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Clinical effect of *DAPK* promoter methylation in gastric cancer
A systematic meta-analysis

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Abstract

Background: The loss of death-associated protein kinase (*DAPK*) gene expression through promoter methylation is involved in many tumors. However, the relationship between *DAPK* promoter methylation and clinicopathological features of gastric cancer (GC) remains to be done. Therefore, we performed a meta-analysis to assess the role of *DAPK* promoter methylation in GC.

Methods: Literature databases were searched to retrieve eligible studies. The pooled odds ratios (ORs) with its 95% confidence intervals (CIs) were calculated using the Stata 12.0 software.

Results: Final 22 available studies with 1606 GC patients and 1508 nonmalignant controls were analyzed. A significant correlation was found between *DAPK* promoter methylation and GC (OR = 3.23, 95% CI = 1.70–6.14, $P < 0.001$), but we did not find any significant association in Caucasian population, and in blood samples in subgroup analyses. *DAPK* promoter methylation was associated with tumor stage and lymph node status (OR = 0.69, 95% CI = 0.49–0.96, $P = 0.03$; OR = 1.50, 95% CI = 1.12–2.01, $P = 0.007$; respectively). However, we did not find that *DAPK* promoter methylation was associated with gender status and tumor histology.

Conclusion: Our findings suggested that *DAPK* promoter methylation may play a key role in the carcinogenesis and progression of GC. In addition, methylated *DAPK* was a susceptible gene for Asian population. However, more studies with larger subjects should be done to further evaluate the effect of *DAPK* promoter methylation in GC patients, especially in blood and Caucasian population subgroup.

Abbreviations: CIs = confidence intervals, *DAPK* = death-associated protein kinase, GC = gastric cancer, IFN-γ = interferon-γ, ORs = odds ratios, TNF-α = tumor necrosis factor-alpha, TSGs = tumor suppressor genes.

Keywords: clinicopathological features, *DAPK*, GC, promoter methylation

1. Introduction

Gastric cancer (GC) is the fifth most common malignancy and the third leading cause of cancer-related deaths worldwide, especially in East Asia.[1] Approximately 90% of stomach tumors are diagnosed as adenocarcinoma, which consist of 2 main histological subtypes: intestinal and diffuse.[2] Although the recent diagnostic and therapeutic opportunities have supported good survival in early gastric cancer patients, numerous patients are typically diagnosed at a late stage, leading to a high mortality rate.[3]

Many factors are correlated with the development of gastric cancer, such as *Helicobacter pylori* (*H. pylori*) infection, dietary and lifestyle factors, genetics, and epigenetic alterations.[4,5] DNA methylation as a major mechanism of epigenetic changes has been proven to involve in the carcinogenesis, progression, and prognosis of GC.[6–9] The gene epigenomic regulation has 2 important components of the molecular mechanism, including the hypermethylation of tumor suppressor genes (TSGs) and hypomethylation of oncogenes.[10,11] Death-associated protein kinase (*DAPK*), a tumor suppressor gene, encoding a calcium/calmodulin-dependent serine/threonine kinase, is localized on human chromosome 9p34 and involves in various apoptosis triggered by tumor necrosis factor-alpha (TNF-α), Fas ligand, and interferon-γ (IFN-γ).[12,13] Moreover, *DAPK* also participates in a range of cellular processes such as growth factor signaling, inflammation and autophagy, as well as the regulation of immune responses.[14,15] The dysfunction of *DAPK* gene via promoter methylation has been linked with many cancers.[16–19]

However, the clinical significance of *DAPK* promoter methylation in GC remains to be determined. Therefore, we performed a meta-analysis to assess the strength of the association of *DAPK* promoter methylation in GC versus noncancerous controls, in relation to sex status, tumor stage, tumor histology, and lymph node status in gastric cancer.

2. Materials and methods

2.1. Literature search

A systematic search of the literature published without language restriction was conducted on PubMed, Embase, EBSCO, and Web of Science databases prior to May 26, 2016. We used the following keywords and search terms: (stomach OR gastric) AND (cancer OR tumor OR neoplasm OR carcinoma) AND (DAPK OR death-associated protein kinase OR DAPK-kinase) AND (methylation OR epigenetic silencing OR epigenetic inactivation). This study was approved by The Institutional Review Board of Ethics Committee of Beijing Hospital.

2.2. Study selection

The eligibility of included studies had to meet the following criteria: (1) gastric cancer patients were histopathologically diagnosed; (2) studies were case-control design or case-series; (3) studies with sufficient information on the DAPK promoter methylation frequency were used to evaluate the correlation between DAPK promoter methylation and GC; (4) articles published as full papers in English were used in this analysis. The studies excluded did not meet inclusion criteria.

2.3. Data extraction

The following data from eligible studies were extracted: the first author's surname, year of publication, methylation detection methodology, ethnicity, sample type, number of methylation, the number of the case and control groups, sample size of the different histology of GC, stage of GC, gender status, and lymph node status. Tumor stages of 0–II were defined as the early stage, and tumor stages of III–IV were defined as the advanced stage.

2.4. Statistical analysis

The meta-analysis was performed using the Stata 12.0 software (Stata Corporation, College Station, TX). The overall odds ratios (ORs) with its 95% confidence intervals (CIs) were calculated to evaluate the strength of the correlation between DAPK promoter methylation and GC. The Cochran Q statistic and I^2 tests were conducted to test the between-study heterogeneity.[20] If I^2 test shows $\geq 50\%$, which indicates strong heterogeneity, a random-effects model was applied; otherwise, a fixed-effects model was used.[21,22] Egger's test was used to assess the possible publication bias.[23]

3. Results

3.1. General characteristics of included studies

A total of 193 publications were identified by the original search as described above. Ultimately, 22 eligible studies[24–45] involving 1606 GC patients and 1508 nonmalignant controls were included in the current meta-analysis based on our inclusion criteria (Fig. 1). Of the included studies, methylation detection methods included 1 study of combined bisulphite restriction analysis (COBRA) [43] and 21 studies of methylation-specific polymerase chain reaction (MSP).[24–42,44,45] Eighteen studies including 1133 cases and 1508 controls assessed the DAPK promoter methylation status in gastric cancer and noncancerous samples.[24–27,29,30,33–36,38–45] Fifteen studies comprising 813 male and 391 female GC patients investigated the relationship between DAPK promoter methylation and gender status.[24,25,27–29,31,32,34,36–38,40–42,44] Nine
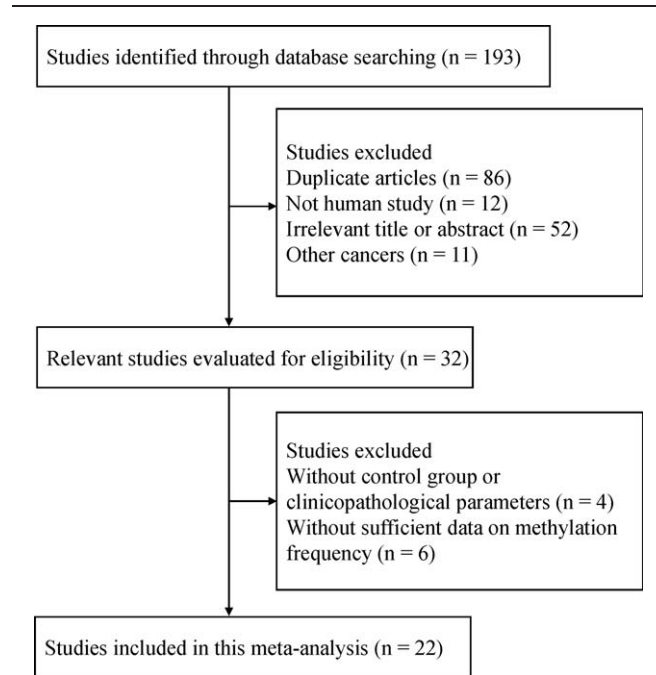

Figure 1. Flow diagram of the literature search strategy.

studies comprising 235 early GC patients and 468 advanced GC patients investigated the association between DAPK promoter methylation and tumor stage.[24,27–29,31,32,36,38,44] Twelve studies involving 607 lymph node-positive patients and 301 lymph node-negative patients investigated the association between DAPK promoter methylation and lymph node status.[24,25,27,29,32,36–38,40–42,44] Nine studies involving 438 intestinal gastric cancer patients and 356 nonintestinal gastric cancer patients investigated the association between DAPK promoter methylation and tumor histology.[25,27,28,31,32,37,38,42,44] The basic characteristics of included studies were presented in Table 1.

3.2. Pooled OR of DAPK promoter methylation in GC and noncancerous samples

A substantial heterogeneity was found in GC and noncancerous samples ($I^2 = 88.6\%$), the random-effects model was conducted. The result demonstrated that GC had significantly higher level of DAPK promoter methylation than nonmalignant samples (OR = 3.23, 95% CI = 1.70–6.14, $P < 0.001$, Fig. 2), which suggested that DAPK promoter methylation may play a key role in the carcinogenesis of GC.

3.3. Subgroup analyses

In addition, subgroup analyses were analyzed to identify the influence of DAPK promoter methylation on the risk of GC based on ethnicity (Asians and Caucasians) and sample type (tissue and blood) (Table 2). Subgroup analysis of ethnicity revealed that DAPK promoter methylation had a significant correlation with the risk of GC in Asian population (OR = 3.25, 95% CI = 1.51–7.01, $P = 0.003$), but not in Caucasian population (OR = 3.06, 95% CI = 0.96–9.70, $P = 0.058$).

Subgroup analysis of sample type showed that there was a statistical association between DAPK promoter methylation and increased GC risk among tissue samples (OR = 3.01, 95%

Table 1

The baseline characteristics of eligible studies.

First author	Country	Ethnicity	Method	Sample	Cancer N (M %)	Control N (M %)	Male M+/N	Female M+/N	Stage 0–II M+/N	Stage III–IV M+/N	Node + M+/N	Node− M+/N	Intestinal M+/N	Nonintestinal M+/N	DAPK expression
Kang et al [45]	Korea	Asians	MSP	Tissue	64 (35.8)	179 (31.3)									Not done
Lee et al [44]	China	Asians	MSP	Blood	54 (48.1)	30 (0)	18/36	8/18	5/11	21/41	22/42	4/12	11/24	15/30	Not done
Lee et al [44]	China	Asians	MSP	Tissue	54 (70.4)	–	26/36	12/18	8/11	30/41	31/42	7/12	17/24	21/30	Not done
Satoh et al [43]	Japan	Asians	COBRA	Tissue	27 (14.8)	2 (0)									Negative
To et al [42]	China	Asians	MSP	Tissue	31 (71)	46 (30.4)	13/16	9/15			17/21	5/10	14/20	8/11	Not done
Waki et al [41]	Japan	Asians	MSP	Tissue	93 (43)	93 (73.1)	27/68	13/25			17/48	22/44			Positive
Sabbioni et al [39]	Italy	Caucasians	MSP	Tissue	21 (90.5)	6 (33.3)									Not done
Kim et al [40]	Korea	Asians	MSP	Tissue	39 (33.3)	39 (66.7)	9/23	4/16			7/24	6/15			Not done
Chan et al [38]	China	Asians	MSP	Tissue	107 (69.1)	23 (0)	52/75	22/32	18/33	56/74	57/76	17/31	40/57	34/50	Not done
Chang et al [37]	Japan	Asians	MSP	Tissue	106 (32.1)	–	27/80	7/26			23/63	11/43	17/62	17/44	Not done
Kato et al [36]	Japan	Asians	MSP	Tissue	81 (22.2)	43 (9.3)	15/58	3/23	5/29	13/52	15/60	3/21			Negative
Kaise et al [35]	Japan	Asians	MSP	Tissue	34 (91.2)	68 (70.6)									Not done
Ksiaa et al [34]	Tunisia	Caucasians	MSP	Tissue	68 (30.9)	53 (24.5)	12/40	9/28							Not done
Zou et al [33]	China	Caucasians	MSP	Tissue	16 (43.7)	61 (6.6)									Not done
Ferrasi et al [32]	Brazil	Caucasians	MSP	Tissue	88 (55.7)	–	33/59	16/29	15/24	34/64	40/71	9/17	22/57	10/31	Not done
Tahara et al [31]	Japan	Asians	MSP	Tissue	146 (87)	–	94/105	33/41	33/35	33/43			71/81	56/65	Not done
Tahara et al [30]	Japan	Asians	MSP	Tissue	125 (83.2)	180 (42.2)									Not done
Hu et al [29]	China	Asians	MSP	Tissue	70 (60)	100 (10)	33/53	9/17	14/31	28/39	33/47	9/23			Not done
Guerfali et al [28]	Tunisia	Caucasians	MSP	Tissue	79 (57)	62 (17.7)	25/47	20/32	6/13	24/43	23/38	11/24	23/45	22/34	Not done
Ye et al [27]	China	Asians	MSP	Tissue	62 (54.8)	412 (48.8)	22/39	12/23	11/28	23/34	23/38	11/24	17/33	17/29	Negative
Nomura et al [26]	Japan	Asians	MSP	Tissue	115 (82.6)	69 (46.4)	19/39	14/30			21/42	12/25	21/35	12/32	Not done
Noreikiene et al [25]	Lithuania	Caucasians	MSP	Tissue	69 (47.8)	42 (28.6)	19/39	9/18	9/20	19/37	17/33	11/24			Not done
Zhang et al [24]	China	Asians	MSP	Blood	57 (49.1)										Not done

"–" stands for no data; COBRA = combined bisulphite restriction analysis, M+ = the number of methylation, MSP = methylation-specific polymerase chain reaction, N = the number of methylation and unmethylation, Node+ = lymph node-positive status, Node− = lymph node-negative status.
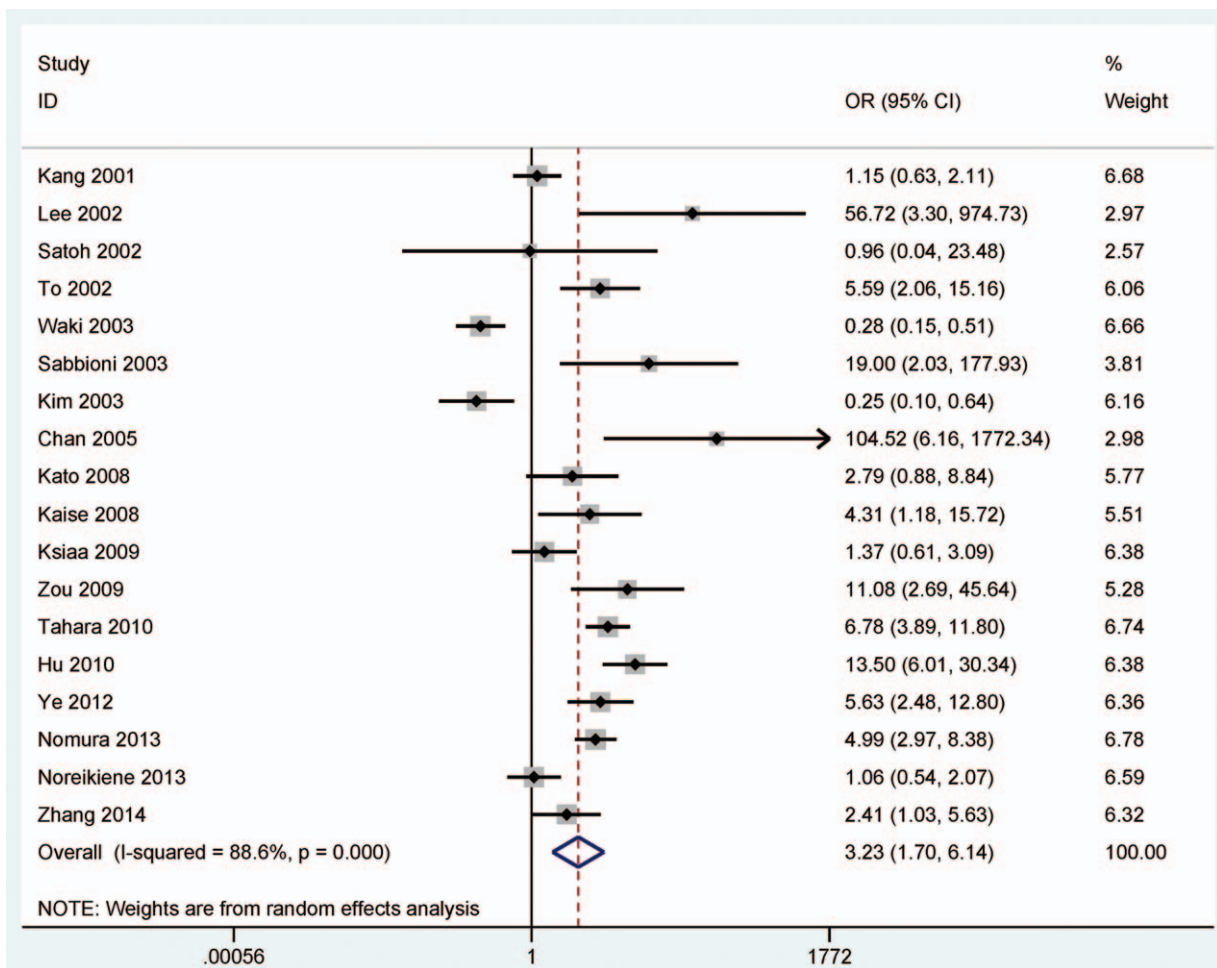
Figure 2. Forest plot of *DAPK* promoter methylation in GC versus nonmalignant controls. *DAPK* = death-associated protein kinase, GC = gastric cancer.

CI = 1.52–5.99, P = 0.002), but not in blood samples (OR = 9.20, 95% CI = 0.30–285.35, P = 0.205). However, the result should be careful as only small subjects in blood and Caucasian population subgroup.

3.4. Association between DAPK promoter methylation and gender status in GC

As shown in Table 3, the pooled OR showed that *DAPK* promoter methylation had a similar OR in male and female under

a fixed-effects model ($I^2 = 0.0\%$, OR = 1.16, 95% CI = 0.89–1.51, P = 0.267), which suggested that *DAPK* promoter methylation was not associated with gender status in GC.

3.5. Association between DAPK promoter methylation and tumor stage

As shown in Fig. 3, the pooled OR revealed that *DAPK* promoter methylation was significantly lower in patients with early gastric cancer than in patients with advanced gastric cancer under a

Table 2

Subgroup analyses in gastric cancer versus nonmalignant controls.

	Studies	Overall OR (95 CI %)	I^2	P	Cancers	Controls	Egger's test (P value)
Total	18	3.23 (1.70–6.14)	88.60%	<0.001	1133	1508	0.319
Subgroup							
Race							
Asians	14	3.25 (1.51–7.01)	90.20%	0.003	959	1319	
Caucasians	4	3.06 (0.96–9.70)	77.60%	0.058	174	189	
Sample							
Tissue	16	3.01 (1.52–5.99)	89.60%	0.002	1022	1436	
Blood	2	9.20 (0.30–285.35)	81.80%	0.205	111	72	

CI = confidence interval, OR = odds ratio.

Table 3

The association of *DAPK* promoter methylation and clinicopathological features.

	Studies	GC patients		Overall OR (95 CI %)	I^2	P	Egger's test - P
Gender	15	Male 813	Female 391	1.16 (0.89–1.51)	0.00%	0.267	0.004
Stage	9	Early stage 235	Advanced stage 468	0.69 (0.49–0.96)	39.40%	0.03	0.076
Node	12	Node + 607	Node − 301	1.50 (1.12–2.01)	16.80%	0.007	0.308
Histology	9	Intestinal 438	Nonintestinal 356	0.97 (0.72–1.32)	0.00%	0.863	0.792

Advanced stage = Stage III–IV, CI = confidence interval, early stage = stage 0–II, ode− = lymph node-negative status, Node+ = lymph node-positive status, OR = odds ratio.

fixed-effects model (I^2 = 39.4%, OR = 0.69, 95% CI = 0.49–0.96, P = 0.03), which indicated that *DAPK* promoter methylation may have an increased risk in patients with advanced gastric cancer.

3.6. Association between DAPK promoter methylation and lymph node status

As shown in Fig. 4, the overall OR revealed that *DAPK* promoter methylation was significantly higher in lymph node-positive patients with gastric cancer than in lymph node-negative patients with gastric cancer under a fixed-effects model (I^2 = 16.8%, OR = 1.50, 95% CI = 1.12–2.01, P = 0.007), which indicated that

DAPK promoter methylation was significantly increased risk of GC in lymph node-positive patients.

3.7. Association between DAPK promoter methylation and tumor histology

As shown in Table 3, the overall OR showed that *DAPK* promoter methylation had a similar OR in intestinal gastric cancer and nonintestinal gastric cancer under a fixed-effects model (I^2 = 0.0%, OR = 0.97, 95% CI = 0.72–1.32, P = 0.863), which suggested that *DAPK* promoter methylation was not correlated with tumor histology.
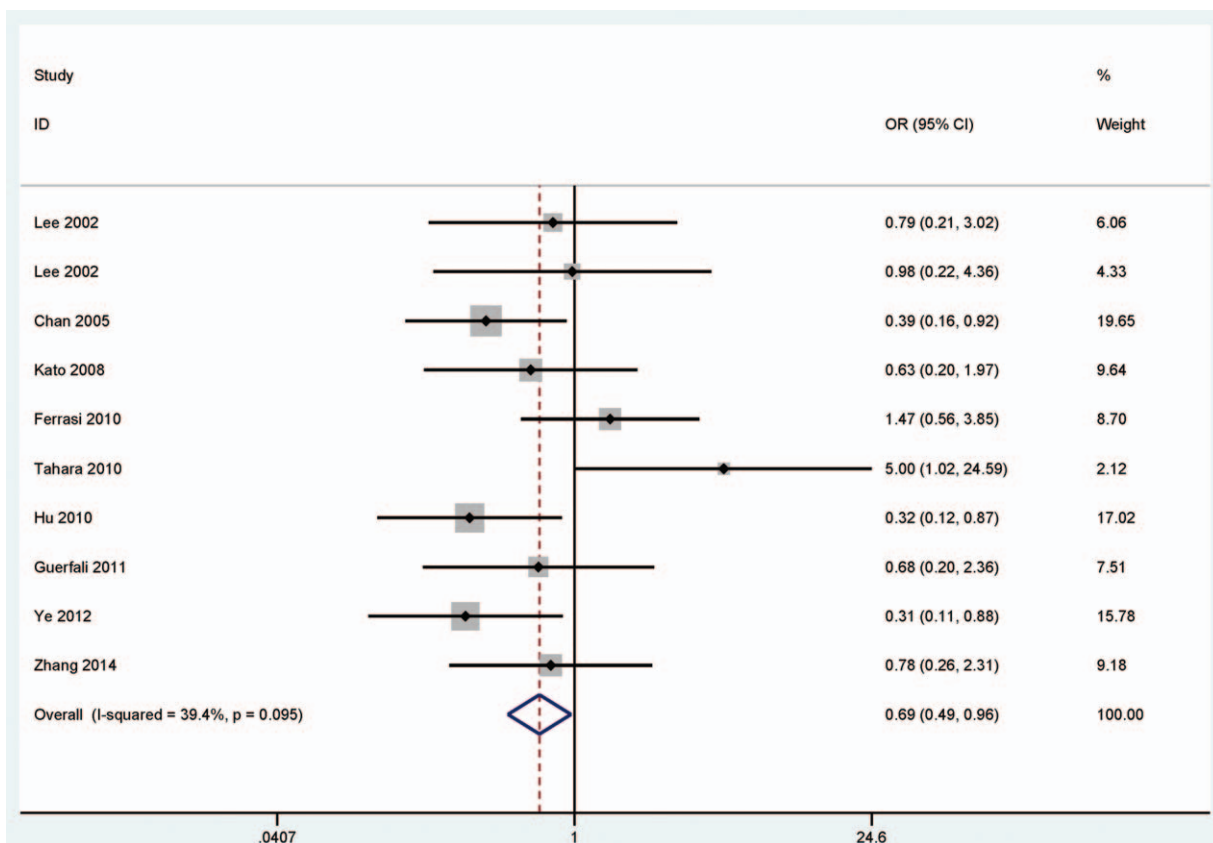

Figure 3. Forest plot of the association between *DAPK* promoter methylation and tumor stage. *DAPK* = death-associated protein kinase.
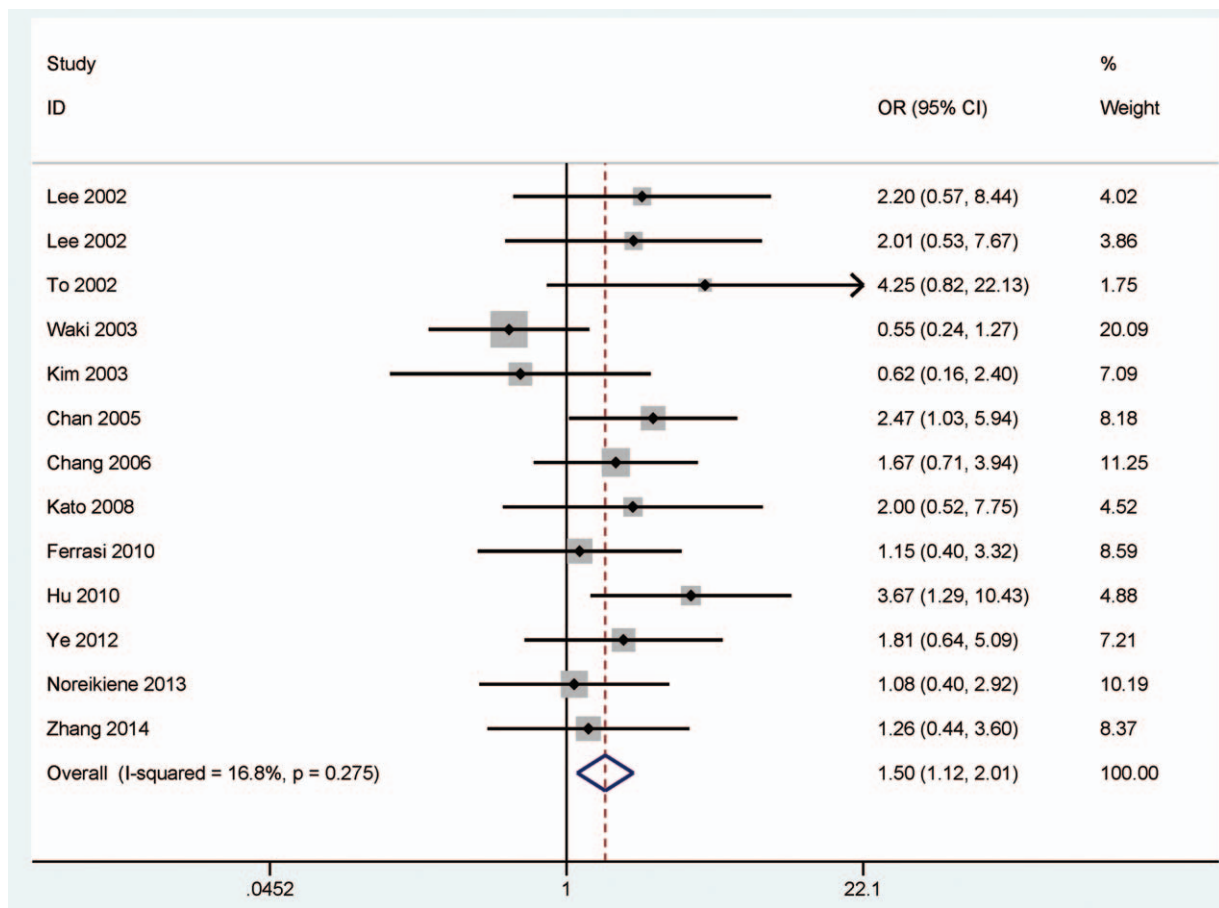
Figure 4. Forest plot of the association between *DAPK* promoter methylation and lymph node status. *DAPK* = death-associated protein kinase.

3.8. Publication bias

Egger's test was conducted to investigate the publication bias in the present analysis (Table 3). The results showed that no evidence of publication bias was observed in GC versus noncancerous controls, in relation to tumor stage, lymph node status, and tumor histology in cancer (all $P > 0.05$), and Egger's test indicated an obvious evidence of publication bias in relation to gender status in cancer ($P = 0.004$).

4. Discussion

The silencing or downregulation of many tumor-related genes by aberrant promoter methylation are implicated in the occurrence and development of cancers including GC.[14,46] In this study, aberrant methylation of tumor suppressor gene *DAPK* was the CpG islands of the promoter region for all eligible studies. However, the results of *DAPK* promoter methylation were inconsistent and controversial in GC and nonmalignant tissues. Two studies reported that the frequency of *DAPK* promoter methylation was significantly lower in GC than in nonmalignant tissues.[40,41] Three studies reported that *DAPK* promoter methylation had a similar level in GC and in nonmalignant tissues.[25,34,45] The remaining other studies reported that *DAPK* promoter methylation had a relatively higher frequency in GC than in nonmalignant samples.[24,26,27,29,30,33,35,36,38,39,42–44] Thus, we first determined whether *DAPK* promoter methylation was significantly correlated with the risk of GC. Next, we

determined whether *DAPK* promoter methylation was associated with clinicopathological features.

Our findings revealed that the *DAPK* promoter methylation rate was significantly higher in GC than nonmalignant samples, which suggested that *DAPK* inactivation via promoter methylation plays an important in the tumorgenesis of GC. No obvious of publication bias was observed, which indicated that our result was reliable. Moreover, we conducted subgroup analyses to find the different association between *DAPK* promoter methylation and subgroups. Subgroup analysis based on ethnic population indicated that Asian population was susceptible to *DAPK* promoter methylation. Subgroup analysis based on sample type indicated that *DAPK* promoter methylation was significantly associated with the risk of GC in tissue subgroup, but not in blood subgroup, indicating that the use of *DAPK* promoter methylation as a noninvasive biomarker based on blood samples could not distinguish gastric cancer and nonmalignant samples. However, the result of blood and Caucasian population subgroup should be cautious because of small sample sizes.

To evaluate the clinical significance of *DAPK* promoter methylation in GC, we further determined whether *DAPK* promoter methylation was correlated with clinicopathological characteristics, including sex status, tumor stage, tumor histology, and lymph node status. Our results showed that *DAPK* promoter methylation was not correlated with gender status and tumor histology. However, we find that *DAPK* promoter methylation had a significant higher OR in lymph node-positive

patients than in lymph node-negative patients. In addition, *DAPK* promoter methylation had a significant higher OR in advanced GC than in early GC. Our findings suggested that *DAPK* promoter methylation may involve in the occurrence and progression of GC. Egger's test showed that there was not significant publication bias in relation to tumor stage and lymph node status.

Compared with the previous meta-analysis of *DAPK* methylation status,[47] our study had some differences. First, our study analyzed nonmalignant samples as the control group, including benign lesions, adjacent tissues, or normal subjects. The previous study did not involve in benign lesions (chronic gastritis and gastric adenoma). Therefore, our study suggests that *DAPK* promoter methylation plays an important role in gastric cancer initiation. Second, our study provided more studies and samples on Caucasian population (our study: 4 studies with 363 samples; the previous study: 3 studies with 273 samples). Third, our study provided more samples in relation to tumor stage (our study: 703 samples; the previous study: 567 samples), in relation to lymph node status (our study: 908 samples; the previous study: 682 samples). Thus, our result suggests that *DAPK* promoter methylation plays an important role in gastric cancer progression. Fourth, our study suggested that no significant association was found in blood subgroup with 2 studies, indicating that the use of *DAPK* promoter methylation as a biomarker based on blood samples could not distinguish GC and nonmalignant samples. The previous study only analyzed the correlation in GC versus normal blood samples. Finally, our study analyzed whether *DAPK* promoter methylation was correlated with gender status and histological type, but the previous study did not evaluated them. In addition, our study included the expression level of *DAPK* to be more informative for clinical diagnosis from *DAPK* promoter methylation to *DAPK* expression to gastric cancer incidence.

Our study had several limitations. First, although we try to search PubMed, Embase, EBSCO, and Web of Science databases as fully as possible, publication bias was found in relation to gender status in GC. Papers with other styles, such as published papers in Chinese and other languages, were excluded due to unreadable content, and unpublished studies and conference abstracts were excluded due to insufficient data. Second, only 2 studies of blood sample were included in the present study, additional studies with larger sample sizes are essential to evaluate whether *DAPK* promoter methylation can become a noninvasive biomarker for GC diagnosis in the future.

In conclusion, our findings indicated that *DAPK* promoter methylation was significantly correlated with increased risk of GC, particularly in Asian subgroup and tissue subgroup. In addition, *DAPK* promoter methylation was also associated with tumor stage and lymph node status. We did not find that *DAPK* promoter methylation was associated with gender status and tumor histology. Further large-scale studies are necessary to provide more insight into the role of *DAPK* promoter methylation in GC patients.

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