

Original Article

Clinical significance of *DAPK* promoter hypermethylation in gastric cancer: a meta-analysis

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Abstract: Death-associated protein kinase (*DAPK*) gene promoter methylation was reported to be associated with gastric cancer (GC) in previous studies. However, the results remained inconsistent. We conducted a systematic literature search of the Embase, PubMed, Cochrane Library, Web of Science, and Chinese Biomedical Database for the relevant articles (up to October 2015). Pooled odds ratios (ORs) and 95% confidence intervals (95% CIs) were used to assess the strength of the association between *DAPK* methylation and GC risk. A total of 24 studies, comprising 3,250 samples, were analyzed in this meta-analysis. Our study revealed that *DAPK* promoter methylation levels were significantly different in the following comparisons: GC tissues vs. adjacent tissues (OR = 3.29, 95% CI = 1.48-7.33, $P = 0.004$), GC tissues vs. normal tissues (OR = 19.91, 95% CI = 11.77-33.69, $P < 0.001$), GC blood samples vs. normal blood samples (OR = 25.11, 95% CI = 3.48-181.36, $P = 0.001$), adjacent tissues vs. normal tissues (OR = 5.67, 95% CI = 3.95-8.12, $P < 0.001$), GC tissues vs. intestinal metaplasia (IM) tissues (OR = 3.29, 95% CI = 1.22-8.86, $P = 0.019$), IM tissues vs. normal tissues (OR = 8.01, 95% CI = 1.34-47.78, $P = 0.022$). In addition, the relationships between *DAPK* promoter methylation and TNM stage, differentiation status and nodal metastasis among GC cases were also identified. Our meta-analysis identified a strong association of *DAPK* promoter hypermethylation with GC risk, provided the evidence that *DAPK* promoter methylation might contribute to tumorigenesis, progression in GC, and might be a promising potential biomarker.

Keywords: Stomach neoplasm, *DAPK*, methylation, meta-analysis

Introduction

Gastric cancer (GC), the second most common cause of cancer death worldwide, remains to be a major health issue in the world, due to its late diagnosis, poor prognosis and inefficient therapy [1, 2]. To date, the etiology and pathophysiology of GC are not fully understood and its early diagnosis is also a great challenge. In the development and progression of GC, multiple factors were documented to play an important role, including inactivation of tumor suppressor genes, and environmental exposures, such as *Helicobacter pylori* infection, smoking [3-5].

Recently, DNA methylation, a common epigenetic phenomenon, was discovered to be linked

to tumor suppressor genes inactivation in GC [3]. Aberrant methylation of CpG islands within the promoter regions of some genes was indicated to occur in the early stages of cancer and had been detected in tumor tissues, corresponding serum and precancerous lesions, such as intestinal metaplasia (IM) tissues in stomach [6], which was thought to be likely to provide novel clues to the research of GC.

Death-associated protein kinase (*DAPK*), a serine/threonine kinase, is involved in apoptosis induced by interferon (IFN)- γ , tumor necrosis factor (TNF)- α and Fas ligand, autophagy and inflammation [7]. The association between *DAPK* promoter methylation and GC has been explored. Several studies suggested that *DAPK* promoter methylation was closely correlated

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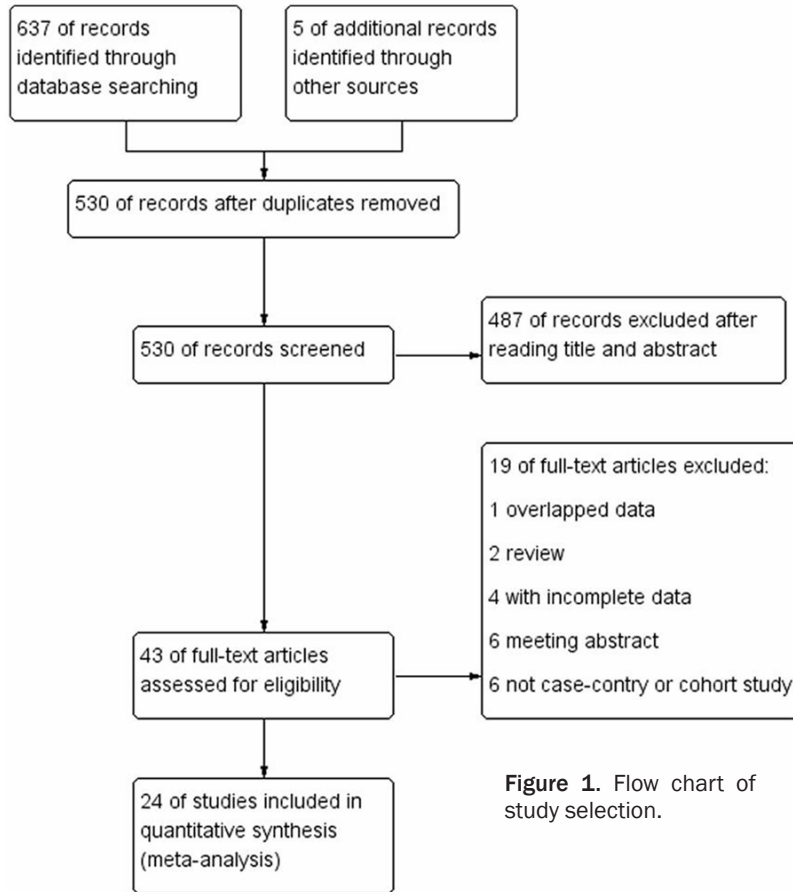


Figure 1. Flow chart of study selection.

with GC, and might serve as a reliable predictor for the development and progression of GC [8-10]. However, the findings were inconsistent [9, 11, 12]. In 2012, Sapari NS et al. reported that *DAPK* promoter methylation frequencies were not significantly different between GC tissues and adjacent normal tissues in their meta-analysis [11]. Therefore, it is necessary to explore the association between *DAPK* promoter methylation and GC risk with more evidence.

Materials and methods

Search strategy

This meta-analysis was performed according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [13]. A computerized literature search was conducted in Embase, PubMed, Cochrane Library, Web of Science, Chinese Biomedical Database (the latest research was retrospective to October 2015) to collect the articles

about the association between *DAPK* promoter methylation and gastric cancer risk. The following search terms were used: “death-associated protein kinase”, “*DAPK*”, “DNA methylation”, “methylation”, “stomach neoplasm”, “gastric neoplasm”, “stomach cancer”, “cancer of stomach” and “gastric cancer”. Meanwhile, reference lists of relevant articles were also collected.

Inclusion and exclusion criteria

Inclusion criteria: (1) The study design must be clinical cohort or case-control study; (2) Raw data available to evaluate the association of *DAPK* methylation with GC risk, or the relevant information could be calculated; (3) All patients diagnosed with GC must be confirmed through

histopathologic examinations; (4) Articles were published in English and Chinese.

Exclusion criteria: (1) Raw data not available for retrieval; (2) Multiple articles based on the same population and published by the same research team, only the latest and/or the largest population study was adopted, others would be excluded; (3) Meeting abstract, case reports, letters, editorials, animal studies, review articles and other meta-analysis were excluded.

Data extraction and quality assessment

Two reviewers (Jianing Xu and Zhihao Liu) independently extracted data, cross-checked, discussed all conflicts, and reached a consensus on all items. The following characteristics were extracted from each study: the first author’s name, years of publication, study location, design of study, age, gender, testing methods for methylation analysis, the frequencies of *DAPK* promoter methylation, and sample types in each study.

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Table 1. General characteristics of the included studies

First author	Year	Study location	Design	Method	Case age Mean/median/ range (years)	Case gender (Male/Female)	Sample type					NOS score
							Tumor (M ⁺ /N)	Adjacent (M ⁺ /N)	Normal (M ⁺ /N)	Blood (M ⁺ /N)	IM (M ⁺ /N)	
To KF [27]	2002	China	case-control	MSP	NA	NA	22/31	NA	0/10	NA	14/36	6
Lee TL [23]	2002	China	case-control	MSP	62.6	36/18	38/54	NA	NA	26/84	NA	7
Sabbioni S [24]	2003	Italy	cohort	MSP	NA	NA	19/21	NA	2/6	7/16	NA	7
Kim WS [21]	2003	Korea	case-control	MSP	54.7	23/16	12/39	26/39	NA	NA	NA	7
Waki T [12]	2003	Japan	case-control	MSP	66 (43-89)	68/25	40/93	68/93	NA	NA	NA	8
Kang GH [19]	2003	Korea	case-control	MSP	NA	NA	45/80	NA	NA	NA	28/57	7
Chan AW [16]	2005	China	cohort	MSP	62	75/32	74/107	NA	0/23	NA	NA	8
Schildhaus HU [25]	2005	Germany	case-control	MSP	NA	7/0	6/7	2/7	NA	NA	NA	6
Yang SH [34]	2007	China	case-control	MSP	57 (36-80)	26/12	31/38	16/38	NA	NA	NA	6
Zhang B [35]	2008	China	case-control	MSP	NA	NA	6/16	NA	0/20	NA	1/14	6
Kato K [20]	2008	Japan	cohort	MSP	NA	58/23	18/81	4/43	NA	NA	NA	7
Kaise M [18]	2008	Japan	case-control	MSP	54.7	NA	NA	31/34	48/68	NA	NA	7
Ksiaa F [22]	2009	Tunisian	case-control	MSP	61.1	40/28	21/68	13/53	NA	NA	NA	7
Kong XY [30]	2009	China	case-control	MSP	62.38 (40-86)	50/16	44/66	7/66	0/20	11/86	NA	7
Zou XP [28]	2009	China	case-control	MSP	NA	NA	7/16	NA	0/20	NA	1/14	6
Jiang XD [29]	2009	China	case-control	MSP	63.0	33/8	28/41	NA	0/20	NA	NA	7
Tahara T [26]	2009	Japan	case-control	MSP	64.4	NA	NA	104/125	76/180	NA	NA	7
Hu SL [17]	2010	China	case-control	MSP	62.11	53/17	42/70	10/70	0/30	NA	NA	8
Shen JJ [33]	2010	China	case-control	MSP	59.5	41/13	49/54	22/54	NA	NA	NA	7
Lin H [31]	2011	China	case-control	MSP	62.5	24/14	20/38	1/20	NA	NA	NA	6
Ye M [8]	2012	China	case-control	MSP	59.8 (33-81)	37/25	34/62	11/62	NA	NA	NA	8
Kupčinskaitė-Noreikienė R [9]	2013	Lithuania	case-control	MSP	64.5	39/30	33/69	32/69	NA	NA	NA	8
Nomura T [10]	2013	Japan	case-control	MSP	66.2	84/31	104/115	95/115	201/412	NA	NA	6
Liu Y [32]	2014	China	case-control	MSP	35-80	28/12	33/40	17/40	NA	NA	NA	7

MSP: methylation specific polymerase chain reaction; M⁺: the number of methylation; N: number of total; Adjacent: normal or non-tumor tissues adjacent to tumor; Normal: normal gastric tissues from healthy people; IM: intestinal metaplasia; NA denotes not applicable.

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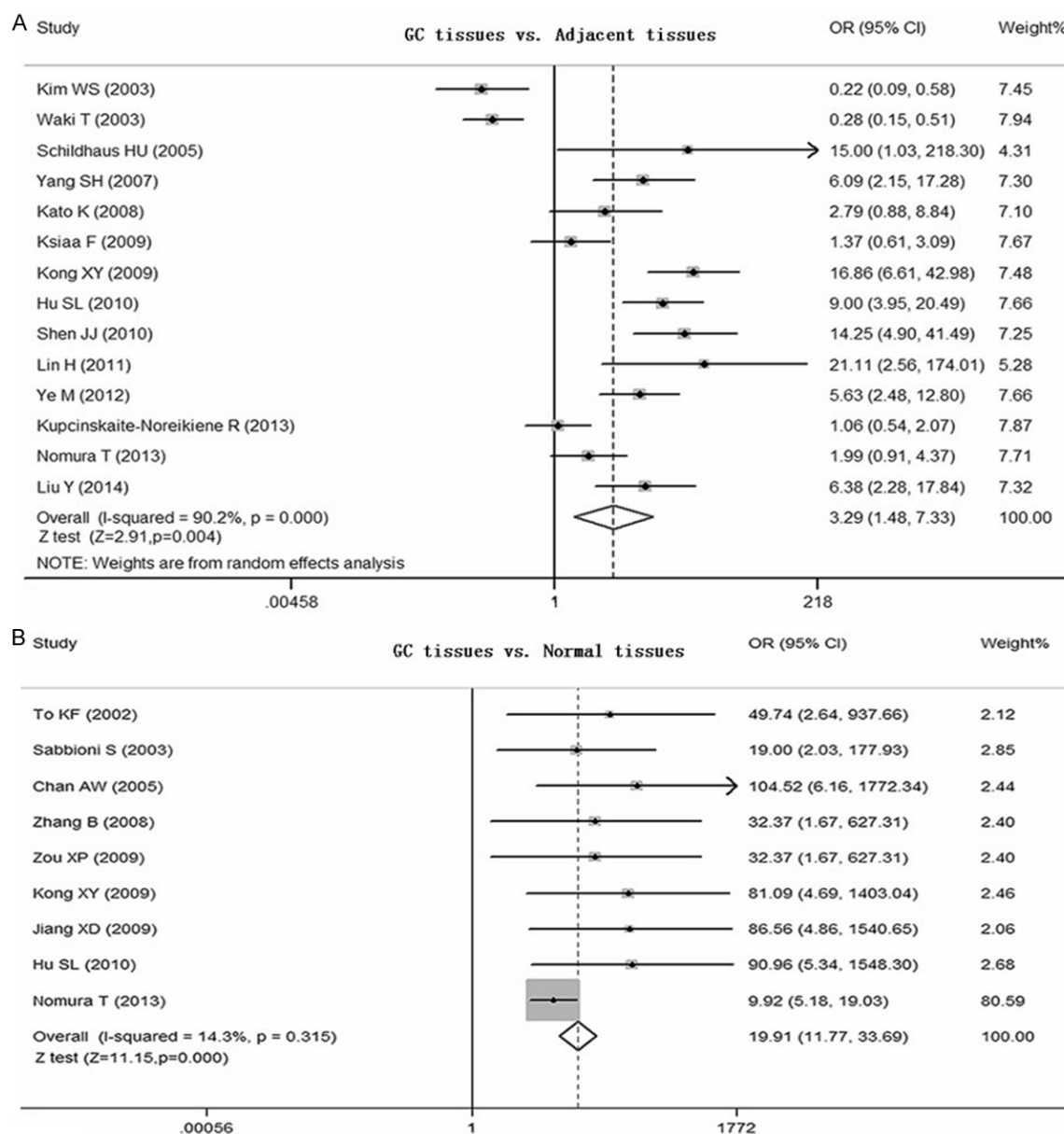


Figure 2. Forest plots of this meta-analysis. A. GC tissues vs. Adjacent tissues; B. GC tissues vs. Normal tissues.

According to the Newcastle-Ottawa Scale (NOS) (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp) criteria, which is widely implemented for observational studies by the Cochrane Collaboration, the quality of the included studies was assessed independently by the same two researchers. NOS scores ranged from 0 to 9 and a score greater than or equal to 7 indicated a good quality.

Statistical analysis

The strength of the association between *DAPK* promoter methylation and risk of GC was mea-

sured by the pooled odds ratios (ORs) with its 95% confidence intervals (95% CIs). The significance of the pooled ORs was determined by the Z test. I^2 statistic was used to assess heterogeneity between study estimates. Substantial heterogeneity exists when I^2 exceeds 50%, and the Q statistic was applied to formally test for heterogeneity ($P < 0.10$ was considered representative of significant statistical heterogeneity). The random effects model was utilized to pool the ORs when heterogeneity among studies existed; otherwise, the fixed effects model was selected. In this meta-analysis, subgroup analysis and meta-regression were further per-

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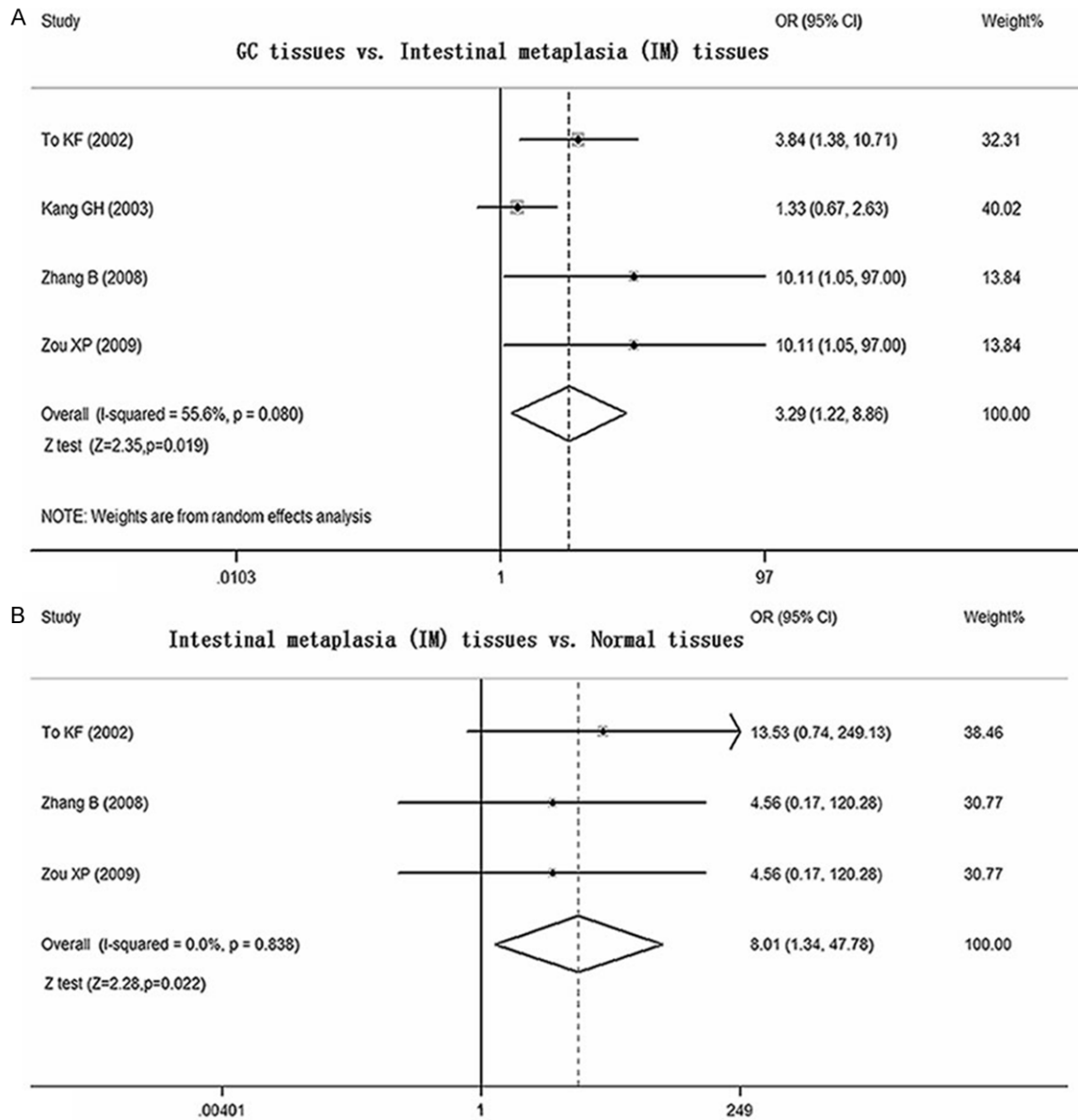


Figure 4. Forest plots of this meta-analysis. A. GC tissues vs. Intestinal metaplasia (IM) tissues; B. Intestinal metaplasia (IM) tissues vs. Normal tissues.

sues were significantly higher than those of adjacent tissues and normal tissues (adjacent: OR = 3.29, 95% CI = 1.48-7.33, $P = 0.004$, $I^2 = 90.2\%$, under the random-effects model; normal: OR = 19.91, 95% CI = 11.77-33.69, $P < 0.001$, $I^2 = 14.3\%$, under the fixed-effects model) (Figure 2). *DAPK* promoter methylation frequencies in GC blood samples were significantly higher than those of normal blood samples under the fixed-effects model (OR = 25.11, 95% CI = 3.48-181.36, $P = 0.001$, $I^2 = 0\%$). *DAPK* promoter methylation frequencies in GC adjacent tissues were significantly higher than

those of normal tissues under the fixed-effects model (OR = 5.67, 95% CI = 3.95-8.12, $P < 0.001$, $I^2 = 0\%$) (Figure 3).

Subsequently, under the random-effects model, we evidenced that *DAPK* promoter methylation frequencies were significantly higher in GC tissues than in intestinal metaplasia (IM) tissues (OR = 3.29, 95% CI = 1.22-8.86, $P = 0.019$, $I^2 = 55.6\%$) as well as in intestinal metaplasia (IM) tissues than in normal tissues under the fixed-effects model (OR = 8.01, 95% CI = 1.34-47.78, $P = 0.022$, $I^2 = 0\%$) (Figure 4).

Table 2. DAPK promoter methylation in relation to TNM stage, differentiation status and nodal metastasis among GC cases

Patient characteristics	No. of studies	Tumor (M*/N)	Odds ratio		Model of meta-analysis	Heterogeneity		P_B/P_E
			OR (95% CI)	P_z		I^2 (%)	P_H	
TNM stage	9							
III-IV		254/380	1.95 (1.32, 2.89)	$P = 0.001$	Fixed-effects model	22.1%	$P = 0.246$	0.754/0.643
I-II		94/187						
Differentiation status	8							
Poorly		186/263	2.17 (1.42, 3.31)	$P < 0.001$	Fixed-effects model	34.0%	$P = 0.157$	0.266/0.194
Moderately/well		95/173						
Nodal metastasis	11							
Present		261/459	1.58 (1.13, 2.21)	$P = 0.007$	Fixed-effects model	44.7%	$P < 0.054$	0.533/0.341
Absent		100/223						

P_z : p value of effect test; P_H : p value of heterogeneity test; P_B : P value of Begg's test; P_E : p value of Egger's test.

In addition, we also conducted an analysis of the relationships between clinicopathologic features and DAPK promoter methylation among GC cases. We found significant relationships between DAPK methylation and TNM stage, differentiation status and nodal metastasis (Table 2). However, there were no significant relationships between DAPK methylation and the following clinicopathologic features: gender, age, Helicobacter pylori (HP) infection, tumor site, tumor depth, tumor size, Lauren's typing, distant metastasis (Data not shown).

Effect of analytical variability and publication bias

During the annotation of studies, considerable heterogeneity was observed among the studies investigating the relationship of DAPK promoter methylation with GC risk between GC tissues and adjacent tissues. To explore the sources of heterogeneity, we conducted subgroup analyses by country (China, [8, 17, 30-34] Japan and Korea, [10, 12, 20, 21] and the others [9, 22, 25]), number of cases (≥ 60 [8-10, 12, 17, 20, 22, 30] and < 60 [21, 25, 31-34]), NOS score (≥ 7 [8, 9, 12, 17, 20-22, 30, 32, 33] and < 7 [10, 25, 31, 34]) and meta-regression. The frequencies of DAPK promoter methylation were significantly higher in GC tissues than in adjacent tissues in China (OR: 9.173, 95% CI: 6.325-13.303, $P < 0.001$, $I^2 = 0\%$), however, significantly lower in GC tissues than in adjacent tissues in Japan and Korea (OR: 0.632, 95% CI: 0.437, 0.914, $P = 0.015$, $I^2 = 88.5\%$), and no statistical difference was identified in the others (OR: 1.324, 95% CI: 0.805-2.178, $P = 0.268$, $I^2 = 44.3\%$). Furthermore, DAPK promoter methylation frequencies were higher in GC

tissues than in adjacent tissues in number of cases < 60 (OR: 5.213, 95% CI: 1.104-24.620, $P = 0.037$, $I^2 = 88.9\%$), and there was no statistical difference in number of cases ≥ 60 (OR: 2.515, 95% CI: 0.952, 6.648, $P = 0.063$, $I^2 = 91.6\%$). And significantly higher methylation frequencies of DAPK promoter in GC tissues than in adjacent tissues in both NOS score < 7 (OR: 4.1285.213, 95% CI: 2.352-7.247, $P < 0.001$, $I^2 = 57.3\%$), and in NOS score ≥ 7 (OR: 2.011, 95% CI: 1.596-2.533, $P < 0.001$, $I^2 = 92.6\%$) (Table 3). The results of meta-regression also indicated that the country of the patients accounted for some of the heterogeneity ($P = 0.048$). However, other factors such as year of publication, number of cases, tissue type and NOS score could not explain the heterogeneity (Table 4).

To test for publication bias, data from the 24 studies analyzed above were further examined. The funnel plots did not reveal any evidence of obvious asymmetry (Figure 5). Furthermore, the consequences of Begg's and Egger's test still did not suggest any statistical evidence of publication bias ($P > 0.05$).

Discussion

DAPK is an apoptosis-related serine/threonine kinase and the suppression of DAPK gene associated with its promoter methylation is thought to be critical in the development and progression of GC [9, 36]. In order to evaluate the exact association of DAPK promoter methylation with GC, we conducted a meta-analysis of 24 studies with a total of 3,250 samples. Our meta-analysis results revealed that DAPK promoter methylation frequencies were signifi-

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Table 3. The total and subgroup analyses for the relationship of *DAPK* promoter methylation with GC between GC tissues and adjacent tissues

	No. of studies	Tumor (M*/N)	Adjacent (M*/N)	Odds ratio		Model of meta-analysis	Heterogeneity		P_B/P_E
				OR (95% CI)	P_Z		I^2 (%)	P_H	
All studies	14	487/840	324/769	3.289 (1.477, 7.326)	$P = 0.004$	Random-effects model	90.2%	$P < 0.001$	0.155/0.060
Subgroup analyses by country									
China	7	253/368	84/350	9.173 (6.325, 13.303)	$P < 0.001$	Fixed-effects model	0%	$P = 0.505$	0.453/0.366
Japan and Korea	4	174/328	193/290	0.632 (0.437, 0.914)	$P = 0.015$	Random-effects model	88.5%	$P < 0.001$	0.497/0.502
The others	3	60/144	47/129	1.324 (0.805, 2.178)	$P = 0.268$	Fixed-effects model	44.3%	$P = 0.166$	0.117/0.047
Subgroup analyses by number of cases									
≥60	8	336/624	240/571	2.515 (0.952, 6.648)	$P = 0.063$	Random-effects model	91.6%	$P < 0.001$	0.013/0.045
<60	6	151/216	84/198	5.213 (1.104, 24.620)	$P = 0.037$	Random-effects model	88.9%	$P < 0.001$	0.573/0.428
NOS score									
≥7	10	326/481	210/589	2.011 (1.596, 2.533)	$P < 0.001$	Random-effects model	92.6%	$P < 0.001$	0.180/0.078
<7	4	161/359	114/180	4.128 (2.352, 7.247)	$P < 0.001$	Random-effects model	57.3%	$P = 0.071$	0.497/0.137

P_Z : p value of effect test; P_H : p value of heterogeneity test; P_B : p value of Begg's test; P_E : p value of Egger's test.

Table 4. Meta-regression analysis for the relationship of *DAPK* promoter methylation with GC between GC tissues and adjacent tissues

Sources	Coefficient (95% CI)	t	P
Year of publication	0.169 (-0.048, 0.386)	1.79	0.111
Tissue type	0.923 (-1.396, 3.322)	0.94	0.374
Study location	1.112 (0.015, 2.209)	-2.34	0.048
Number of cases	0.272 (-1.469, 2.014)	0.36	0.728
NOS score	0.703 (-1.162, 2.568)	0.87	0.410

Tissue type includes frozen and paraffin-embedded tumor tissue.

cantly higher in GC tissues than in normal tissues under the fixed effects model. Consistent with our study, Hu SL et al. [17] reported that *DAPK* promoter methylation frequencies in tumor tissues were 60.0% (42 of 70) and in 30 histologically normal gastric tissues were 0%, suggesting that *DAPK* promoter hypermethylation may play a key role in the development of GC.

Through random effects model, our meta-analysis showed that *DAPK* promoter methylation frequencies in GC tissues were higher than those of adjacent tissues, which was also documented in previous studies [8, 22]. Due to the high heterogeneity, we performed specific subgroup analyses [country (China, Japan and Korea, and the others), number of cases (≥ 60 and < 60), NOS score (≥ 7 and < 7)] and meta-regression. Notably, we discovered that the country contributed to the high heterogeneity, and it might be a confounding factor for the association of *DAPK* methylation and GC. However, several studies reported that *DAPK* methylation frequencies were lower in GC tissues than corresponding adjacent tissues, [12, 21] and the same result was shown in the Japan-Korea subgroup in our meta-analysis (OR: 0.632, 95% CI: 0.437-0.914, $P = 0.015$, $I^2 = 88.5\%$). Nevertheless, the results of the Japan-Korea subgroup in our meta-analysis should be explained with particular caution due to high heterogeneity. Besides, the adjacent tissues in above studies [12, 21] were adjacent non-tumor tissues but not normal stomach tissues, thus early pre-malignant events in these non-tumor tissues might exist, which might increase *DAPK* methylation frequencies, [28, 37] and other factors such as age, sample size might also influence the final result. In addition, a published meta-analysis [11] showed that there was no significant correlation of *DAPK* promoter methylation with GC between tumor

and adjacent normal tissues from GC subjects [OR (95% CI): 1.37 (0.21, 9.04)]. The conflicting results might result from the small number of studies analyzed; the previous meta-analysis just covered the articles published in pubmed before October 27, 2011.

In some studies, aberrant promoter methylation in blood samples was detected in a host of GC subjects [23, 30]. Consistently, the present meta-analysis revealed that *DAPK* promoter methylation frequencies in blood samples were higher from GC subjects than those of non-cancer subjects, suggesting that there were significant concordance of the *DAPK* methylation changes between tumors and blood samples. DNA methylation in blood is clinically promising and may serve as a new tool for screening and surveillance of GC. However, the exact mechanism how tumor DNA gets into blood is not clear, additional studies are necessary to be performed in the future. Furthermore, aberrant *DAPK* methylation has also been detected in precancerous gastric lesions such as intestinal metaplasia (IM), [27, 28] and tends to accumulate along the multistep pathway of GC, [19] showing an increasing tendency. In our meta-analysis, *DAPK* promoter methylation frequencies were significantly higher in GC tissues compared with IM tissues as well as in IM tissues compared with normal tissues, which were consistent with the previous studies. The increasing tendency of *DAPK* methylation levels from normal tissues to GC suggests that *DAPK* methylation appears to be an early event in GC multistep progression and may be used in cancer early detection and risk prediction. In addition, through the analysis of the relationships between clinicopathologic features and *DAPK* methylation among GC cases, significant relationships of *DAPK* promoter methylation with TNM stage, differentiation status and nodal metastasis were documented in our meta-analysis. Consistently, increased methylation of *DAPK* in GC patients with moderate/well differentiation, or present nodal metastases, or in stage III-IV was also identified in previous studies, [8, 17] revealing that *DAPK* promoter methylation may be involved in the progression of GC. In short, our findings revealed that *DAPK* promoter methylation was closely correlated with GC, and could be a potential biomarker for early diagnosis, risk prediction, prognosis assessment of GC.

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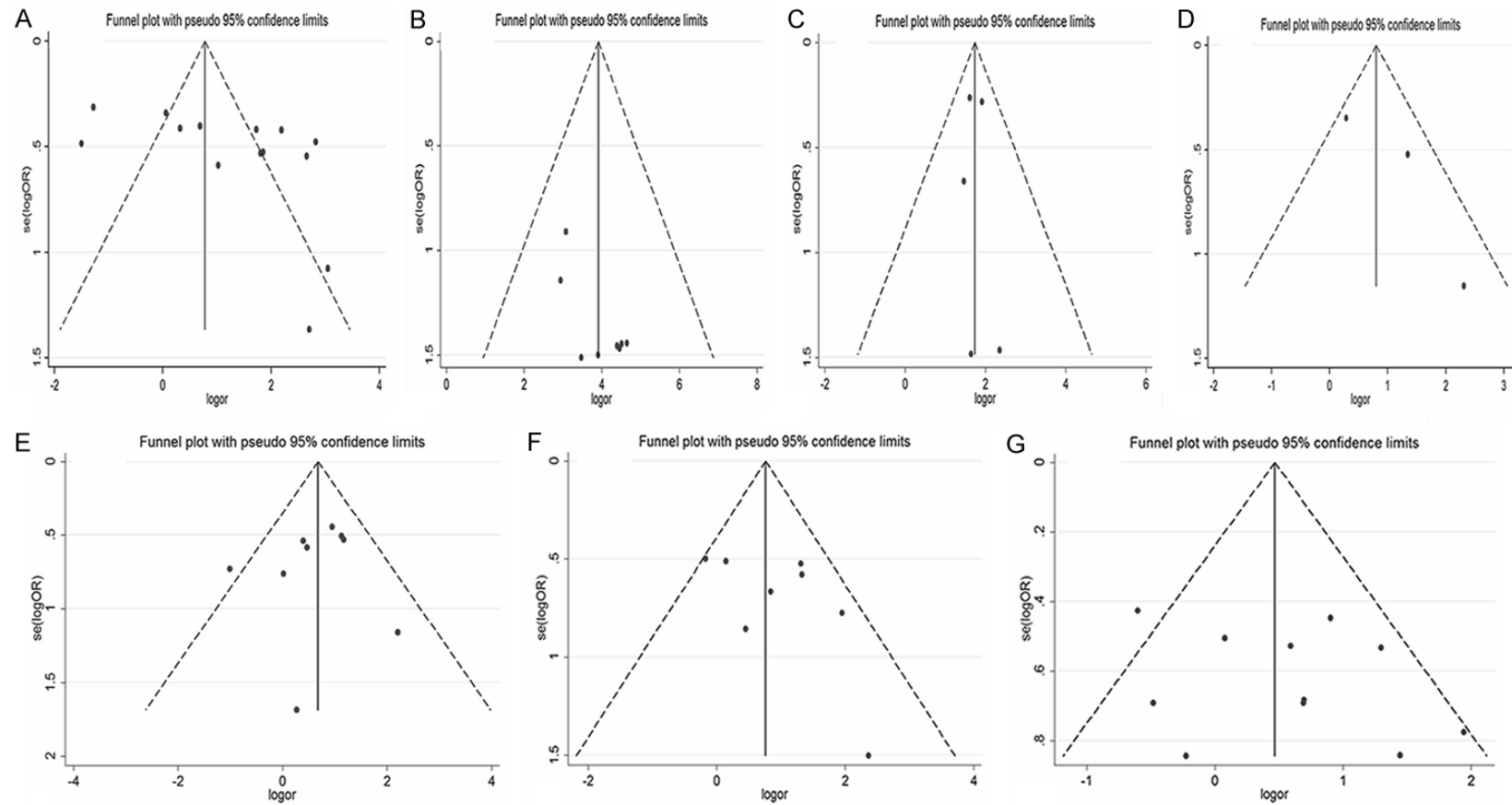


Figure 5. Funnel plots of this meta-analysis. A. GC tissues vs. Adjacent tissues; B. GC tissues vs. Normal tissues; C. Adjacent tissues vs. Normal tissues; D. GC tissues vs. Intestinal metaplasia (IM) tissues; E. TNM stage: III-IV vs. I-II; F. Differentiation status: Poorly vs. Moderately/Well; G. Nodal metastasis: Present vs. Absent.

Additionally, the relationships between *DAPK* methylation status and survival outcome of GC subjects had also been observed. However, meta-analyses could not be performed on this series of studies due to the limited number of studies and the irregular reporting of hazard ratios. Thus, we summarized their data as follows. A total of 3 studies [12, 16, 20] investigated the association of *DAPK* methylation status with survival outcome of GC patients with conflicting results. Chan AW et al. reported that *DAPK* hypermethylation was correlated with poorer event-free survival (event refers to development of recurrence, metastasis or death of disease, log-rank test $P = 0.0141$) [16]. Kato K et al. also showed that shorter overall survival was observed in GC patients with methylation of *DAPK* ($P = 0.0454$). And among the patients who received chemotherapy, time to progression was significantly shorter in the patients with *DAPK* methylation compared with the patients without methylation ($P = 0.0464$) [20]. These results suggested that the subjects with *DAPK* methylation had a worse survival than those without methylation, and *DAPK* methylation might response to chemotherapy. However, Waki T et al. reported that no significant influence was found between *DAPK* methylation status and event-free survival rates [12]. The inconsistent results may be due to several factors, such as the influence of follow-up period, sample size and other confounding variables, and much further work is required to confirm these findings.

In this meta-analysis, a strict inclusion criterion was applied; however, there were several limitations. First, our relatively strict inclusion criteria might have introduced selection bias, although little evidence of publication bias was observed. Second, some studies were excluded due to failure to acquire complete data either from the original text or from the authors, which could have produced selective bias. Third, the analyzed CpG sites might not stand for the whole promoter region. Finally, the conclusions drawn from some analyses might be limited due to a low statistic power from the small sample size.

In conclusion, our study identified a strong association between *DAPK* promoter hypermethylation and GC risk, and provided the evidence that *DAPK* promoter methylation might contribute to tumorigenesis and progression in GC, and may be a promising potential biomark-

er for early diagnosis, risk prediction, prognosis assessment and chemotherapy choice for GC. More large-scale and well-designed studies are needed in future.

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Disclosure of conflict of interest

None.

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