

META-ANALYSIS: ASSOCIATION BETWEEN PROMOTER HYPERMETHYLATION OF DAPK (DEATH-ASSOCIATED PROTEIN KINASE) AND CERVICAL CANCER

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ABSTRACT

Purpose: Death-associated protein kinase (DAPK or DAPK1) is an important tumor suppressor protein that involved in the regulation of cell activities. The aberrant methylation of *DAPK* promoter has been reported in patients with cervical cancer. However, the association between of DAPK1 and cervical cancer was not always unification, in previous studies. Therefore, in current study, a meta-analysis was performed for association of between *DAPK* gene's promoter hypermethylated and cervical cancer.

Methods: A systematic literature analysis was conducted based on the previous studies published in PubMed, PubMed Central (NCBI), Google by using following keywords: cervical cancer, cervical carcinoma, Methylation, by the end of January, 2018. The association between *DAPK* promoter methylation and cervical cancer was evaluated by odds ratio (ORs) and 95% confidence intervals (CI). To evaluate the potential sources of heterogeneity, the meta-regression analysis and subgroup analysis were conducted.

Results: A total of 21 case-control studies which relevant to the association between *DAPK1* gene's promoter methylation frequency and cervical cancer, that including 1600 cancer cases and 1011 control cases (non-cancerous cases). The analysis results indicated that the characteristic of candidate gene's promoter methylation increased the cervical cancer risk through the calculation of OR value (OR = 21.25; 95% CI = 8.73 – 52.97; $p < 0.001$; Random effect model). The association between *DAPK1* gene's promoter hypermethylation was confirmed in all the subgroups analyses, including materials and assays methods, ethnicity. Furthermore, this association is higher in cervical squamous cell carcinoma than cervical adenocarcinoma and is a characteristic of late-stage disease.

Conclusion: The hypermethylated *DAPK1* gene's promoter was also one of etiological factor, lead to the cervical tumorigenesis.

Keywords: Cervical cancer; DAPK1; Methylation; Meta-analysis.

1. Introduction

In addition to the infection with oncogenic human papillomavirus, which is the most significant risk factor in the etiology of cervical

cancer, the epigenetic alterations could result in heritable gene silencing without changes to genetic sequences and are recognized as the important cause of human cervical cancer

(Burd, 2003; Lu *et al.*, 2012; Yang, 2013). The epigenetic molecular mechanisms associated with human cervical cancer comprise of a variety of alterations, including the silencing of tumor suppressor genes (TSGs) by hypermethylation of CpG islands (Lu *et al.*, 2012; Yang, 2013). Many cellular pathways, including DNA repair, cell cycle, apoptosis, etc., are proven to be inactivated by the hypermethylation of CpG islands located in the promoter regions of many TSGs (Esteller, 2002; Le *et al.*, 2017). Among the involved TSGs, the *Death-associated protein kinase 1* (*DAPK1*) gene, located at 9q21.33, encodes the 160-kD calmodulin dependent serine-threonine kinase involved in multiple cellular signaling pathways that trigger cell survival, apoptosis and autophagy (Raveh, 2001; Cai *et al.*, 2017). The decreased expression of *DAPK* is associated with the methylation of gene promoter has been frequently reported in various types of human cancers, including cancer of cervix (Leung *et al.*, 2008; Niklinska *et al.*, 2009; Wang *et al.*, 2016; Cai *et al.*, 2017). However, there are significant differences in the frequency of *DAPK* promoter in patients with cervical cancer, due to different populations, sample sizes, methods for methylation analysis. Moreover, whether or not the methylation frequency of *DAPK* gene's promoter is correlated with clinicopathological characteristics, including sex, stages, and invasion remain debated. Thus, we performed the systematic review and meta-analysis to summarize the current previous studies and evaluate the relationship between the methylation status of *DAPK* promoter and cervical cancer as an epigenetic marker of cervical cancer risk.

2. Materials and Methods

Search strategy, inclusion and exclusion criteria of literature

The systematic review of relevant literature was conducted by using many keywords for the literature search: methylation, *DAPK1*, *Death-*

associated protein kinase 1, cervical cancer, etc. on four electronic databases: PUBMED, Web of Science, Embase database, updated to December, 2018. The aim of the first selection was to identify studies that relevant to establish the association between *DAPK* promoter methylation and cervical cancer. Accordingly, the studies were included if they satisfied the following inclusion criteria: (1) The investigation/ identification of *DAPK* promoter methylation, which correlated with cervical cancer; (2) The correlation between *DAPK* promoter methylation and clinicopathological features of FDB; (3) cohort design studies of *DAPK* promoter methylation and cervical cancer; (4) the identification method was not excluded. Additionally, only studies written in English were included for current study. Types of case reports, abstracts, review, and letter to editor were eliminated.

Data extraction, quality assessment and statistical analysis

Two of the Authors independently reviewed all the eligible studies. The data were abstracted the following information in a standard format, including the name of first author, year of publication, region of study subjects, age of patients, source/type of samples, detection method, clinical features of cervical cancer, sample size of case and control groups. Finally, the third reviewer independently reviewed the relevant extract data from the eligible studies.

In current study, all data were analyzed using Medcalc®2018. The frequency of *DAPK* promoter methylation was observed in both case and control study. The strength of the association between *DAPK* promoter methylation and cervical cancer was evaluated by odds ratio (OR) and risk relative (RR) with 95% confidence intervals (95% CIs). The degree of association between *DAPK* promoter methylation and clinical features was also evaluated by OR and RR with 95% CIs. Heterogeneity across studies was measured

using the Cochran's Q-test (Higgins, Thompson, 2002). The random-effects model was employed when the p value was less than 0.05 in the Q-test, indicating the presence of substantial heterogeneity. The Cochran's Q-test only indicates the presence of heterogeneity, thus, we also reported I^2 statistic, which estimated the percentage of outcome variability that can be attributed to heterogeneity across studies (Higgins, Thompson, 2002). Furthermore, subgroups analyses were conducted by histological stage (Squamous cell carcinoma - SCC, Adenocarcinoma - AC), source/type of samples (Biopsy, scrape, and urine), detection methods (Methylation Specific PCR - MSP, Realtime quantitative MSP - QMSP), ethnicity (Asian, European, American,

and African).

3. Results

Identified study and data characteristics

A total of 158 studies were initially identified by systematic literature search. After the duplicates and non-relevant studies were excluded by considering the title and abstract of studies, 61 articles with potentially relevant studies were further investigated by examining full text. Finally, 23 studies, including 21 case-control studies and 2 cohort studies, were included in current meta-analysis after excluding studies that not met the inclusion criteria. The detail processes of the systematic review and selections are given as PRISMA flow chart, illustrated in Fig. 1.

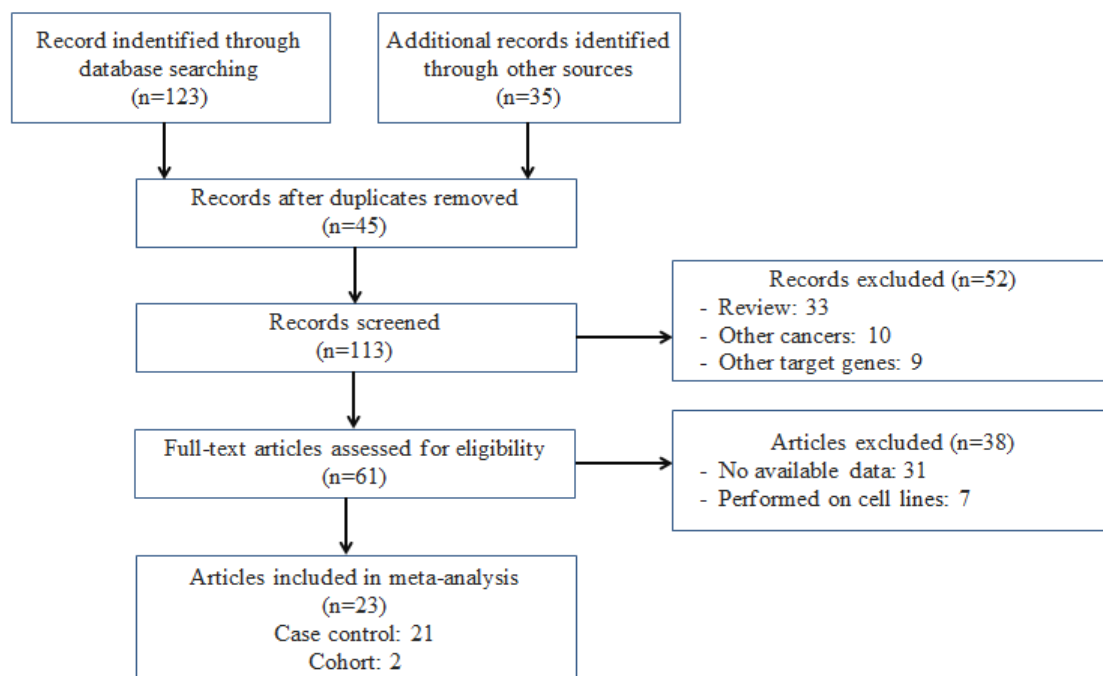


Figure 1. Flow chart of processes included in current meta-analysis

Out of the 23 studies included, 21 studies with 1600 cases and 1011 controls were combined to calculate the pooled OR between *DAPK* promoter methylation and cervical cancer. The 21 case - control studies encompassed the publication years from 2001-2017, 11 from Asian countries (counting for 52.38%), 5 from European countries

(counting for 23.81%), 3 from American countries (counting for 14.29%), and 2 from Africa (counting for 9.52%), were included in the systematic review and analysis. The methylation detection methods consisted of 15 studies of using MSP (counting for 71.43%), 5 studies of using QMSP (counting for 23.81%), and 1 study of using Realtime PCR

(counting for 4.76%), to explore *DAPK* promoter methylation in cervical cancer and corresponding to controls. The type of case samples consisted of 15 studies of using Biopsy - tumor tissues (counting for 71.43%), 5 studies of using scrape sample (counting for 23.81%), and 1 study of using urine (counting

for 4.76%). The type of controls consisted of 20 studies of using normal/benign biopsy sample (counting for 95.24%), and 1 study of using healthy urine sample (counting for 4.76%). The detail characteristic of included studies were summarized in Table 1.

Table 1

Detail characteristic of studies included in the systematic review and meta-analysis of *DAPK* promoter methylation and cervical cancer

First Author	Year	Country	Ethnicity	Method	Types of case	Cases		Types of control	Control	
						Total	Events		Total	Events
Case - control studies										
Truong	2017	Vietnamese	Asian	MSP	Scrape	61	27	NCT	48	1
Banzai	2014	Japan	Asian	MSP	Biopsy	53	40	NCT	24	1
Niyaki	2012	China	Asian	MSP	Biopsy	30	19	NCT	30	1
Sun	2012	China	Asian	MSP	Biopsy	331	181	NCT	336	157
Huang	2011	Taiwan	Asian	MSP	Scrape	26	13	NCT	15	3
Missaoui	2011	Tunisia	African	MSP	Biopsy	42	22	NCT	8	0
Kim	2010	Korea	Asian	MSP	Biopsy	69	50	NCT	41	11
Yang	2010	Netherlands	European	QMSP	Biopsy	60	31	BCT	20	5
Flatley	2009	UK	European	MSP	Scrape	42	17	NCT	40	0
Iliopoulos	2009	Greece	European	RMethyLight	Biopsy	61	41	NCT	15	0
Leung	2008	China	Asian	MSP	Biopsy	107	60	NCT	27	0
Zhao	2008	China	Asian	MSP	Biopsy	112	45	NCT	20	0
Feng	2007	Senegalese	African	QMSP	Urine	63	31	U	16	1
Shivapurkar	2007	USA	American	QMSP	Biopsy	45	24	NCT	12	0
Jeong	2006	Korea	Asian	MSP	Biopsy	78	35	NCT	24	1
Wisman	2006	Netherland	European	QMSP	Scrape	30	19	NCT	19	0
Feng	2005	USA	American	MSP	Biopsy	176	69	NCT	140	3
Gustafson	2004	USA	American	MSP	Biopsy	28	7	NCT	11	0
Reesink-Peters	2004	Netherlands	European	QMSP	Scrape	48	35	NCT	41	2
Yang	2004	HK	Asian	MSP	Biopsy	85	51	NCT	100	0
Dong	2001	Korea	Asian	MSP	Biopsy	53	27	NCT	24	0
Cohort studies										
Kalantari	2014	USA	American	MSP	Scrape	408	317	NA	NA	NA
Henken	2007	Netherlands	European	MS-MLPA	Biopsy	24	15	NA	NA	NA

Note: MSP: Methylation specific PCR; QMSP: Realtime quantitative MSP - QMSP; RMethyLight: Real-time MethyLight; NCT: normal cervical tissue; BCT: benign cervical tissue; U: Urine of healthy woman; NA: non-analysis.

Among the 2 included cohort studies consisted 432 cases, from American countries (1 of 2 studies, counting for 50%) and European countries (1 of 2 studies, counting for 50%), to estimate the frequency of *DAPK* promoter methylation by using MSP (1 of 2 studies, counting for 50%) and MS-MLPA (1 of 2 studies, counting for 50%). The type of case samples consisted of 1 studies of using Scrape samples (counting for 50%), and 1 study of using Biopsy - tumor tissues (counting for 50%). The detail characteristic of included studies were summarized in Table 1.

Meta-analysis

Association between *DAPK1* promoter methylation and cervical cancer

In current meta-analysis, the heterogeneity among included 21 case-control studies was significant for Cochran's Q-test ($p < 0.001$), thus, the random effect model was applied to evaluate the strength of the association between *DAPK* promoter methylation and cervical cancer. The association was estimated by calculation of OR value with 95% confidence intervals (95% CIs). As the results, we found that the frequency of *DAPK* promoter methylation were 52.75% (844 of 1600 cases), 18.40% (186 of 1011 controls) for cases and controls, respectively. Moreover, we found that *DAPK* promoter methylation was significantly associated with an increased cervical risk with a pooled OR of 21.51 (95% CI = 8.73-52.97) due to the significant heterogeneity ($I^2 = 85.64\%$, $p < 0.001$), based on the random effect model (Fig 2).

Subgroup analysis

In current study, subgroups analyses were performed by source of sample types, methylation detection method and ethnicity. The association between *DAPK1* promoter methylation and cervical cancer was observed in each subgroup (Table 2). The significant association between *DAPK* promoter methylation and cervical cancer was identified including MSP and QMSP method. The ORs were 21.41 (95% CI = 7.03-65.19) in MSP subgroup and 20.45 (95% CI = 5.61-74.51) in QMSP subgroup, based on the random effect model, while I^2 were high with 87.60% and 58.34%, respectively. The subgroup analysis by source of samples, particularly focused on biopsy and scrape samples, reported that ORs were 20.30 (95% CI = 6.87-60.00) in case of using biopsy, based on the random effect model, and 25.65 (95% CI = 11.25-58.44) in case of using Scrape sample, based on the fixed effect model. According to subgroup analysis by the ethnicity, significant association between methylation status and cervical cancer was found among the Asian region and the non-Asian region, including American, European and African region. In detail, the ORs were 20.93 (95% CI = 5.77-75.97) in Asian region, 26.34 (95% CI = 5.08-136.62) in European region, based on the random effect model, 25.48 (95% CI = 9.16-70.92) in American region, and 15.88 (95% CI = 2.92-86.41) in African region while I^2 were high with 85.50%, moderate with 68.91%, and low with both 0%, respectively.

Table 2Subgroup analysis in the meta-analysis of *DAPK* promoter methylation and cervical cancer

Variables	N	Test of association			Test of heterogeneity		
		OR (95% CI)	Z	P-value	Model	Ph	I ² (%)
Materials							
Biopsy	15	20.30 [6.87-60.00]	5.44	< 0.001	R	<0.001	87.11
Scrape	5	25.65 [11.25-58.44]	7.72	< 0.001	F	<0.001	47.96
Urine	1	14.53 [18.07-116.74]	2.52	0.01	-	-	-
Methods							
MSP	15	21.41 [7.03-65.19]	5.39	< 0.001	R	<0.0001	87.60
QMSP	6	20.45 [5.61-74.51]	4.57	< 0.001	R	0.03	58.34
Ethnicity							
Asian	11	20.934 [5.77-75.97]	4.63	< 0.001	R	<0.001	85.50
European	5	26.34 [5.08-136.62]	3.90	< 0.001	R	0.001	68.91
American	3	25.48 [9.16-70.92]	6.20	< 0.001	F	0.72	0.00
Africa	2	15.88 [2.92-86.41]	3.2	0.001	F	0.89	0.00

4. Discussion

DNA hypermethylation of CpG islands located in the promoter of TSGs resulting in silencing the expression of TSGs that plays a crucial role in the carcinogenesis of tumor. The *DAPK* promoter methylation mainly induced the loss of *DAPK* functions, provides a unique mechanism that links suppression of

apoptosis to metastasis of tumor, including cervical cancer (Inbal *et al.*, 1997; Leung *et al.*, 2008; Niklinska *et al.*, 2009; Wang *et al.*, 2016; Cai *et al.*, 2017). The current results suggested that individuals with the *DAPK* methylation are associated with cervical cancer by calculating of pooled OR of 21.51 (95% CI = 8.73-52.97).

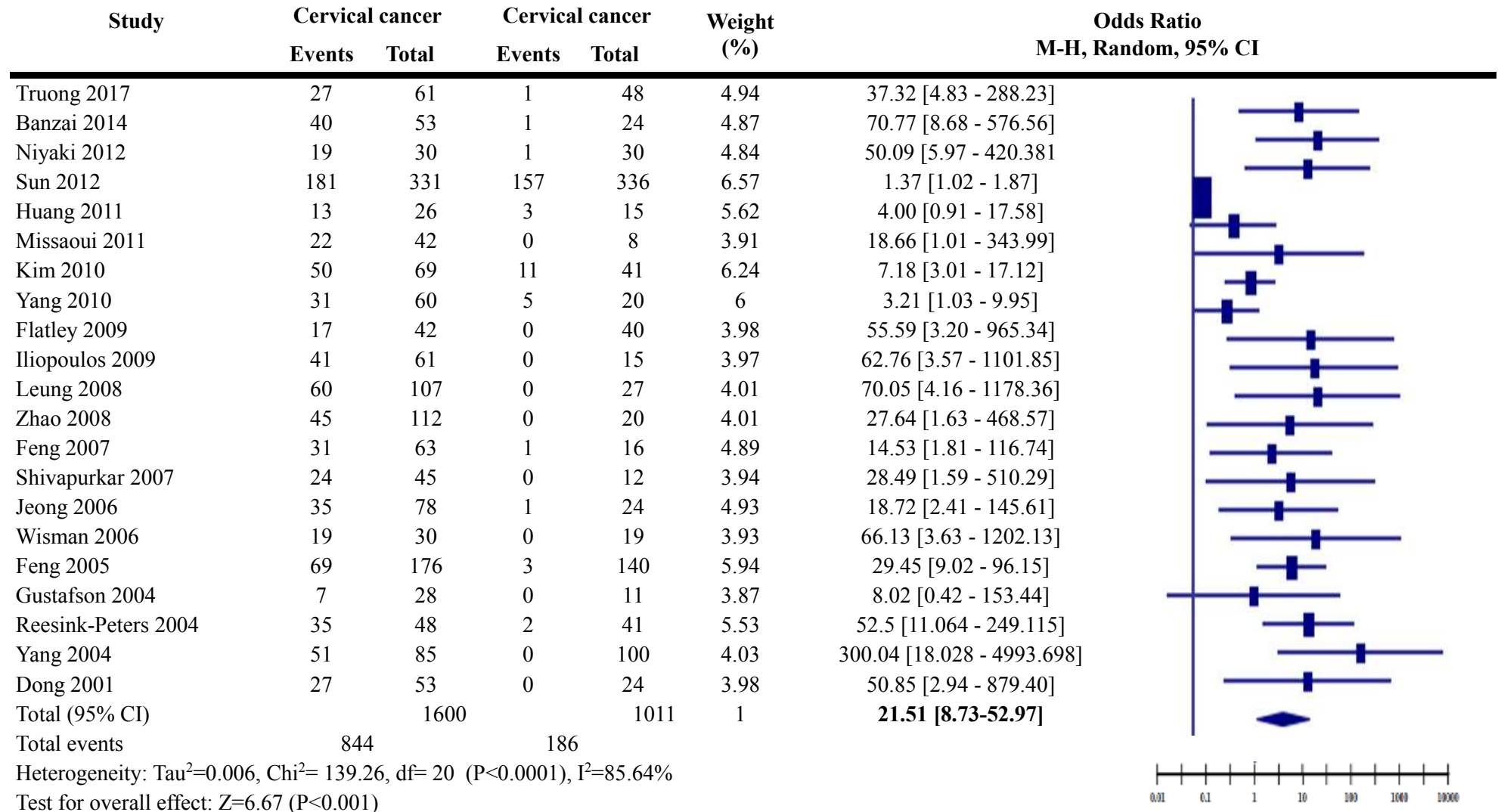


Figure 2. Forest plot of *DAPK1* promoter methylation and cervical cancer by calculating of OR value based on random effect model

In our study reported that a meta-analysis on 21 published studies on 2611 samples, including 1600 cancerous samples and 1011 non-cancerous sample. As the results, we reported that the *DAPK* would appear the promising methylated gene for cervical screening, which was similar to the previous study of Agodi *et al.* (2015) summarized the results of 20 published studies relevant to *DAPK* methylation, from 2001 to 2004, on 1929 samples. Because of the moderate heterogeneity between studies, the subgroup analyses by methylation detection method, sources of cancerous samples used to detect the *DAPK* methylation were performed. In the methylation detection method, a significant association between *DAPK* methylation and cervical cancer was observed between the MSP and QMSP in random-effect method. The higher OR was observed in MSP method (MSP: OR = 21.41, 95% CI = 7.03-65.19; QMSP: OR = 20.45, 95%

CI = 5.61-74.51). It could be explained that MSP method was considered to be “gold standard” method for methylation detection within reported results as methylated and/or unmethylated on the DNA sequence. The subgroup analysis by sources of samples revealed a significant association in subgroups, in detail, the OR was highly observed in both biopsy and scrape samples and the heterogeneity in scrape subgroup was moderately low ($I^2 = 47.96\%$). Finally, the *DAPK* methylation was the significant association revealed to ethnicity towards European country.

5. Conclusion

The methylation of *DAPK* was reported as the epigenetic characteristic in many human cancers, including cervical cancer. The present meta-analysis provides evidence to conclude that the significant strong correlation between *DAPK* tumor suppressor gene hypermethylation in cervical cancer■

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