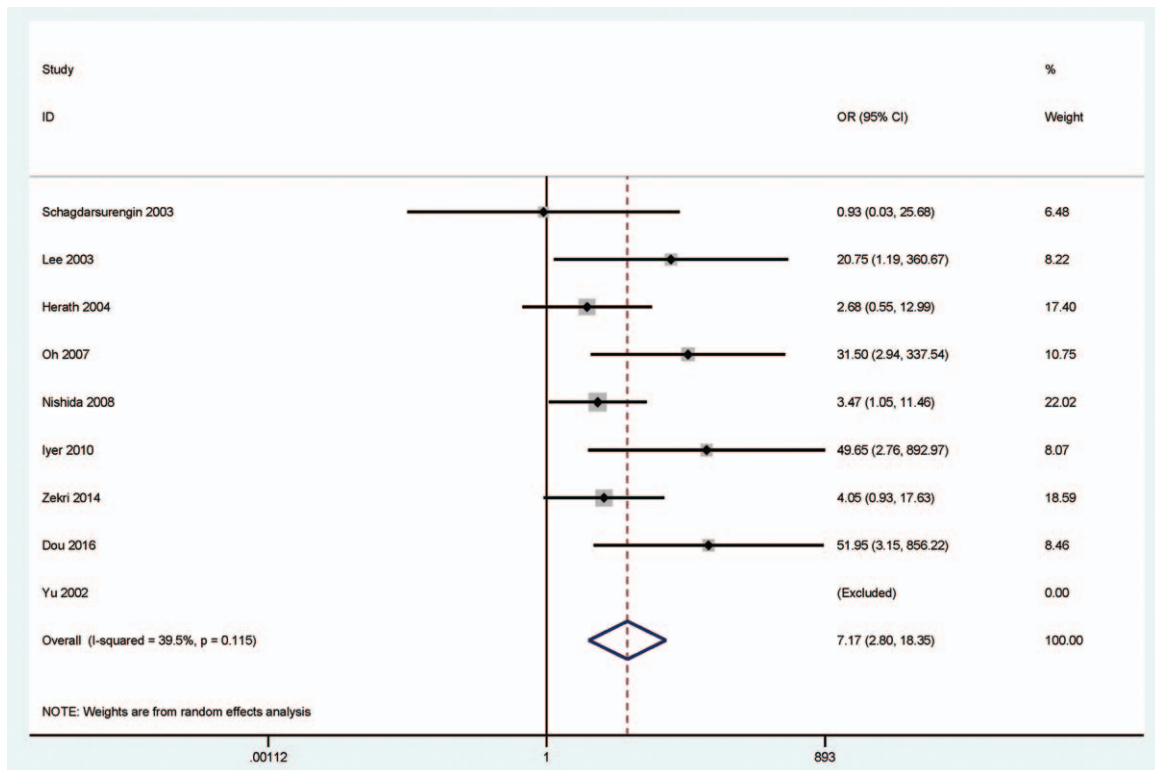
Our findings showed that methylated *CDH13*, *PCDH10*, *CDH3*, and *CDH15* may be associated with an increased risk of HCC. However, the results regarding the methylation of other *CDH* genes should be carefully considered, as only small sample sizes were included in the current study.

**3.4. Subgroup analyses of the *CDH1* hypermethylation in cancer and control groups**

Subgroup analyses based on ethnicity (Asians and Caucasians), sample types (tissue and blood), and detection methods (Southern blot and methylation-specific polymerase chain reaction [MSP]) were conducted to detect the various correlations with *CDH1* hypermethylation under a random-effects model (Table 1).



**Figure 3.** Forest plot for the association of *CDH1* hypermethylation status from 12 studies showing the pooled OR under a random-effects model in 598 HCC versus 471 adjacent samples, OR=2.42, 95% CI=1.01 to 5.83. CDH1 = E-cadherin, CI=confidence interval, HCC=hepatocellular carcinoma, OR=odds ratio.



**Figure 4.** Forest plot for the relationship of *CDH1* hypermethylation status from 9 studies showing the pooled OR under a random-effects model in 494 HCC versus 166 normal samples, OR=7.17, 95% CI=2.80 to 18.35. CDH1 = E-cadherin, CI=confidence interval, HCC=hepatocellular carcinoma, OR=odds ratio.



**Table 1****The summary of the ORs for CDH1 gene methylation in HCC versus controls.**

CDH1	Studies	Pooled OR (95% CI)	I <sup>2</sup>	P	Cases	Controls
HCC vs benign samples						
Method						
Southern blot	3	1.63 (0.21–12.96)	88.30%	.643	97	172
MSP	10	4.83 (2.50–9.35)	49.30%	<.001	497	409
Race						
Asians	9	3.90 (1.20–12.66)	87.40%	.023	474	501
Caucasians	4	2.70 (0.75–9.68)	55.00%	.127	120	80
Sample						
Tissue	11	3.44 (1.16–10.23)	83.40%	.026	385	392
Blood	2	3.35 (0.47–24.16)	83.90%	.23	209	189
HCC vs adjacent samples						
Race						
Asians	6	4.72 (0.67–33.36)	87.40%	.12	306	228
Caucasians	5	2.52 (1.06–5.99)	44.3	.037	231	182
HCC vs normal samples						
Race						
Asians	4	32.44 (7.03–149.64)	0.00%	<.001	297	81
Caucasians	4	4.45 (1.53–13.01)	24.80%	.006	136	65
Sample						
Tissue	7	4.44 (2.11–9.35)	0.00%	<.001	283	88
Blood	2	50.82 (6.80–379.93)	5.00%	<.001	211	78
CDH13						
HCC vs adjacent samples	3	4.11 (1.04–16.23)	61.20%	.044	125	125
PCDH10						
HCC vs adjacent and normal samples	2	5.00 (2.18–11.45)	0.00%	<.001	62	56
CDH3						
HCC vs adjacent samples	1	65.00 (3.78–1118.41)	NA	.004	47	47
CDH5						
HCC vs adjacent samples	1	NA	NA	NA	47	47
CDH8						
HCC vs adjacent samples	1	7.47 (0.38–148.77)	NA	.188	47	47
CDH11						
HCC vs adjacent samples	1	12.29 (0.66–228.97)	NA	.093	47	47
CDH15						
HCC vs adjacent samples	1	117.09 (6.82–2011.85)	NA	.001	47	47

CDH1 = E-cadherin, CDH3 = P-cadherin, CDH5 = VE-cadherin, CDH8 = Cadherin 8, type 2, CDH11 = OB-cadherin, CDH13 = H-cadherin, CDH15 = M-cadherin, CI = confidence interval, HCC = hepatocellular carcinoma, MSP = methylation-specific polymerase chain reaction, NA = not applicable, OR = odds ratio, PCDH10 = protocadherin-10.

When HCC samples were compared with benign samples, the results related to ethnicity showed that *CDH1* hypermethylation was only significantly associated with HCC in the Asian population ( $P=.023$ ), and not the Caucasian population ( $P=.127$ ). The results regarding sample types showed that *CDH1* hypermethylation was only significantly associated with HCC in the tissue subgroup ( $P=.026$ ), and not in the blood subgroup ( $P=.23$ ). Subgroup analysis based on the testing method demonstrated that *CDH1* hypermethylation exhibited a significant association in the MSP subgroup ( $P<.001$ ), but not in the Southern blot subgroup ( $P=.643$ ).

When HCC samples were compared with adjacent tissue samples, subgroup analysis of ethnic populations showed that *CDH1* hypermethylation was correlated with the Caucasian population subgroup ( $P=.037$ ), but not with the Asian population subgroup ( $P=.12$ ).

When HCC samples were compared with normal samples, subgroup analysis of ethnic populations showed that *CDH1* hypermethylation presented a significant association in Asians and Caucasians (OR=32.44,  $P<.001$ ; OR=4.45,  $P=.006$ , respectively). Subgroup analysis based on sample types showed that *CDH1* hypermethylation exhibited a significant association in tissue and blood samples (OR=4.44;  $P<.001$ ; OR=50.82,

$P<.001$ , respectively). Although the analysis of blood samples only involved in 2 studies, the obtained OR was significantly higher than that for tissue samples, indicating that *CDH1* hypermethylation may be a potential noninvasive biomarker.

### 3.5. Meta-regression analysis of CDH1 hypermethylation in cancer and control groups

Meta-regression analysis was performed to explain the sources of heterogeneity (Table 2). In the comparison of HCC and benign samples, the results revealed no detectable heterogeneity related

**Table 2****Meta-regression analysis of CDH1 hypermethylation in HCC versus controls.**

HCC vs benign samples	Coefficient (95% CI)	T	P
Detection method	-1.885 (-4.296, 0.525)	-1.8	.109
Ethnicity	-1.309 (-3.773, 1.155)	-1.22	.256
Sample type	0.985 (-1.642, 3.613)	0.86	.412
HCC vs adjacent samples			
Sample type	-0.504 (-2.175, 1.167)	-0.68	.512

CDH1 = E-cadherin, CI = confidence interval, HCC = hepatocellular carcinoma.

to ethnicity, sample type, or testing method (all  $P > .05$ ). In the comparison of HCC and adjacent tissues, the result of the ethnicity failed to explain the source ( $P > .05$ ). Therefore, sensitivity analyses were required.

### 3.6. Sensitivity analysis of the *CDH1* hypermethylation in cancer and controls

Next, a sensitivity analysis was performed to assess the influence of an individual study on the pooled OR and the effect of omitting a single study on its stability. In the comparison of HCC and benign samples, when we removed 2 studies (Kanai et al 2000, Japan,<sup>[54]</sup> and Schagdarsurengin et al 2003, Germany<sup>[50]</sup>) and recalculated the pooled OR from the remaining studies (OR = 5.41, 95% CI = 3.25–9.01,  $P < .001$ ), a very low heterogeneity was observed ( $I^2 = 29.4\%$ ). In the comparison of HCC and adjacent tissues, when we deleted 3 studies (Su et al 2007, China<sup>[41]</sup>; Herath et al 2004, Australia<sup>[47]</sup>; and Yuan et al 2006, China<sup>[42]</sup>) and recalculated the overall OR (OR = 3.31, 95% CI = 1.64–6.67,  $P = .001$ ), a decreased heterogeneity was observed ( $I^2 = 41.8\%$ ). The results of the sensitivity analyses showed that the pooled OR for *CDH1* hypermethylation was not significantly altered, indicating stability of our results.

### 3.7. Correlation of *CDH1* hypermethylation with clinicopathological features of HCC patients

We further determined whether *CDH1* hypermethylation was associated with clinicopathological characteristics in HCC patients. Our findings revealed that a hypermethylated *CDH1* gene did not present a significant association in relation to gender status, tumor grade, clinical stage, HBV status, or HCV status in HCC (all  $P > .1$ ) (Table 3).

### 3.8. Publication bias

Possible publication bias was detected using Egger test (Figure S1, <http://links.lww.com/MD/B656> and Table 3). A slight publication bias was only found in relation to HCV status in HCC ( $P = .039$ ). However, there was no obvious publication bias in the cancer or control groups in relation to gender status, tumor grade, pathological stage, or HBV status in cancer (all  $P > .05$ ).

## 4. Discussion

The hypermethylation of TSGs and hypomethylation of oncogenes play crucial roles in the initiation and development

of HCC.<sup>[56,57]</sup> DNA methylation of CpG islands is one of the important causes of the downregulation of gene expression in cancer.<sup>[58]</sup> Reduction of the expression of the TSG *CDH1* may be an important event in cancer invasion and metastasis.<sup>[12,59]</sup> *CDH13* expression in human cancer cells can inhibit their invasion and markedly reduce their proliferation.<sup>[60,61]</sup> *PCDH10* has been demonstrated to act as a TSG in some cancers and plays a key role in proliferation inhibition, apoptosis induction, and invasion repression.<sup>[62,63]</sup>

However, the results are still inconsistent and controversial. Different methylation rates of the *CDH1* gene are reported in different studies, ranging from 0%<sup>[51]</sup> to 100%.<sup>[31]</sup> Yu et al<sup>[51]</sup> reported that the *CDH13* gene displayed a similar methylation rate in HCC tissues and adjacent tissues. Two studies reported that the *CDH13* methylation rate in HCC was significantly higher than in adjacent tissue samples.<sup>[35,39]</sup> In addition, we found that the *CDH1* gene showed a significantly different methylation frequency in HCC samples than in benign and adjacent samples. For example, 2 studies reported that the *CDH1* gene showed a significantly lower methylation rate in HCC than in benign samples.<sup>[50,54]</sup> Su et al and Herath et al reported that adjacent tissues exhibited a higher methylation frequency of the *CDH1* gene than HCC tissue samples.<sup>[41,47]</sup> Therefore, our study is the first to evaluate the role of *CDH* gene methylation in HCC risk. Moreover, we are the first to determine whether *CDH1* hypermethylation is associated with clinicopathological characteristics in HCC.

The results regarding *CDH1* hypermethylation demonstrated that HCC showed a significantly higher OR than that benign, adjacent, and normal samples, suggesting that *CDH1* hypermethylation may play a crucial role in the tumorigenesis of HCC. Regarding our finding that the OR of *CDH1* hypermethylation was lower in the comparison of HCC samples and adjacent samples (adjacent samples: OR = 2.42; benign samples: OR = 3.40; and normal samples: OR = 7.17), the possible reason may have been impure adjacent specimens contaminated by HCC cells. In addition, methylated *CDH13*, *PCDH10*, *CDH3*, and *CDH15* were associated with an increased risk of HCC, while no significant association was found between methylated *CDH8* and *CDH11* and HCC. However, the results regarding methylated *CDH13*, *PCDH10*, *CDH3*, *CDH15*, *CDH8*, and *CDH11* should be considered carefully because of the small number of subjects included in this analysis.

Subgroup analyses of *CDH1* hypermethylation were performed to identify various associations in the current study. When HCC samples were compared with benign samples, the results related to ethnicity suggested that only Asians were

**Table 3**

**The association of *CDH1* hypermethylation with clinicopathological features in HCC patients.**

	Studies	Pooled OR (95 CI %)	$I^2$	$P$	HCC patients		Egger $p$
					Male	Female	
Gender	5	0.81 (0.44–1.50)	0.00%	.504	216	57	0.744
Tumor grade	7	1.04 (0.55–1.97)	0.00%	.909	Grade 1–2 205	Grade 3 67	0.428
Tumor stage	6	0.83 (0.57–1.22)	0.00%	.35	Stage 1–2 367	Stage 3–4 188	0.053
HBV	12	1.37 (0.80–2.36)	24.60%	.257	HCV+ 411	Negative 178	0.822
HCV	7	0.99 (0.54–1.79)	0.00%	.963	HCV+ 157	Negative 88	0.039

CDH1 = E-cadherin, CI = confidence interval, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, OR = odds ratio.

susceptible to *CDH1* hypermethylation. Subgroup analysis of sample types showed that the *CDH1* gene only exhibited a significant methylation frequency in the tissue subgroup. Subgroup analysis of detection methods revealed that only MSP was a sensitive method of methylation detection for identifying *CDH1* hypermethylation. When HCC samples were compared with adjacent tissue samples, an analysis of ethnic populations showed that hypermethylated *CDH1* was a susceptibility gene for the Caucasian population. When HCC samples were compared with normal samples, the results regarding race showed that the *CDH1* gene was a susceptibility gene for Asians and Caucasians, with the Asian population (OR=32.44) showing a higher OR than the Caucasian population subgroup (OR=4.45), suggesting that hypermethylated *CDH1* may be a stronger susceptibility gene for Asians. According to subgroup analysis of sample types, there was a significant correlation between hypermethylated *CDH1* and HCC in both tissue and blood samples, and the pooled OR for blood samples (OR=50.82) was significantly higher than that for tissue samples (OR=4.44), which suggested that hypermethylated *CDH1* may be a useful noninvasive biomarker for blood detection. Due to the limitations of the available studies, we could not further evaluate the diagnostic capacity of *CDH1* hypermethylation as a promising biomarker for HCC diagnosis. The results should be interpreted with caution, as only small sample sizes were involved, especially in the analysis of the Southern blot subgroup.

Additionally, *CDH1* hypermethylation exhibited substantial heterogeneity between the cancer and benign or adjacent samples. Next, a meta-regression analysis was performed to identify the sources of heterogeneity. Our results showed that the meta-regression analysis could not reveal the sources of heterogeneity. Subsequently, sensitivity analyses were performed to evaluate the changes in the pooled OR and heterogeneity by omitting 2 studies<sup>[50,54]</sup> comparing cancer and benign samples or by deleting 3 studies<sup>[41,42,47]</sup> comparing cancer and adjacent samples. The results showed that the overall OR of hypermethylated *CDH1* was not significantly altered, and no significant heterogeneity was observed, suggesting that the results regarding *CDH1* hypermethylation were stable and reliable.

Finally, the relationship of *CDH1* hypermethylation with clinicopathological parameters was determined in the present study. Our findings showed that *CDH1* hypermethylation was not correlated with gender status, tumor grade, tumor stage, HBV status, or HCV status in HCC.

Several limitations of the present meta-analysis should be acknowledged. First, a slight publication bias was detected in relation to HCV status in cancer, with papers with positive results being more frequently published than papers with negative results. Second, only studies published in English were included in our analyses; potentially high-quality articles published in other languages were excluded because of the limitations of our language abilities, which may have led to selection bias. Third, results for methylated *CDH13*, *PCDH10*, *CDH3*, *CDH15*, *CDH8*, and *CDH11* that were based on smaller sample sizes were analyzed in the current study; thus, additional studies with larger sample sizes are essential to further validate the obtained results.

In conclusion, our findings suggested that *CDH1* hypermethylation is involved in HCC hepatocarcinogenesis. However, *CDH1* hypermethylation was not found to be correlated with clinicopathological features. Moreover, methylated *CDH13*, *PCDH10*, *CDH3*, and *CDH15* are associated with an increased

risk of HCC, while methylated *CDH8* and *CDH11* were not observed to be associated with a risk of HCC. Hypermethylated *CDH1* may become a potential noninvasive biomarker based on blood sample analysis. Further large-scale studies are required to strengthen the findings of our study in the future.

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