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ORIGINAL RESEARCH

Clinicopathological significance and potential drug target of CDH1 in breast cancer: a meta-analysis and literature review

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Abstract: *CDH1*, as a tumor suppressor gene, contributes sporadic breast cancer (BC) progression. However, the association between *CDH1* hypermethylation and BC, and its clinicopathological significance remains unclear. We conducted a meta-analysis to investigate the relationship between the *CDH1* methylation profile and the major clinicopathological features. A detailed literature was searched through the electronic databases PubMed, Web of Science™, and EMBASE™ for related research publications. The data were extracted and assessed by two reviewers independently. Odds ratios (ORs) with corresponding confidence intervals (CIs) were calculated and summarized respectively. The frequency of *CDH1* methylation was significantly higher in invasive ductal carcinoma than in normal breast tissues (OR =5.83, 95% CI 3.76–9.03, $P<0.00001$). *CDH1* hypermethylation was significantly higher in estrogen receptor (ER)-negative BC than in ER-positive BC (OR =0.62, 95% CI 0.43–0.87, $P=0.007$). In addition, we found that the *CDH1* was significantly methylated in HER2-negative BC than in HER2-positive BC (OR =0.26, 95% CI 0.15–0.44, $P<0.00001$). However, *CDH1* methylation frequency was not associated with progesterone receptor (PR) status, or with grades, stages, or lymph node metastasis of BC patients. Our results indicate that *CDH1* hypermethylation is a potential novel drug target for developing personalized therapy. *CDH1* hypermethylation is strongly associated with ER-negative and HER2-negative BC, respectively, suggesting *CDH1* methylation status could contribute to the development of novel therapeutic approaches for the treatment of ER-negative or HER2-negative BC with aggressive tumor biology.

Keywords: methylation, estrogen receptor, HER2, triple-negative breast cancer

Introduction

Breast Cancer (BC) is the most common malignancy among women in most western countries.[1] The development of BC involves a progression, starting with atypical hyperplasia, followed by intermediate stages until the invasive carcinoma, and finally into metastatic disease. A series of epigenetic and genetic changes contribute to this multistep process of BC onset and progression. Epigenetics refers to heritable changes in gene activity and expression that do not involve changes to underlying DNA sequence and has recently gained significant attention of researchers.[2] Epigenetic alterations occur in transformed cells and includes global hypomethylation, focal hypermethylation, histone modifications, and nucleosomal remodeling.[3] Aberrant DNA hypermethylation is a commonly observed epigenetic modification in human malignancies, including BC.[4–6] The *CDH1* gene encodes a transmembrane glycoprotein E-cadherin that maintains Ca^{2+}-dependent cell–cell adhesion in epithelial tissues and therefore plays an important role in tumorigenesis as a tumor suppressor gene.[7–10] Alteration in E-cadherin expression

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has been observed in several types of malignancies, including ovarian cancer,[11] prostate cancer,[12] hepatocellular carcinoma,[13] and BC.[14] Recent evidence indicate that reduced E-cadherin expression is associated with pathological features, such as poor differentiation, infiltrative growth, lymph node metastasis, and poor prognosis.[15–17] However, the association of between *CDH1* promoter hypermethylation and BC, and its clinicopathological significance, remains under investigation. We conducted a meta-analysis to investigate the relationship between *CDH1* methylation status in BC and the major clinicopathological features including estrogen receptor (ER), progesterone receptor (PR) and HER2.

Methods
Literature search and selection of studies
We conducted a systematic search of the literature published in English and Chinese prior to November 2014. The electronic databases included PubMed, Web of Science™, and EMBASE™. The search was conducted using the following key words: "*CDH1* or *E-cadherin* methylation" and "breast cancer" or "breast carcinoma" for relative articles. There were 84 articles identified from PubMed, 143 articles from Web of Science, and 101 articles from EMBASE. A total of 328 articles were screened using article titles and abstracts. Reference lists of identified articles were searched manually for further relevant articles (Figure 1).

The following inclusion criteria were applied: 1) studies that reported the association between *CDH1* methylation and the clinicopathological significance of BC and 2) studies that investigated the frequency of *CDH1* methylation in different ER, PR, as well as HER2 statuses of BC. After screening by article titles and abstracts, 25 relevant articles were included for full text review. The exclusion criteria were as follows: 1) reviews; 2) studies in which CDH1 protein expression was investigated; 3) studies in which cell lines or mice were utilized; and 4) studies in which same populations or overlapping data were used. Finally, eleven articles were selected for inclusion in this meta-analysis. The variables from eleven relevant studies were listed in Table 1.

Data extraction and study assessment
Two independent reviewers (RH and PD) extracted the following data: first author's name, year of publication, geographical location, sample size of the different histologic categories of BC, grade of BC, stage of BC, and ER, PR, and HER2 status. Any disagreement was resolved by discussion between two authors (RH and PD). If they could not reach a consensus, a third author (YD) was consulted.

Statistics analysis
The meta-analysis was conducted using Review Manager 5.2 (software update; The Nordic Cochrane Centre, Copenhagen, Denmark). Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. The assessment of statistical heterogeneity was done by using the Cochran's Q statistic and I^2 tests. If the I^2 value was below 50%, a fixed effects model was used, and if the I^2 value was 50% or greater, a random effects model was used. The analysis was conducted to evaluate the association of the frequency of *CDH1* methylation with invasive ductal carcinoma (IDC). Furthermore, we compared the frequency of *CDH1* methylation in different ER, PR, and HER2 statuses as well as in different grades and stages. All *P*-values were two sided, with $P < 0.05$ considered statistically significant. Publication bias was determined using funnel plot analysis.

Results
CDH1 was more frequently methylated in IDCs than in normal breast tissues (pooled OR =5.83, 95% CI 3.76–9.03,

Table 1 Main characteristics of included studies

Author	Year	Country	Sample		Stage (I + II/III)	Lymph node (0/+)	Grade (I + II/III)	ER status (−/+)	HER2 status (−/+)
			Normal	IDC					
Asiaf et al[36]	2014	India	128	128	89/39	79/49	108/20	39/89	
Karray-Chouayekh et al[37]	2010	Tunisia		78	21/30	36/42	61/17	31/47	51/13
Hoque et al[50]	2009	Italy	12	45					
Zou et al[51]	2009	New Zealand	19	20					
Sunami et al[52]	2008	USA		130	102/8	76/54		65/65	78/29
Prasad et al[38]	2008	India		50		15/35	27/23		
Li et al[53]	2006	Australia		193		83/71		54/134	142/30
Shinozaki et al[27]	2005	USA	10	151		102/43		37/114	
Caldeira et al[54]	2005	Brazil		71	53/18		53/18	45/26	63/8
Hu et al[55]	2002	People's Republic of China		23	18/5		15/8	5/10	10/3
Toyooka et al[28]	2002	USA	10	51					

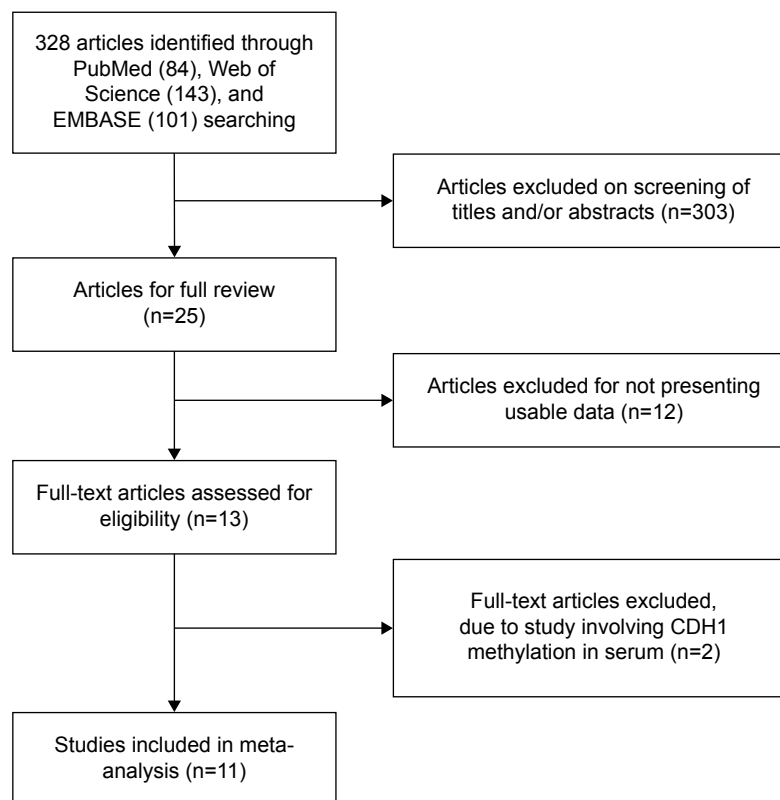**Abbreviations:** ER, estrogen receptor; IDC, invasive ductal carcinoma.

Figure 1 Schematic flow diagram for selection of included studies.

z=7.88, P<0.00001, I^2=46%, P=0.10) (Figure 2). *CDH1* hypermethylation was significantly higher in ER-negative than in ER-positive patients with BC (OR =0.62, 95% CI 0.43–0.87, z=2.72, P=0.007, I^2=39%, P=0.13) (Figure 3). *CDH1* promoter was significantly more methylated in HER2-negative patients than in HER2-positive patients with BC (OR =0.26, 95% CI 0.15–0.44, z=4.97, P<0.00001, I^2=0%, P=0.57) (Figure 4). The methylation

frequency was similar between PR-positive and -negative patients with BC (OR =0.76, 95% CI 0.49–1.15, z=1.30, P=0.19, I^2=0%, P=0.45) (Figure 5). The frequency of *CDH1* hypermethylation was not significantly higher in grade III than in grade I/II (OR =1.46, 95% CI 0.86–2.49, z=1.40, P=0.16, I^2=11%, P=0.35) (Figure 6). The frequency of *CDH1* hypermethylation was not significantly changed between late stage and early stage of BC (OR =1.09, 95%
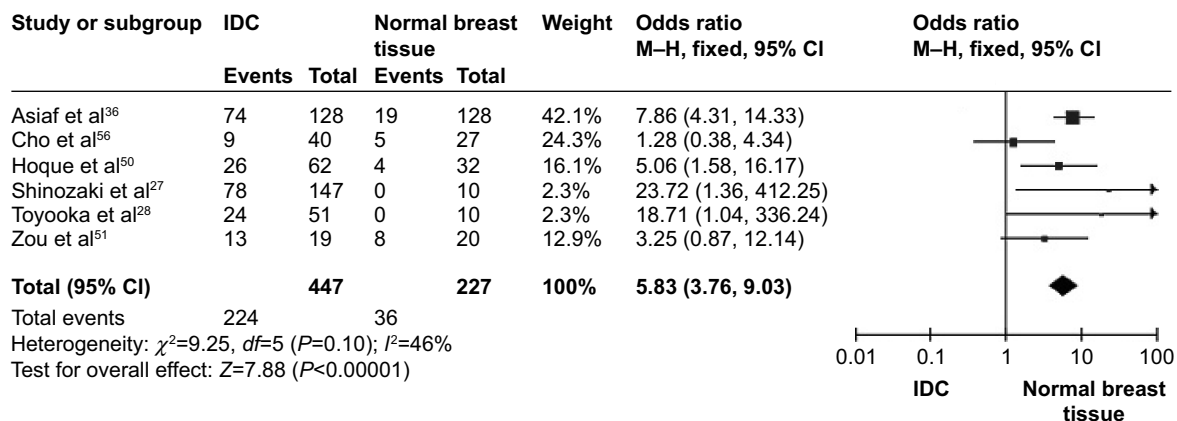

Study or subgroup	IDC		Normal breast tissue		Weight	Odds ratio M–H, fixed, 95% CI	Odds ratio M–H, fixed, 95% CI
	Events	Total	Events	Total			
Asiaf et al[36]	74	128	19	128	42.1%	7.86 (4.31, 14.33)	
Cho et al[56]	9	40	5	27	24.3%	1.28 (0.38, 4.34)	
Hoque et al[50]	26	62	4	32	16.1%	5.06 (1.58, 16.17)	
Shinozaki et al[27]	78	147	0	10	2.3%	23.72 (1.36, 412.25)	
Toyooka et al[28]	24	51	0	10	2.3%	18.71 (1.04, 336.24)	
Zou et al[51]	13	19	8	20	12.9%	3.25 (0.87, 12.14)	
Total (95% CI)		447		227	100%	5.83 (3.76, 9.03)	
Total events	224		36				

Heterogeneity: χ^2=9.25, df=5 (P=0.10); I^2=46%
Test for overall effect: Z=7.88 (P<0.00001)

0.01 0.1 1 10 100
IDC   Normal breast tissue

Figure 2 Forest plot for *CDH1* hypermethylation in IDC and normal breast tissue.
Abbreviations: CI, confidence interval; IDC, invasive ductal carcinoma; M–H, Mantel–Haenszel.
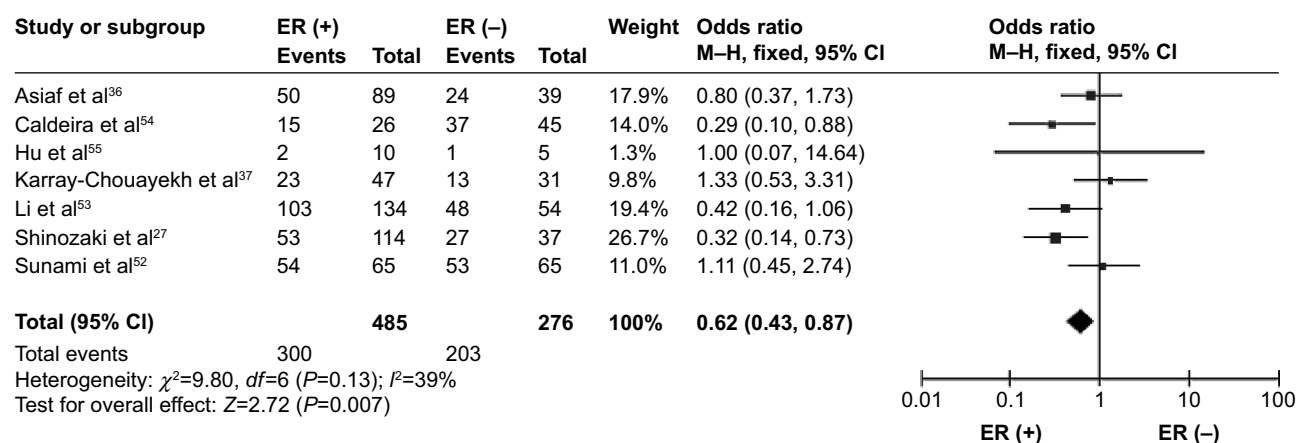
| Study or subgroup | ER (+) | | ER (–) | | Weight | Odds ratio | Odds ratio |
	Events	Total	Events	Total		M–H, fixed, 95% CI	M–H, fixed, 95% CI
Asiaf et al[36]	50	89	24	39	17.9%	0.80 (0.37, 1.73)	
Caldeira et al[54]	15	26	37	45	14.0%	0.29 (0.10, 0.88)	
Hu et al[55]	2	10	1	5	1.3%	1.00 (0.07, 14.64)	
Karray-Chouayekh et al[37]	23	47	13	31	9.8%	1.33 (0.53, 3.31)	
Li et al[53]	103	134	48	54	19.4%	0.42 (0.16, 1.06)	
Shinozaki et al[27]	53	114	27	37	26.7%	0.32 (0.14, 0.73)	
Sunami et al[52]	54	65	53	65	11.0%	1.11 (0.45, 2.74)	
Total (95% CI)		**485**		**276**	**100%**	**0.62 (0.43, 0.87)**	
Total events	300		203				

Heterogeneity: χ^2=9.80, df=6 (P=0.13); I^2=39%
Test for overall effect: Z=2.72 (P=0.007)

0.01 0.1 1 10 100
ER (+)   ER (–)

Figure 3 Forest plot for *CDH1* hypermethylation in ER-positive and -negative BC.
Abbreviations: BC, breast cancer; CI, confidence interval; ER, estrogen receptor; M–H, Mantel–Haenszel.
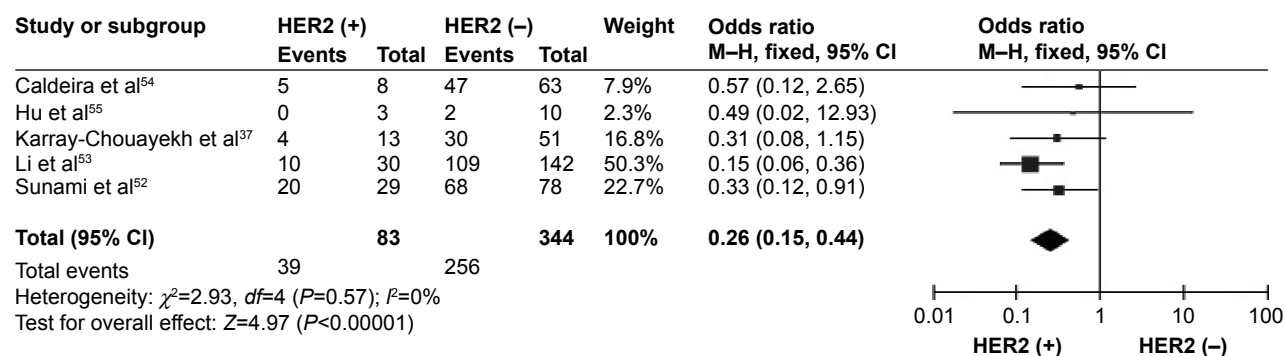
| Study or subgroup | HER2 (+) | | HER2 (–) | | Weight | Odds ratio | Odds ratio |
	Events	Total	Events	Total		M–H, fixed, 95% CI	M–H, fixed, 95% CI
Caldeira et al[54]	5	8	47	63	7.9%	0.57 (0.12, 2.65)	
Hu et al[55]	0	3	2	10	2.3%	0.49 (0.02, 12.93)	
Karray-Chouayekh et al[37]	4	13	30	51	16.8%	0.31 (0.08, 1.15)	
Li et al[53]	10	30	109	142	50.3%	0.15 (0.06, 0.36)	
Sunami et al[52]	20	29	68	78	22.7%	0.33 (0.12, 0.91)	
Total (95% CI)		**83**		**344**	**100%**	**0.26 (0.15, 0.44)**	
Total events	39		256				

Heterogeneity: χ^2=2.93, df=4 (P=0.57); I^2=0%
Test for overall effect: Z=4.97 (P<0.00001)

0.01 0.1 1 10 100
HER2 (+)   HER2 (–)

Figure 4 Forest plot for *CDH1* hypermethylation in HER2-positive and -negative BC.
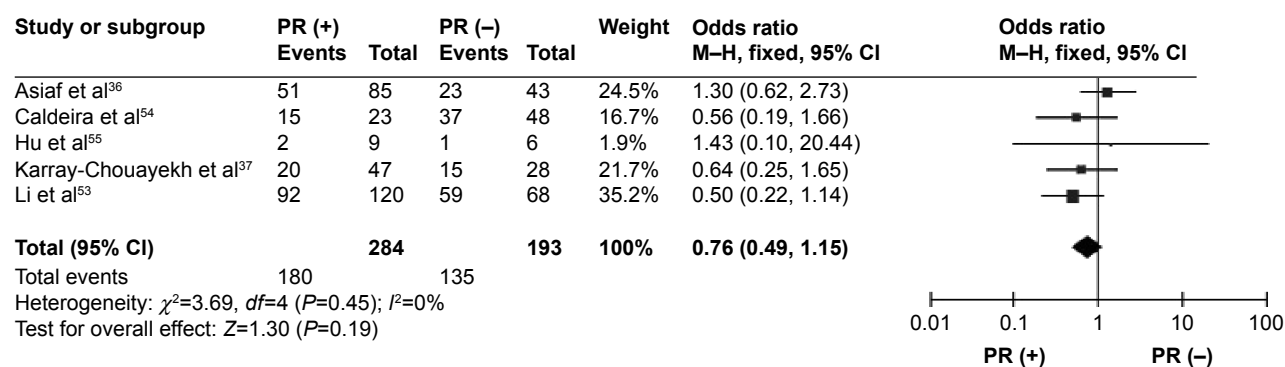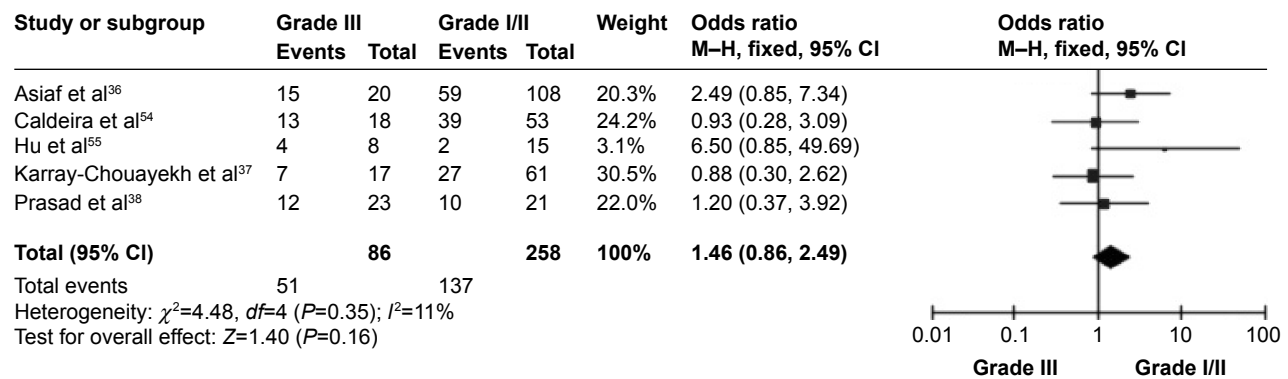**Abbreviations:** BC, breast cancer; CI, confidence interval; M–H, Mantel–Haenszel.

| Study or subgroup | PR (+) | | PR (–) | | Weight | Odds ratio | Odds ratio |
	Events	Total	Events	Total		M–H, fixed, 95% CI	M–H, fixed, 95% CI
Asiaf et al[36]	51	85	23	43	24.5%	1.30 (0.62, 2.73)	
Caldeira et al[54]	15	23	37	48	16.7%	0.56 (0.19, 1.66)	
Hu et al[55]	2	9	1	6	1.9%	1.43 (0.10, 20.44)	
Karray-Chouayekh et al[37]	20	47	15	28	21.7%	0.64 (0.25, 1.65)	
Li et al[53]	92	120	59	68	35.2%	0.50 (0.22, 1.14)	
Total (95% CI)		**284**		**193**	**100%**	**0.76 (0.49, 1.15)**	
Total events	180		135				

Heterogeneity: χ^2=3.69, df=4 (P=0.45); I^2=0%
Test for overall effect: Z=1.30 (P=0.19)

0.01 0.1 1 10 100
PR (+)   PR (–)

Figure 5 Forest plot for *CDH1* hypermethylation in PR-positive and -negative BC.
Abbreviations: BC, breast cancer; CI, confidence interval; PR, progesterone receptor; M–H, Mantel–Haenszel.

Study or subgroup	Grade III		Grade I/II		Weight	Odds ratio M–H, fixed, 95% CI	Odds ratio M–H, fixed, 95% CI
	Events	Total	Events	Total			
Asiaf et al[36]	15	20	59	108	20.3%	2.49 (0.85, 7.34)	
Caldeira et al[54]	13	18	39	53	24.2%	0.93 (0.28, 3.09)	
Hu et al[55]	4	8	2	15	3.1%	6.50 (0.85, 49.69)	
Karray-Chouayekh et al[37]	7	17	27	61	30.5%	0.88 (0.30, 2.62)	
Prasad et al[38]	12	23	10	21	22.0%	1.20 (0.37, 3.92)	
Total (95% CI)		**86**		**258**	**100%**	**1.46 (0.86, 2.49)**	
Total events	51		137				

Heterogeneity: χ^2=4.48, df=4 (P=0.35); I^2=11%
Test for overall effect: Z=1.40 (P=0.16)

(scale: 0.01 0.1 1 10 100; Grade III — Grade I/II)

Figure 6 Forest plot for *CDH1* hypermethylation in different grades of BC.
Abbreviations: BC, breast cancer; CI, confidence interval; M–H, Mantel–Haenszel.

CI 0.63–1.88, z=0.30, P=0.77, I^2=41%, P=0.16) (Figure 7). The frequency of *CDH1* hypermethylation was similar between BC patients with positive lymph node metastasis and those with negative lymph node metastasis (OR =1.21, 95% CI 0.59–2.47, z=0.53, P=0.60, I^2=71%, P=0.007) (Figure 8).

The assessment of quality of the included articles was done using the Newcastle–Ottawa Quality Assessment Scale.[18] This scale allocates a maximum of 9 points for the quality of selection, comparability, exposure, and outcomes for study participants. The Newcastle–Ottawa scale scores ranged from 0 to 9 (a score of 7 or more indicates a good quality). Of the studies, three scored 9 points, three scored 8 points, and five scored 7 points. Hence, the studies were of a relatively high quality (data not shown). We further conducted analyses to determine the result stability in the choice of a single study; the finding suggested no single study had the effect on the pooled ORs, indicating the stability of our analyses. There were no publication biases in the meta-analysis according to the largely symmetric funnel plots (Figure 9).

Discussion

The *CDH1* gene encodes E-cadherin protein, which is a 120 kDa glycoprotein consisting of an extracellular domain of five tandem repeated domains, a cytoplasmic domain, and a single transmembrane domain.[19,20] The extracellular domain binds to cadherin on adjacent cells and forms cell–cell adhesion, whereas the cytoplasmic domain binds to catenin and activates the Wnt signaling cascade.[21] *CDH1* hypermethylation is one of the mechanisms that cause gene silencing and results in reduced E-cadherin expression. Lack of E-cadherin leads to the dysfunction of the cell–cell adhesion system, resulting in a loss of cell–cell adhesion, release of cytoplasmic β-catenin, increase in Wnt signaling, and increased tumor invasiveness.[22] *CDH1* hypermethylation and its reduced expression have been observed in several of malignancies, including gastric, ovarian, lung, and BC.[23–26] In the present study, we compared the frequency of *CDH1* hypermethylation between IDC and normal breast tissues. Our results showed *CDH1* promoter hypermethylation was significantly higher in IDC than in
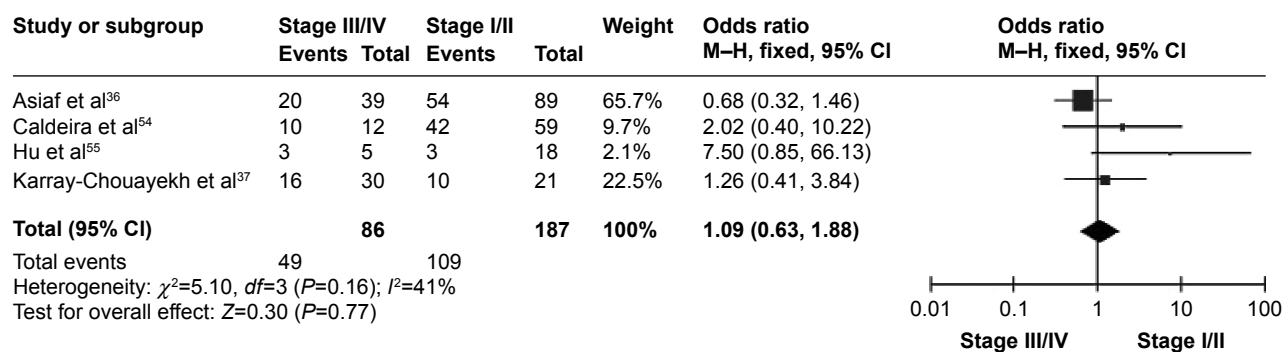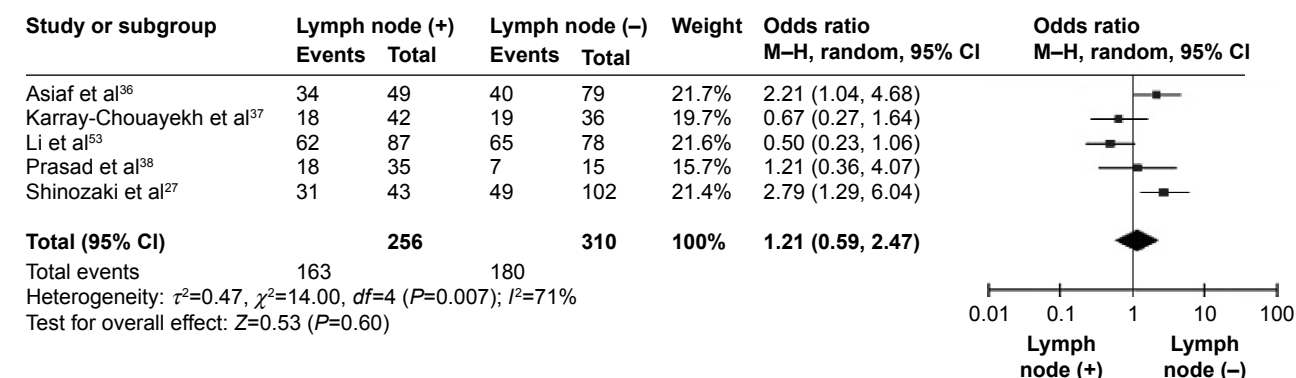
Study or subgroup	Stage III/IV		Stage I/II		Weight	Odds ratio M–H, fixed, 95% CI	Odds ratio M–H, fixed, 95% CI
	Events	Total	Events	Total			
Asiaf et al[36]	20	39	54	89	65.7%	0.68 (0.32, 1.46)	
Caldeira et al[54]	10	12	42	59	9.7%	2.02 (0.40, 10.22)	
Hu et al[55]	3	5	3	18	2.1%	7.50 (0.85, 66.13)	
Karray-Chouayekh et al[37]	16	30	10	21	22.5%	1.26 (0.41, 3.84)	
Total (95% CI)		**86**		**187**	**100%**	**1.09 (0.63, 1.88)**	
Total events	49		109				

Heterogeneity: χ^2=5.10, df=3 (P=0.16); I^2=41%
Test for overall effect: Z=0.30 (P=0.77)

(scale: 0.01 0.1 1 10 100; Stage III/IV — Stage I/II)

Figure 7 Forest plot for *CDH1* hypermethylation in different stages of BC.
Abbreviations: BC, breast cancer; CI, confidence interval; M–H, Mantel–Haenszel.

Study or subgroup	Lymph node (+)		Lymph node (–)		Weight	Odds ratio M–H, random, 95% CI	Odds ratio M–H, random, 95% CI
	Events	Total	Events	Total			
Asiaf et al[36]	34	49	40	79	21.7%	2.21 (1.04, 4.68)	
Karray-Chouayekh et al[37]	18	42	19	36	19.7%	0.67 (0.27, 1.64)	
Li et al[53]	62	87	65	78	21.6%	0.50 (0.23, 1.06)	
Prasad et al[38]	18	35	7	15	15.7%	1.21 (0.36, 4.07)	
Shinozaki et al[27]	31	43	49	102	21.4%	2.79 (1.29, 6.04)	
Total (95% CI)		**256**		**310**	**100%**	**1.21 (0.59, 2.47)**	
Total events	163		180				

Heterogeneity: $\tau^2=0.47$, $\chi^2=14.00$, $df=4$ ($P=0.007$); $I^2=71\%$
Test for overall effect: $Z=0.53$ ($P=0.60$)

Figure 8 Forest plot for *CDH1* hypermethylation in lymph node-positive and -negative metastasis of BC.
Abbreviations: BC, breast cancer; CI, confidence interval; M–H, Mantel–Haenszel.

normal breast tissue, which is in agreement with previous studies.[27,28] There was a 5.83 times increased risk to BC in the subjects with methylated *CDH1* promoter. The major treatments of BC include chemotherapy, hormone therapy, and target therapy, and drug target therapy has been gaining more attention recently. *CDH1*, as a suppressor gene, is a potential novel drug target, and its hypermethylation could be reversed through demethylation. Inhibitors of DNA methylation (DNMTis), such as 5-Aza-CdR and 5-fluoro-2′-deoxycytidine, have been applied to human lung cancer and BC cells, and currently 5-fluoro-2′-deoxycytidine is in clinical trials for the treatment of BC and other solid tumors.[29–31] Lopes et al induced E-cadherin expression in triple-negative MDA-MB-231 cells with $1\alpha,25(OH)_2D_3$, through *CDH1* promoter demethylation.[32] They observed that $1\alpha,25(OH)_2D_3$ promoted differentiation of MDA-MB-231 cells by inducing de novo E-cadherin expression, an effect that was time- and dose-dependent. These preclinical studies showed the therapeutic potential of restoration of the tumor suppressor gene through epigenetic modulation, which may decrease the aggressiveness of BC. Therefore, *CDH1* hypermethylation is a potential novel drug target in the development of personalized therapy.

Epithelial-to-mesenchymal transition (EMT) is an important process in embryonic development and also plays a particularly important role during tumor progression.[33] Loss of E-cadherin induces EMT, and the nonpolarized mesenchymal cells are highly motile and invasive, therefore allowing tumor invasion and metastatic spread.[34,35] Previous reports showed inconsistent results of the association between *CDH1* hypermethylation and clinicopathologic parameters in BC, due to small sample size and different ethnicity.[36,37] We pooled eleven studies and included 940 patients in the present study. Our data showed that *CDH1* hypermethylation

was not significantly associated with grade, stage, or lymph node metastasis in BC, with a high-power sample compared with previous studies.[37,38]

About 70% of BCs are ER-positive.[39] The ER is responsible for estrogen-induced mitogenic signaling in epithelial cells in the breast and plays a crucial role in BC onset and progression. When estradiol (E2) binds to ER, the ER undergoes conformational changes and forms dimers, which attract coactivators and corepressors. This complex regulates hundreds of genes involved in a variety of processes, including proliferation, differentiation, survival, invasion, and metastasis.[40,41] The ER status of breast provides prognostic information but more importantly, is a predictor of response to endocrine therapy.[42] We investigated *CDH1* methylation status in ER-positive versus -negative BC in the present study, showing significantly higher frequency of *CDH1* hypermethylation in ER-negative BC than in ER-positive BC. Interestingly, a few studies reported that loss of β-catenin membranous expression and nuclear accumulation of β-catenin are associated with ER-negative status and reduced CDH1 expression,[43,44] along with aggressive tumor phenotype and poor patient outcome in BC.[44,45] *CDH1* methylation is one of the mechanisms to regulate E-cadherin expression in the ER-negative subtype of BC. In addition, HER2 is a transmembrane tyrosine kinase receptor, located on chromosome 17q21. HER2, encoded by the *ERBB2/HER2* oncogene, belongs to a family of epidermal growth factor receptors (EGFRs) structurally related to EGFR.[46] This oncogene is overexpressed in 20%–30% of BC and has been recognized as a marker of poor prognosis, including increased metastasis potential and decreased overall survival.[47] Inhibition of HER2 activity with antibodies has been applied to treat HER2-positive BC.[48] In present study, the frequency of *CDH1* hypermethylation was significantly
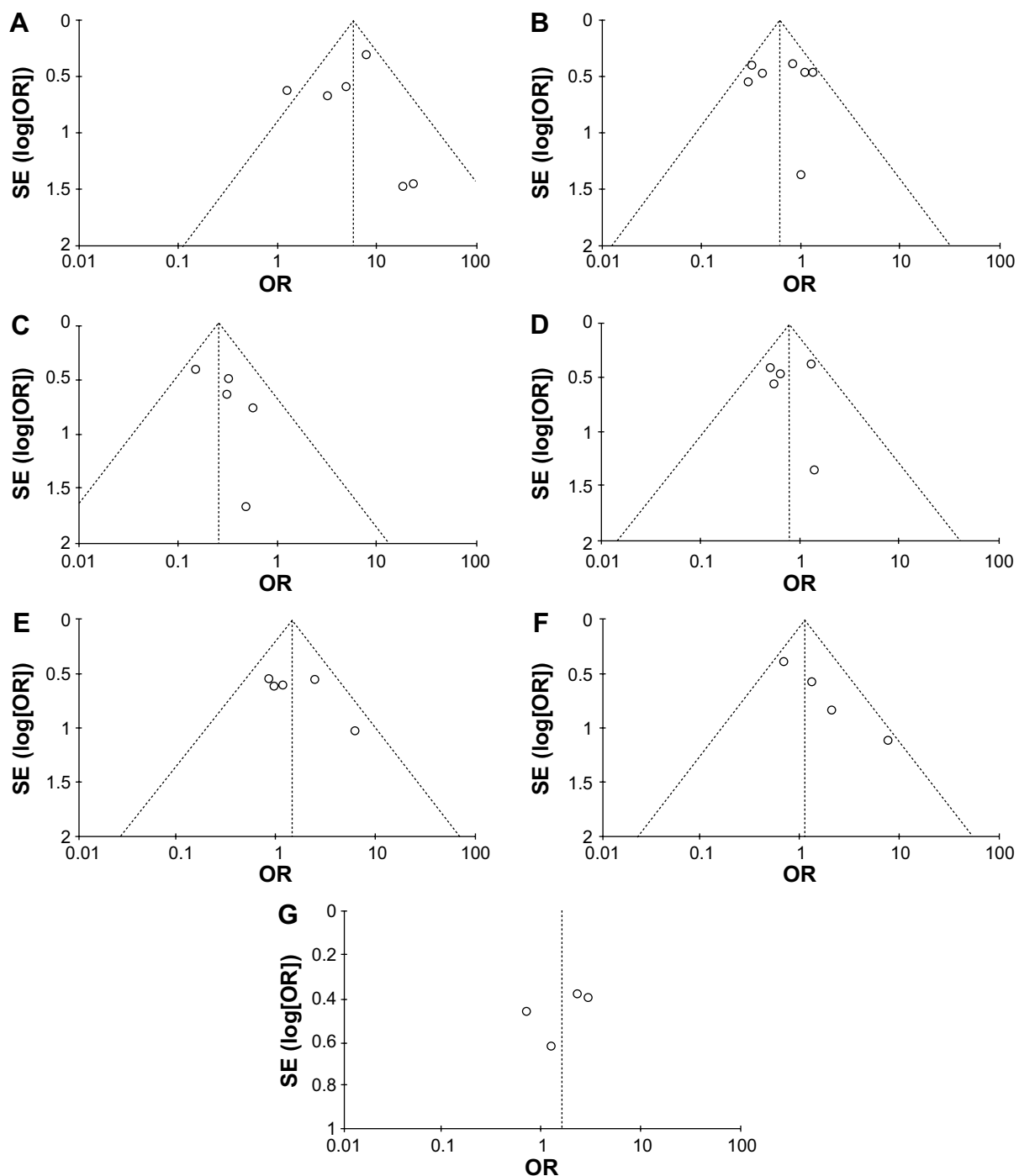
Figure 9 Funnel plots for publication bias.
Notes: (**A**) *CDH1* methylation in IDC and normal breast tissue; (**B**) *CDH1* methylation in ER-positive and -negative BC; (**C**) *CDH1* methylation in HER2-positive and -negative BC; (**D**) *CDH1* methylation in PR-positive and -negative BC; (**E**) *CDH1* methylation in different grades of BC; (**F**) *CDH1* hypermethylation in different stages of BC; and (**G**) *CDH1* hypermethylation in lymph node-positive and -negative metastasis of BC.
Abbreviations: BC, breast cancer; ER, estrogen receptor; IDC, invasive ductal carcinoma; PR, progesterone receptor; OR, odds ratio.

higher in HER2-negative BC than in HER2-positive BC. Our results showed that *CDH1* hypermethylation was increased in HER2-negative and ER-negative BCs, respectively, but was not significantly associated with PR status. In the future, *CDH1* hypermethylation should be investigated in triple-negative BCs, which are HER2-negative, ER-negative, and PR-negative. As we know, about 85% of triple-negative BCs are basal-like subtypes, which are associated with poor clinical outcome.[49] *CDH1* hypermethylation could contribute to the development of a new treatment strategy

for triple-negative BC, especially in the basal-like subtypes. Finally, our study only selected the published articles and did not include some relevant unpublished papers, which may have resulted in certain publication bias. Therefore, the results should be interpreted carefully.

Conclusion

In conclusion, *CDH1* hypermethylation was shown to be significantly higher in IDC than in normal breast tissue, indicating that *CDH1* is a potential novel drug target in the development of personalized therapy. *CDH1* hypermethylation is strongly associated with ER-negative and HER2-negative BC, respectively, suggesting *CDH1* methylation status could contribute to the development of novel therapeutic approaches of ER-negative and or HER2-negative BC, which is a subtype of BC with poor patient outcome.

Disclosure

The authors report no conflicts of interest in this work.

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