RESEARCH ARTICLE

# DAPK Promoter Methylation and Bladder Cancer Risk: A Systematic Review and Meta-Analysis

Lihe Dai☯, Chong Ma☯, Zhensheng Zhang, Shuxiong Zeng, Anwei Liu, Shijie Tang, Qian Ren, Yinghao Sun, Chuanliang Xu*

Department of Urology, Changhai Hospital, Second Military Medical University, Shanghai, China

☯ These authors contributed equally to this work.
* xuchuanliang@vip.126.com

## Abstract

### Background

Methylation of tumor suppressor gene promoter leads to transcription inactivation and is involved in tumorigenesis. Several studies demonstrate a potential association between the Death-Associated Protein Kinase (DAPK) gene promoter methylation and bladder cancer risk, tumor stage and histological grade. Due to inconsistent results of these studies, we performed this meta-analysis to ascertain the association.

### Methods

Studies were retrieved from the PubMed, Embase, Web of Science and the Cochrane Library databases. Study selection and data extraction were executed by two reviewers independently. Meta-analysis was performed using Stata 13.0 and Review Manager 5.3 software.

### Results

A total of 21 articles involving 15 case control and 8 case series studies were included in this meta-analysis. DAPK promoter methylation was associated with bladder cancer risk (OR: 5.81; 95%CI = 3.83–8.82, P<0.00001). The frequency of DAPK promoter methylation was equal in bladder cancer tissue and paired adjacent normal tissue (OR: 0.87; 95%CI = 0.31–2.48, P = 0.794). Furthermore, DAPK promoter methylation was associated with higher histological grade (OR: 1.52; 95%CI = 1.10–2.09, P = 0.011) but not associated with tumor stage (OR: 1.12; 95%CI = 0.67–1.87, P = 0.668).

### Conclusions

The result suggests that DAPK promoter methylation is significantly increased in bladder cancer patients compared to normal controls. DAPK promoter methylation could serve as a biomarker for bladder cancer detection and management.

## Introduction

Bladder cancer (BCa) is the 11th most commonly diagnosed cancer in the world [1]. There will be an estimated 76,960 new cases and 16,390 deaths in the US in 2016[2]. BCa is the fourth most common cancer of men and twelfth of women in the US [2]. About 75% patients are diagnosed as non-muscle invasive BCa with a high recurrence but low progression rate. The five-year survival is aproximately 90% [3, 4]. Muscle invasive BCa frequently progresses to metastasis with five-year survival less than 50% [5]. Cystoscopy with biopsy, the current standard for diagnosis and surveillance of BCa, is an invasive method that causes discomfort to patients. Urine cytology is non-invasive but yields low sensitivity in detecting low-grade lesions [6]. Recently, efforts have been made to develop biomarker for non-invasive diagnosis of BCa.

DNA methylation is involved in transcriptional silencing of tumor suppressor gene, being a common epigenetic event in early phase of tumorigenesis [7, 8]. Inactivation of tumor suppressor gene by promoter methylation has been frequently observed in BCa [9–11]. The Death-Associated Protein Kinase (DAPK) gene is located on chromosome 9p34. It encodes Ca+/calmodulin-regulated serine/threonine kinase, which induces apoptosis and suppresses tumor growth [12, 13]. DAPK promoter methylation was reported to be associated with varies cancers including BCa [14]. Down regulation of DAPK expression by promoter methylation was observed in both tissue and cell lines of BCa [15, 16]. DAPK promoter methylation was detected in the urine and blood of BCa patients, making it a potential non-invasive diagnostic biomarker [17–19]. In addition, DAPK promoter methylation was associated with tumor stage and histological grade [16, 20–22]. However, there were inconsistent results among different studies in evaluating the association between DAPK promoter methylation and BCa risk, tumor stage and histological grade. Thus, we conducted a meta-analysis to ascertain the association.

## Materials and Methods

This meta-analysis was designed according to the latest version of PRISMA checklist for meta-analysis (**S1 File**).

### Publication selection

Studies were identified via a search of PubMed, EMBASE, Web of Science and the Cochrane Library databases updated on 8, March, 2016 using the following key words: ("methylation" or "hypermethylation") and ("bladder cancer" or "bladder neoplasm" or "bladder tumor" or "bladder carcinoma" or "bladder carcinogenesis" or "urothelial carcinoma") and ("DAPK or "Death-associated protein kinase"). References of these publications were manually reviewed in order to retrieve additional studies.

### Inclusion and exclusion criteria

Studies were reviewed by two authors (LHD and CM) independently. Studies met the following inclusion criteria were eligible for the meta-analysis: (1) Purpose of study was to evaluate DAPK promoter methylation in either tissue, urine or blood of BCa patients and normal controls or to assess the association between DAPK promoter methylation and tumor stage or histological grade, (2) types of sample from BCa patients and normal controls should be homogeneous, (3) Detection method of DAPK promoter methylation was based on methylation specific polymerase chain reaction (MSP), (4) Articles published in English. When several studies

with overlapping data were observed, only those with a larger sample size were included. Studies without sufficient data after contacting with the original author were excluded.

## Data collection

Information extracted from each included study were author, year of publication, ethnicity of individuals involved, type of sample, method for methylation examination, pathological stage, histological grade, frequency of DAPK promoter methylation in BCa patients, normal controls and adjacent normal bladder tissue. The normal controls were defined as those without a historical or current diagnose of BCa, including healthy people, those with benign urological diseases and those with tumors of the other kinds. Studies assessing DAPK promoter methylation in both tissue and urine of BCa patients and normal controls were treated as two. Histological grade $\geq 2$ was defined as high grade, otherwise low grade. Tumor stage $\geq 2$ T2 was classified as high stage, otherwise low stage. Data collection was carried out by two reviewers independently (LHD and CM). Different opinions were settled by discussion.

## Quality assessment of individual study

Quality of individual study was assessed in accordance with the Newcastle-Ottawa Scale (NOS) assessment[23] separately by two authors. Articles contain case control studies were scored according to selection, comparability and exposure. Assessment was made by scoring with stars ranging from zero to nine. Article scored with six or more stars were qualified.

## Statistical Analysis

The pooled odds ratios (ORs) and corresponding 95% confidence intervals (CIs) of different studies were calculated to compare dichotomous statistics between studies. To assess heterogeneity across the studies, Cochrane's Q test[24] and $I^2$ statistic[25, 26] were calculated. If the studies were shown to be homogeneous with P>0.05 and $I^2$ values < 50%, the fixed-effects model (the Mantel-Haenszel method) were selected. Otherwise, a random-effects model (the DerSimonian and Laird method) was applied. In addition, a sensitivity analysis was performed to assess the stability of the results. The potential publication bias was examined in a funnel plot visually and the degree of asymmetry was tested by Egger's test[27]. This meta-analysis was performed using the software STATA version 13.0 (Stata Corporation, TX, USA) and Review Manager 5.3(Cochrane Collaboration, Oxford, UK). All P-values were based on two-sided tests and a P<0.05 was considered statistically significant.

## Results

### Study selection and characteristics

The strategies for study selection and the results were presented in **Fig 1**. The study by Motlagh et al [28] examined DAPK promoter methylation in the tissue from BCa patients and the blood from healthy individuals, thus excluded. All of the 7 articles without sufficient data after contacting the authors were case series studies. The result of NOS assessment showed all case control studies were qualified for this meta-analysis (**S1 Table**). Finally, 21 articles [15–22, 29–41] were enrolled including 15 case control and 8 case series studies.

The frequency of the DAPK promoter methylation was evaluated in a total of 1,247 samples from BCa patients and 405 from normal controls in 15 case control studies. Among them nine involved Caucasians and six Asians. Six studies applied MSP method and nine studies used qMSP. MethyLight was classified as qMSP method [42]. DAPK promoter methylation was assessed in tissue only, in urine only, in both tissue and urine and in blood in 14, 3, 2 and 2

studies respectively. DAPK promoter methylation was assessed in 233 paired BCa tissue and adjacent normal bladder tissue in five studies. In addition, the association between DAPK promoter methylation and tumor stage and histological grade were evaluated in 13 case control and 4 case series studies. Characteristics of included studies were listed in Table 1.

## The correlation of DAPK promoter methylation and BCa

**1. DAPK promoter methylation in BCa patients and normal controls with stratified analysis.** Altogether, DAPK hypermethylation was associated with increased risk of BCa (OR: 5.81; 95%CI = 3.83–8.82, P<0.00001, fixed-effects model) (Fig 2). We validated the result in stratified analysis by type of sample, ethnicity and detection method (Table 2).

**2. DAPK promoter methylation in paired BCa tissue and adjacent normal bladder tissue.** Then, we investigated the level of DAPK promoter methylation in BCa tissue and adjacent normal bladder tissue. In pooled meta-analysis, the frequency of DAPK promoter methylation was equal in paired BCa tissue and adjacent normal bladder tissue (OR: 0.87; 95% CI = 0.31–2.48, P = 0.794, random-effects model) (Fig 3).

**3. The association between DAPK promoter methylation and tumor stage or histological grade of BCa.** There was no association between DAPK promoter methylation and tumor stage (OR: 1.12; 95%CI = 0.67–1.87, P = 0.668, random-effects model) (Fig 4). On the contrary, DAPK promoter methylation was associated with higher histological grade (OR: 1.52; 95%CI = 1.10–2.09, P = 0.011, fixed-effects model) (Fig 5).
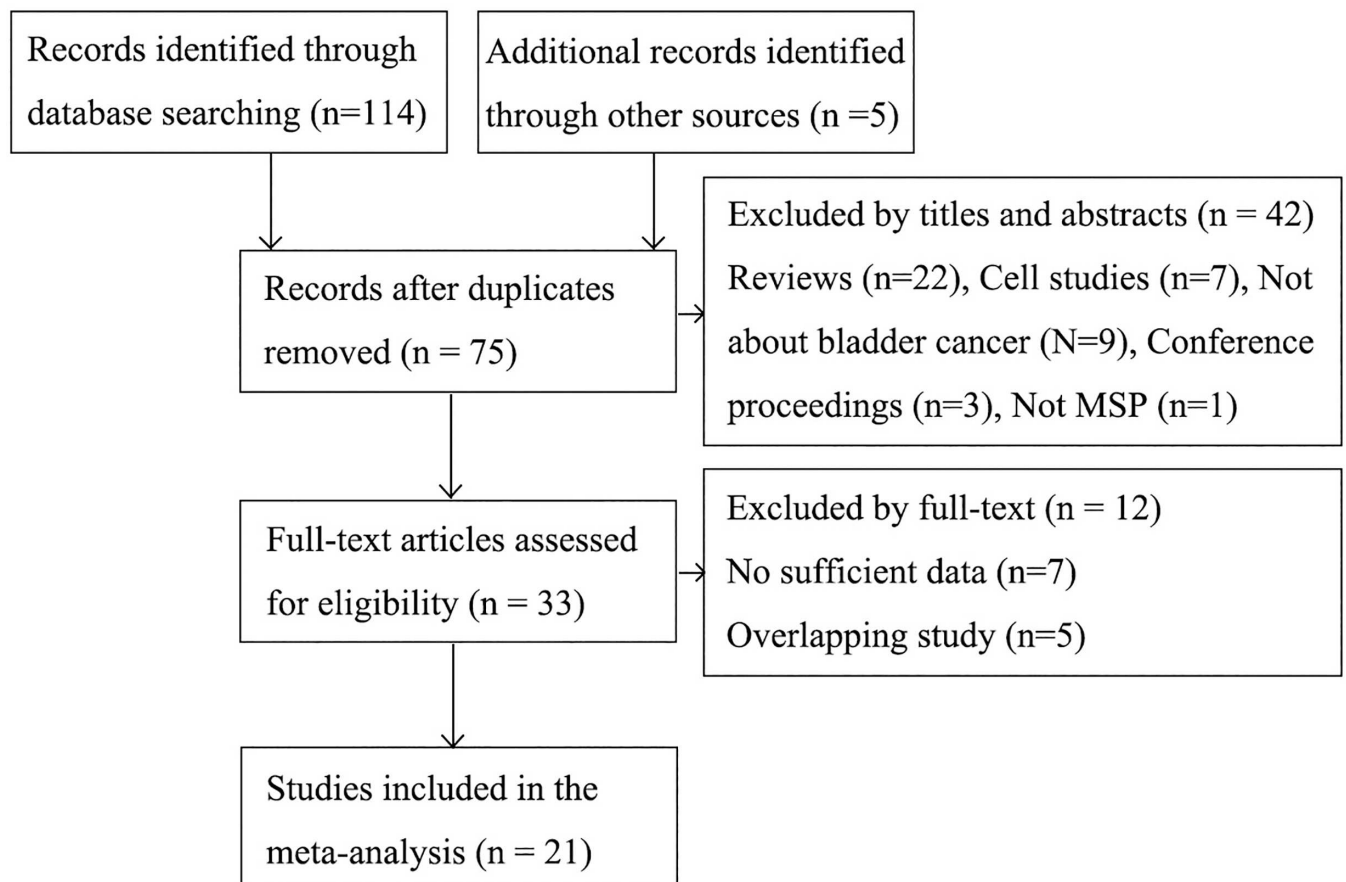


**Fig 1. Selection of studies in the meta-analysis.**

**Table 1. Characteristics of the Included Studies**

| First author | Year | Ethnicity | Sample type | Control type | Method | Sample size | Case | | Control | | Low stage | | High stage | | Low grade | | High grade | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | M+ | U | M+ | U | M+ | U | M+ | U | M+ | U | M+ | U |
| Chan-t[a][21] | 2002 | Asian | tissue | non-BCa | MSP | 102 | 60 | 42 | 0 | 7 | - | - | - | - | - | - | - | - |
| Chan-u[b][21] | 2002 | Asian | urine | non-BCa | MSP | 22 | 10 | 12 | 0 | 17 | 8 | 10 | 2 | 2 | 5 | 4 | 5 | 8 |
| Friedrich[15] | 2004 | Caucasian | urine | non-BCa | MethyLight | 37 | 8 | 29 | 0 | 20 | 4 | 11 | 4 | 18 | - | - | - | - |
| | | | tissue | ANT | qMSP | 33 | 9 | 24 | 19 | 14 | - | - | - | - | - | - | - | - |
| Nakagawa[29] | 2005 | Asian | tissue | non-BCa | MSP | 63 | 18 | 45 | 1 | 7 | 10 | 34 | 5 | 14 | - | - | - | - |
| Christoph[30] | 2006 | Caucasian | tissue | non-BCa | qMSP | 110 | 81 | 29 | 4 | 16 | 57 | 9 | 24 | 20 | - | - | - | - |
| Yates[31] | 2006 | Caucasian | urine | non-BCa | qMSP | 35 | 2 | 33 | 3 | 66 | - | - | - | - | - | - | - | - |
| Yates[32] | 2007 | Caucasian | tissue | ANT | qMSP | 23 | 4 | 19 | 1 | 22 | 14 | 56 | 7 | 14 | 1 | 19 | 20 | 51 |
| Ellinger[33] | 2008 | Caucasian | blood | non-BCa | qMSP | 42 | 1 | 41 | 0 | 39 | 0 | 11 | 1 | 30 | - | - | - | - |
| Jarmalaite[34] | 2008 | Caucasian | tissue | non-BCa | MSP | 58 | 16 | 42 | 1 | 1 | 10 | 28 | 6 | 14 | 1 | 9 | 15 | 33 |
| Wolff[20] | 2008 | Caucasian | tissue | non-BCa | qMSP | 253 | 11 | 242 | 0 | 8 | 5 | 184 | 6 | 58 | 1 | 98 | 10 | 144 |
| | | | tissue | ANT | qMSP | 43 | 0 | 43 | 3 | 40 | - | - | - | - | - | - | - | - |
| Hellwinkel[35] | 2008 | Caucasian | tissue | ANT | qMSP | 39 | 24 | 15 | 26 | 13 | - | - | - | - | - | - | - | - |
| Brait[36] | 2008 | Caucasian | tissue | non-BCa | qMSP | 32 | 7 | 25 | 0 | 5 | 85 | 70 | 29 | 26 | - | - | - | - |
| Sobti[37] | 2010 | Asian | tissue | non-BCa | MSP | 103 | 39 | 64 | 4 | 44 | 24 | 33 | 15 | 31 | - | - | - | - |
| Jablonowski [17] | 2011 | Caucasian | blood | non-BCa | MSP | 42 | 27 | 15 | 0 | 36 | - | - | - | - | 21 | 9 | 6 | 6 |
| Chen-t[a][19] | 2011 | Asian | tissue | non-BCa | qMSP | 210 | 114 | 96 | 0 | 2 | - | - | - | - | 30 | 28 | 84 | 68 |
| Chen-u[b][19] | 2011 | Asian | urine | non-BCa | qMSP | 30 | 8 | 22 | 2 | 17 | 6 | 19 | 2 | 2 | 3 | 9 | 5 | 13 |
| Vinci[18] | 2011 | Caucasian | urine | non-BCa | qMSP | 108 | 27 | 81 | 10 | 95 | - | - | - | - | 15 | 50 | 12 | 31 |
| | | | tissue | ANT | qMSP | 85 | 41 | 44 | 26 | 59 | - | - | - | - | 22 | 28 | 19 | 16 |
| Maruyama[38] | 2001 | Caucasian | tissue | - | MSP | 98 | 4 | 94 | - | - | 2 | 40 | 2 | 54 | - | - | - | - |
| Tada[16] | 2002 | Asian | tissue | - | MSP | 55 | 16 | 39 | - | - | - | - | - | - | 0 | 5 | 16 | 34 |
| Friedrich[39] | 2005 | Caucasian | tissue | - | MethyLight | 105 | 25 | 80 | - | - | - | - | - | - | 1 | 22 | 24 | 58 |
| Neuhausen[40] | 2006 | Caucasian | tissue | - | MSP | 88 | 28 | 60 | - | - | 5 | 8 | 23 | 52 | 1 | 2 | 27 | 58 |
| Park[22] | 2010 | Asian | tissue | - | MSP | 64 | 18 | 46 | - | - | 9 | 40 | 9 | 6 | 6 | 17 | 12 | 29 |
| Jarmalaite[41] | 2010 | Caucasian | tissue | - | MSP | 21 | 4 | 17 | - | - | - | - | - | - | 2 | 3 | 2 | 14 |

Abbreviation: non-BCa, non-bladder cancer; ANT: adjacent normal tissue; M+, methylated; U, unmethylated.

[a]tissue of BCa patients and control in studies evaluating DAPK promoter methylation in both tissue and urine.

[b]urine of BCa patients and control in studies evaluating DAPK promoter methylation in both tissue and urine.

doi:10.1371/journal.pone.0167228.t001

**4. Sensitivity Analyses.** Sensitivity analysis showed that the pooled ORs ranged from 4.25 (95%CI: 2.26–8.01) to 5.36 (95%CI: 2.93–9.78) (Fig 6), indicating that none of the studies dramatically changed the pooled ORs. The results of the other meta-analysis turned out to be reliable and stable after sensitivity analysis (S1 Fig) except for studies of the association between DAPK promoter methylation and histological grade (Fig 7). In omitting Friedrich's [39] study, which was the only one applying MethyLight technology in this set, the result turned out that DAPK promoter methylation was not associated with histological grade (OR: 1.38; 95%CI: 0.99–1.91, P = 0.054, fixed-effects model). The same result was seen when random-effects model was applied (OR: 1.38; 95%CI: 0.89–2.14, p = 0.155).

**5. Publication Bias.** Funnel plots of all the studies above were listed in Fig 8. No publication bias was observed, as the shape of the funnel plots seems to show no evident asymmetry in each meta-analysis. It is further validated by the Egger's test (P>0. 05) (data not shown).

## Discussion

DNA methylation plays an important role in epigenetic transcriptional control and genome stability [43]. Methylation induced silencing of tumor suppressor gene has been documented in varies types of cancer [14, 44]. In our meta-analysis, DAPK promoter methylation, detected either in urine, blood or tissue, in Asian or Caucasian, using MSP or qMSP method, was significantly associated with increased risk for BCa.

Previous studies demonstrated that the frequency of DAPK promoter methylation was associated with tumor stage [22, 37] and histological grade [17, 20], while others reported no association [15, 30, 35, 40]. This meta-analysis indicated that there was no significant association between DAPK promoter methylation and stage. Though it appeared that DAPK promoter methylation was associated with higher histological grade under fixed-effects model, the result changed in sensitivity analysis and after adopting random-effects model. Thus, we may carefully draw the conclusion whether there was an association between DAPK promoter methylation and histological grade of BCa. In addition, DAPK promoter methylation frequency was equivalent in paired BCa tissue and adjacent normal bladder tissue. We may
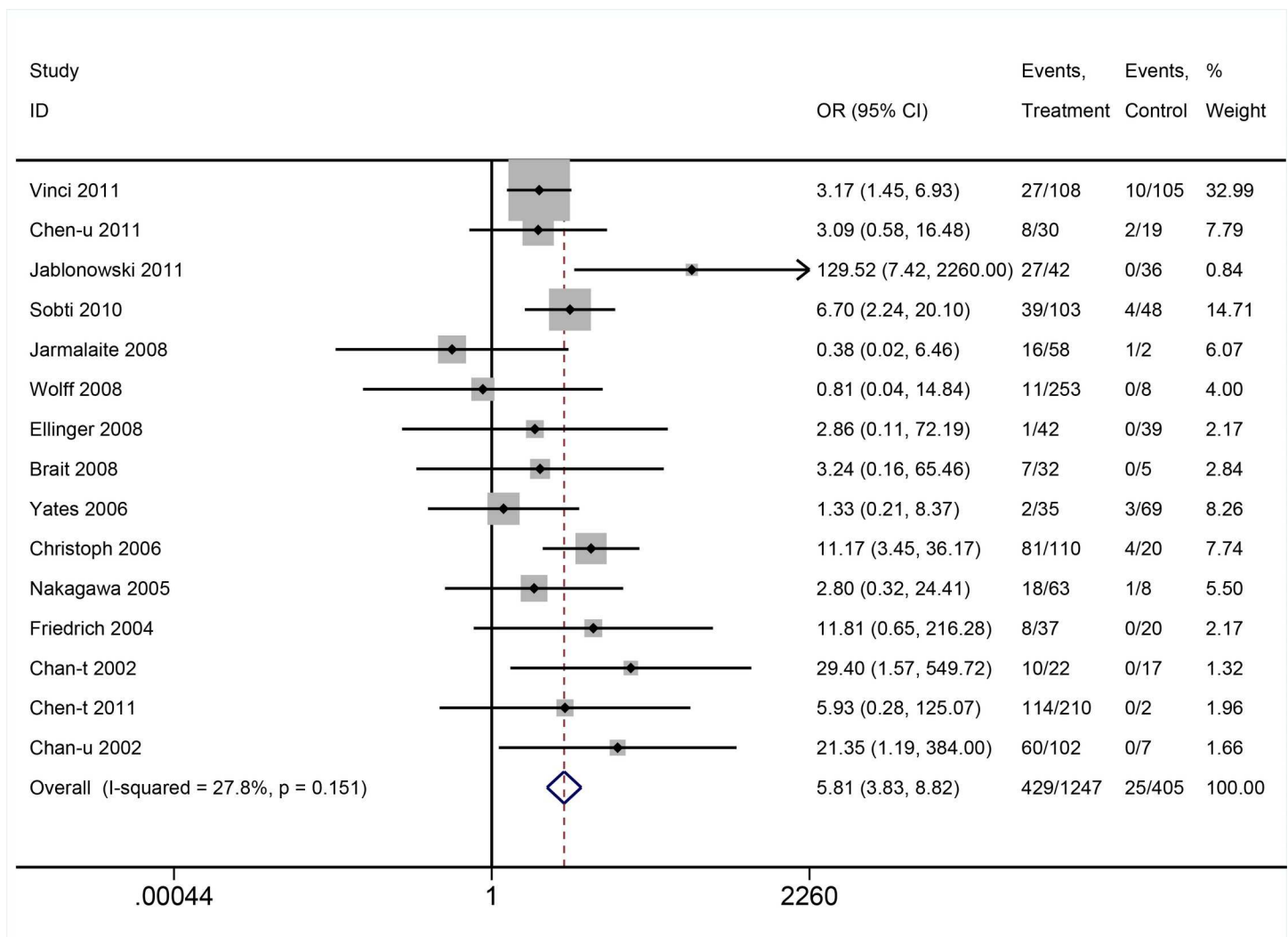


| Study ID | OR (95% CI) | Events, Treatment | Events, Control | % Weight |
|---|---|---|---|---|
| Vinci 2011 | 3.17 (1.45, 6.93) | 27/108 | 10/105 | 32.99 |
| Chen-u 2011 | 3.09 (0.58, 16.48) | 8/30 | 2/19 | 7.79 |
| Jablonowski 2011 | 129.52 (7.42, 2260.00) | 27/42 | 0/36 | 0.84 |
| Sobti 2010 | 6.70 (2.24, 20.10) | 39/103 | 4/48 | 14.71 |
| Jarmalaite 2008 | 0.38 (0.02, 6.46) | 16/58 | 1/2 | 6.07 |
| Wolff 2008 | 0.81 (0.04, 14.84) | 11/253 | 0/8 | 4.00 |
| Ellinger 2008 | 2.86 (0.11, 72.19) | 1/42 | 0/39 | 2.17 |
| Brait 2008 | 3.24 (0.16, 65.46) | 7/32 | 0/5 | 2.84 |
| Yates 2006 | 1.33 (0.21, 8.37) | 2/35 | 3/69 | 8.26 |
| Christoph 2006 | 11.17 (3.45, 36.17) | 81/110 | 4/20 | 7.74 |
| Nakagawa 2005 | 2.80 (0.32, 24.41) | 18/63 | 1/8 | 5.50 |
| Friedrich 2004 | 11.81 (0.65, 216.28) | 8/37 | 0/20 | 2.17 |
| Chan-t 2002 | 29.40 (1.57, 549.72) | 10/22 | 0/17 | 1.32 |
| Chen-t 2011 | 5.93 (0.28, 125.07) | 114/210 | 0/2 | 1.96 |
| Chan-u 2002 | 21.35 (1.19, 384.00) | 60/102 | 0/7 | 1.66 |
| Overall (I-squared = 27.8%, p = 0.151) | 5.81 (3.83, 8.82) | 429/1247 | 25/405 | 100.00 |

.00044   1   2260

**Fig 2. The pooled OR from 15 studies including 1,247 samples form BCa patients and 405 from normal controls.** OR: 5.81; 95%CI = 3.83–8.82, P<0.00001.

doi:10.1371/journal.pone.0167228.g002

**Table 2. Stratified analysis of the association between DAPK promoter methylation and bladder cancer risk**

| Variables | p[a] | OR | 95%CI | Heterogeneity | | |
|---|---|---|---|---|---|---|
| | | | | X | P | I |
| **Total** | 15 | 5.81 | 3.83–8.82 | 19.38 | 0.151 | 27.8% |
| **Ethnicity** | | | | | | |
|   Asian | 6 | 6.80 | 3.25–14.24 | 3.07 | 0.689 | 0.0% |
|   Caucasian | 9 | 4.02 | 1.56–10.33 | 15.67 | **0.047[b]** | 49.0% |
| **Method** | | | | | | |
|   qMSP | 9 | 4.03 | 2.40–6.78 | 6.57 | 0.584 | 0.0% |
|   MSP | 6 | 9.95 | 4.76–20.81 | 10.80 | 0.055 | 53.7% |
| **Material** | | | | | | |
|   Urine | 5 | 3.89 | 2.13–7.09 | 4.03 | 0.402 | 0.8% |
|   Blood | 2 | 38.23 | 5.55–263.37 | 3.18 | 0.075 | 68.5% |
|   Tissue | 8 | 5.90 | 3.09–11.26 | 7.95 | 0.337 | 11.9% |

[a]Number of comparisons.
[b]Random-effects model.

doi:10.1371/journal.pone.0167228.t002
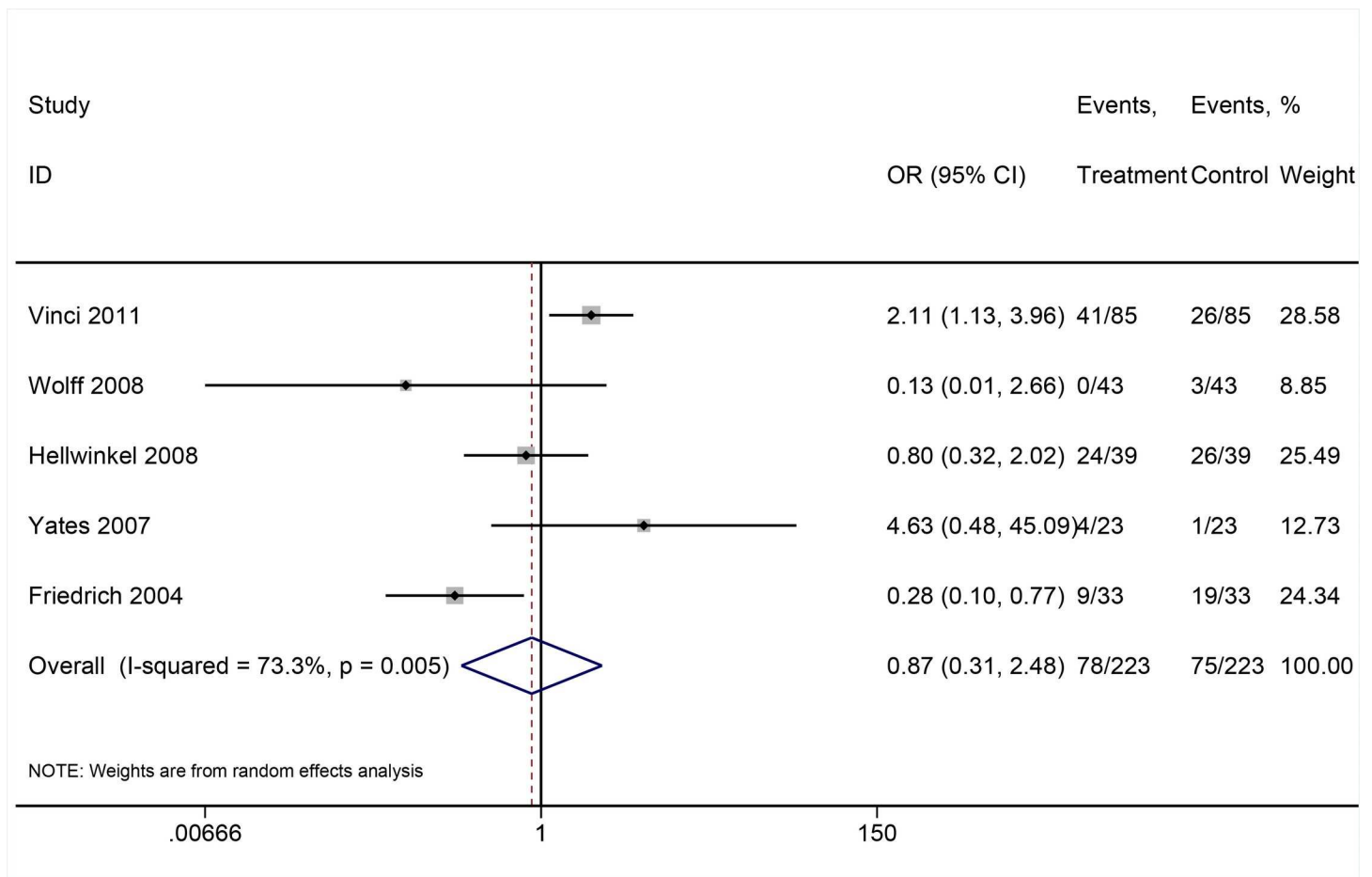


**Fig 3. The pooled OR from 5 studies including 223 paired BCa tumor tissue and adjacent normal bladder tissue.** OR: 0.87; 95%CI = 0.31–2.48, P = 0.794.

doi:10.1371/journal.pone.0167228.g003

| Study ID | OR (95% CI) | Events, Treatment | Events, Control | % Weight |
|---|---|---|---|---|
| Chen-u 2011 | 3.17 (0.36, 27.57) | 2/4 | 6/25 | 4.02 |
| Chen-t 2011 | 0.92 (0.50, 1.70) | 29/55 | 85/155 | 11.68 |
| Sobti 2010 | 0.67 (0.30, 1.50) | 15/46 | 24/57 | 10.41 |
| Park 2010 | 6.67 (1.89, 23.52) | 9/15 | 9/49 | 7.60 |
| Jarmalaite 2008 | 1.20 (0.36, 3.98) | 6/20 | 10/38 | 7.96 |
| Wolff 2008 | 3.81 (1.12, 12.93) | 6/64 | 5/189 | 7.82 |
| Ellinger 2008 | 1.13 (0.04, 29.81) | 1/31 | 0/11 | 2.09 |
| Yates 2007 | 2.00 (0.68, 5.89) | 7/21 | 14/70 | 8.66 |
| Christoph 2006 | 0.19 (0.08, 0.48) | 24/44 | 57/66 | 9.68 |
| Neuhausen 2006 | 0.71 (0.21, 2.40) | 23/75 | 5/13 | 7.83 |
| Nakagawa 2005 | 1.21 (0.35, 4.20) | 5/19 | 10/44 | 7.72 |
| Friedrich 2004 | 0.61 (0.13, 2.95) | 4/22 | 4/15 | 6.05 |
| Chan 2002 | 1.25 (0.14, 10.94) | 2/4 | 8/18 | 4.00 |
| Maruyama 2001 | 0.74 (0.10, 5.49) | 2/56 | 2/42 | 4.48 |
| Overall (I-squared = 57.5%, p = 0.004) | 1.12 (0.67, 1.87) | 135/476 | 239/792 | 100.00 |

NOTE: Weights are from random effects analysis

.0335     1     29.8

**Fig 4. The pooled OR from 14 studies assessing the association between DAPK promoter methylation and tumor stage.** OR: 1.12; 95%CI = 0.67–1.87, P = 0.668.

doi:10.1371/journal.pone.0167228.g004

postulate that DAPK promoter methylation is an early or pre-cancerous event in BCa tumorigenesis. Based on these findings, DAPK promoter methylation may serve as an effective biomarker for early diagnose and surveillance of BCa. Due to the reversibility of DNA methylation [45], therapeutic target based on DAPK promoter methylation may be developed.

The DAPK gene is involved in the p53-dependent apoptosis pathway and has been found to be inactivated via promoter methylation in lung cancer [46, 47]. DAPK promoter methylation was associated with decreased expression of DAPK protein and mRNA in BCa tissue [16, 37]. It was also observed in BCa cell lines to be associated with mRNA down-expression [48]. DAPK mRNA and protein re-expressed after treated with 5-aza-2'-deoxycytidine, inhibiting growth and inducing apoptosis of BCa cell line [48, 49]. Arsenic exposure induced DAPK promoter methylation and transcription inactivation in urothelial cancer [50, 51]. Additionally, smoking increased the risk of DAPK promoter methylation in BCa patients [18, 37]. In this sense, we may postulate that there is a correlation between DAPK promoter methylation and carcinogen exposure of BCa, which further supports its role in BCa tumorigenesis.
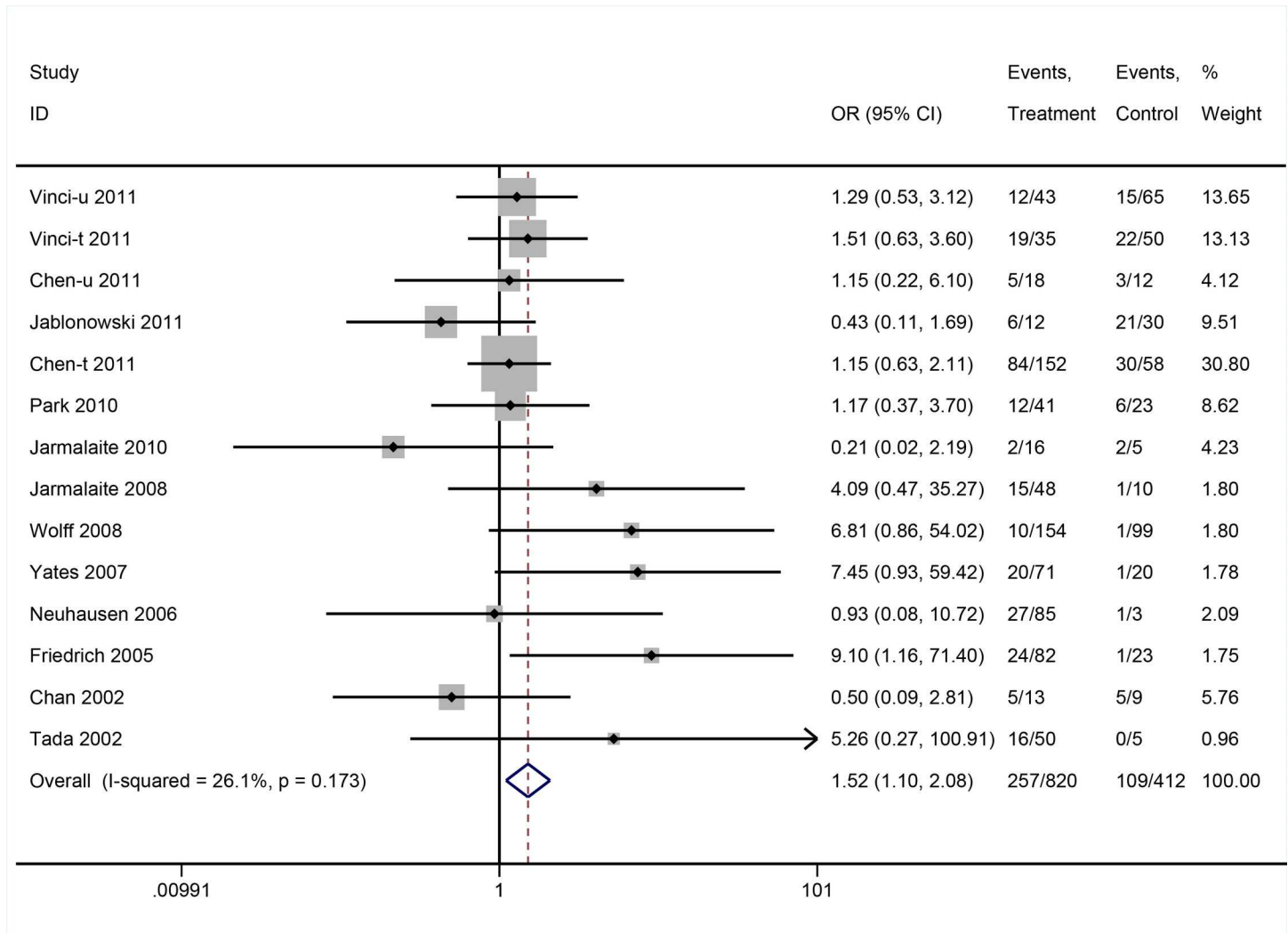
| Study ID | OR (95% CI) | Events, Treatment | Events, Control | % Weight |
|---|---|---|---|---|
| Vinci-u 2011 | 1.29 (0.53, 3.12) | 12/43 | 15/65 | 13.65 |
| Vinci-t 2011 | 1.51 (0.63, 3.60) | 19/35 | 22/50 | 13.13 |
| Chen-u 2011 | 1.15 (0.22, 6.10) | 5/18 | 3/12 | 4.12 |
| Jablonowski 2011 | 0.43 (0.11, 1.69) | 6/12 | 21/30 | 9.51 |
| Chen-t 2011 | 1.15 (0.63, 2.11) | 84/152 | 30/58 | 30.80 |
| Park 2010 | 1.17 (0.37, 3.70) | 12/41 | 6/23 | 8.62 |
| Jarmalaite 2010 | 0.21 (0.02, 2.19) | 2/16 | 2/5 | 4.23 |
| Jarmalaite 2008 | 4.09 (0.47, 35.27) | 15/48 | 1/10 | 1.80 |
| Wolff 2008 | 6.81 (0.86, 54.02) | 10/154 | 1/99 | 1.80 |
| Yates 2007 | 7.45 (0.93, 59.42) | 20/71 | 1/20 | 1.78 |
| Neuhausen 2006 | 0.93 (0.08, 10.72) | 27/85 | 1/3 | 2.09 |
| Friedrich 2005 | 9.10 (1.16, 71.40) | 24/82 | 1/23 | 1.75 |
| Chan 2002 | 0.50 (0.09, 2.81) | 5/13 | 5/9 | 5.76 |
| Tada 2002 | 5.26 (0.27, 100.91) | 16/50 | 0/5 | 0.96 |
| Overall (I-squared = 26.1%, p = 0.173) | 1.52 (1.10, 2.08) | 257/820 | 109/412 | 100.00 |

**Fig 5. The pooled OR from 14 studies assessing the association between DAPK promoter methylation and histological grade.** OR: 1.52; 95% CI = 1.10–2.09, P = 0.011.

doi:10.1371/journal.pone.0167228.g005

Our study has several limitations. First, most of the studies were retrospective, information and selection biases and the other confounders may not be fully controlled. Second, seven studies were excluded due to insufficient data in evaluating the association between DAPK promoter methylation and tumor stage and histological grade, which may lead to publication bias and influence the reliability of our conclusion. Third, as regard to source of control, we failed to distinguish between the healthy and those with different types of benign urological diseases. Fourth, while only studies published in English were included, studies of high quality in the other languages may be excluded.

## Conclusions

In conclusion, our meta-analysis demonstrated that DAPK promoter methylation is significantly associated with BCa tumorigenesis. Further well-designed prospective studies with larger sample size may provide more valid evidence of the role of DAPK promoter methylation in BCa tumorigensis and disease management.
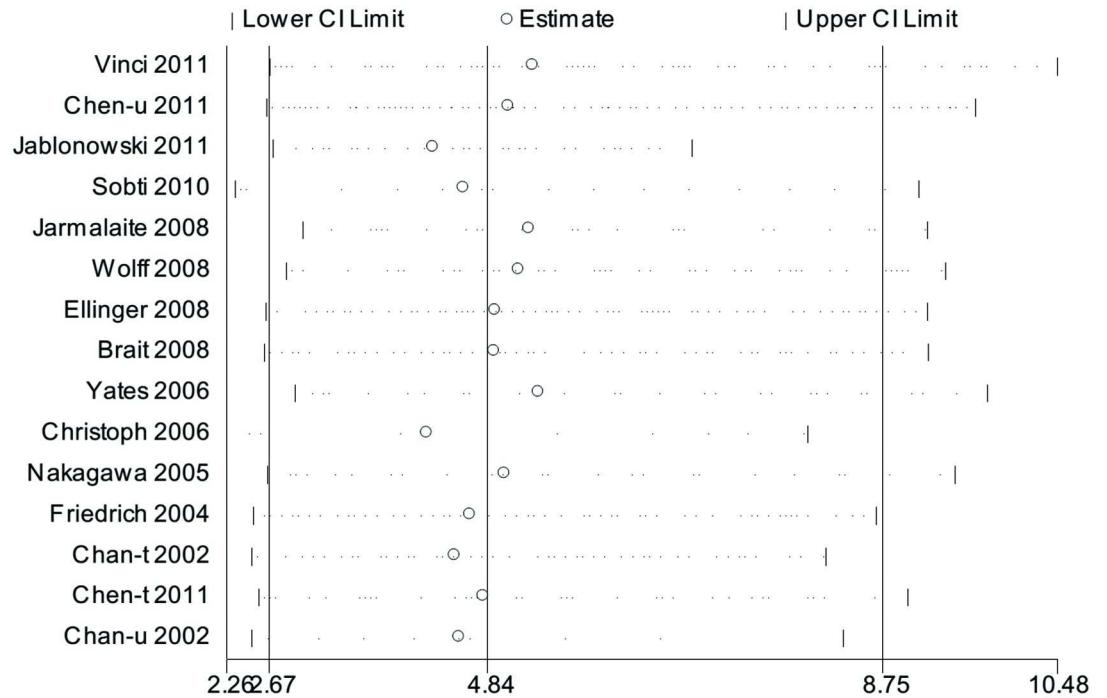
**Fig 6. Sensitivity analysis from 15 studies comparing DAPK promoter methylation in BCa patients and normal controls.**

doi:10.1371/journal.pone.0167228.g006

| Study Omitted | Estimate 95%CI | Lower CI Limit | Upper CI Limit |
|---|---|---|---|
| Vinci 2011 | 5.2989264 | 2.678909 | 10.48137 |
| Chen-u 2011 | 5.0590539 | 2.646712 | 9.670122 |
| Jablonowski 2011 | 4.3139544 | 2.71306 | 6.859489 |
| Sobti 2010 | 4.6141329 | 2.336394 | 9.11243 |
| Jarmalaite 2008 | 5.2634821 | 3.012462 | 9.196546 |
| Wolff 2008 | 5.1615486 | 2.842393 | 9.372942 |
| Ellinger 2008 | 4.9280262 | 2.641908 | 9.19239 |
| Brait 2008 | 4.9177108 | 2.628696 | 9.199953 |
| Yates 2006 | 5.3584976 | 2.934736 | 9.784012 |
| Christoph 2006 | 4.2502508 | 2.25515 | 8.010389 |
| Nakagawa 2005 | 5.0196552 | 2.660421 | 9.471034 |
| **Friedrich 2004** | **4.6772208** | **2.517476** | **8.689814** |
| Chan-t 2002 | 4.5253754 | 2.501839 | 8.185587 |
| Chen-t 2011 | 4.8094549 | 2.569962 | 9.000466 |
| Chan-u 2002 | 4.5705438 | 2.498958 | 8.359432 |
| Combined | 4.8354828 | 2.672644 | 8.7486 |

**Fig 7. Sensitivity analysis from 14 studies assessing the association between DAPK promoter methylation and histological grade.**

doi:10.1371/journal.pone.0167228.g007
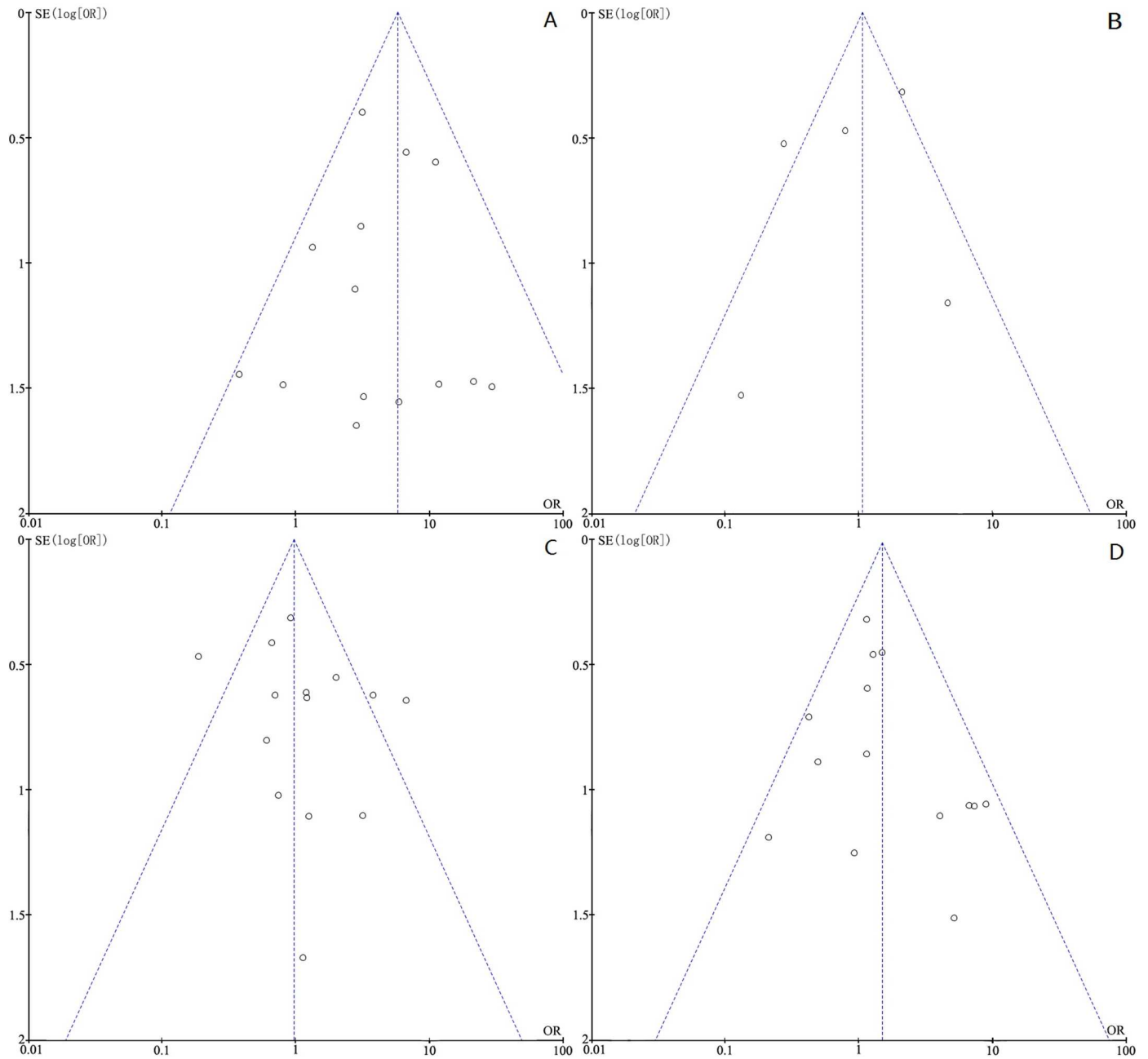
Fig 8. The funnel plots were virtually symmetrical, suggesting there were no publication bias in this meta-analysis. (A)The funnel plot from 15 studies comparing DAPK promoter methylation in BCa patients and normal controls. (B)The funnel plot from 5 studies with paired BCa tissue and adjacent normal bladder tissue. (C)The funnel plot from 14 studies assessing the association between DAPK promoter methylation and tumor stage. (D)The funnel plot from 14 studies assessing the association between DAPK promoter methylation and histological grade.

doi:10.1371/journal.pone.0167228.g008

## Supporting Information

**S1 File. PRISMA 2009 Checklist.**
(DOC)

**S1 Table. Quality assessment of case control studies according to the Newcastle-Ottawa Scale.**
(DOCX)

**S1 Fig. Sensitivity analysis of the other three meta-analysis in this paper.** S1A
Fig. Sensitivity analysis from studies of DAPK hypermethylation in matched bladder cancer
tissue and adjacent normal tissue. S1B Fig. Sensitivity analysis from studies of association
between DAPK hypermethylation and tumor stage. S1C Fig. Sensitivity analysis from studies
of association between DAPK hypermethylation and tumor grade.
(DOCX)

## Author Contributions

**Conceptualization:** LHD CLX.

**Data curation:** LHD CM AWL.

**Formal analysis:** LHD SJT.

**Investigation:** LHD CM SJT.

**Methodology:** ZSZ QR.

**Project administration:** ZSZ.

**Resources:** LHD CM SXZ.

**Software:** SXZ.

**Supervision:** YHS CLX.

**Validation:** CM SXZ QR.

**Visualization:** LHD ZSZ.

**Writing – original draft:** LHD CM.

**Writing – review & editing:** LHD CM CLX.

## References

1. Witjes JA, Comperat E, Cowan NC, De Santis M, Gakis G, Lebret T, et al. EAU guidelines on muscle-invasive and metastatic bladder cancer: summary of the 2013 guidelines. European urology. 2014 Apr; 65(4):778–92. doi: 10.1016/j.eururo.2013.11.046 PMID: 24373477

2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016 Jan-Feb; 66(1):7–30. Epub 2016/01/09. eng. doi: 10.3322/caac.21332 PMID: 26742998

3. Babjuk M, Burger M, Zigeuner R, Shariat SF, van Rhijn BW, Comperat E, et al. EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2013. European urology. 2013 Oct; 64 (4):639–53. doi: 10.1016/j.eururo.2013.06.003 PMID: 23827737

4. Prout GR Jr., Barton BA, Griffin PP, Friedell GH. Treated history of noninvasive grade 1 transitional cell carcinoma. The National Bladder Cancer Group. The Journal of urology. 1992 Nov; 148(5):1413–9. PMID: 1433540

5. Soloway MS. Bladder cancer: Lack of progress in bladder cancer—what are the obstacles? Nature reviews Urology. 2013 Jan; 10(1):5–6. doi: 10.1038/nrurol.2012.219 PMID: 23165404

6. Lokeshwar VB, Habuchi T, Grossman HB, Murphy WM, Hautmann SH, Hemstreet GP, 3rd, et al. Blad-der tumor markers beyond cytology: International Consensus Panel on bladder tumor markers. Urology. 2005 Dec; 66(6 Suppl 1):35–63. Epub 2006/01/10. eng.

7. Laird PW. The power and the promise of DNA methylation markers. Nature reviews Cancer. 2003 Apr; 3(4):253–66. doi: 10.1038/nrc1045 PMID: 12671664

8. Clark SJ, Melki J. DNA methylation and gene silencing in cancer: which is the guilty party? Oncogene. 2002 Aug 12; 21(35):5380–7. Epub 2002/08/03. eng. doi: 10.1038/sj.onc.1205598 PMID: 12154400

9. Marsit CJ, Karagas MR, Schned A, Kelsey KT. Carcinogen exposure and epigenetic silencing in bladder cancer. Annals of the New York Academy of Sciences. 2006 Sep; 1076:810–21. doi: 10.1196/annals.1371.031 PMID: 17119258

10. Kandimalla R, van Tilborg AA, Zwarthoff EC. DNA methylation-based biomarkers in bladder cancer. Nature reviews Urology. 2013 Jun; 10(6):327–35. Epub 2013/05/01. eng. doi: 10.1038/nrurol.2013.89 PMID: 23628807

11. Gao T, Wang S, He B, Pan Y, Song G, Gu L, et al. The association of RAS association domain family Protein1A (RASSF1A) methylation states and bladder cancer risk: a systematic review and meta-analysis. PloS one. 2012; 7(11):e48300. Pubmed Central PMCID: PMC3491061. Epub 2012/11/10. eng. doi: 10.1371/journal.pone.0048300 PMID: 23139773

12. D'Cruz CM, Gunther EJ, Boxer RB, Hartman JL, Sintasath L, Moody SE, et al. c-MYC induces mammary tumorigenesis by means of a preferred pathway involving spontaneous Kras2 mutations. Nature medicine. 2001 Feb; 7(2):235–9. doi: 10.1038/84691 PMID: 11175856

13. Schulz WA, Goering W. DNA methylation in urothelial carcinoma. Epigenomics. 2016 Sep 14. Epub 2016/09/15. Eng.

14. Esteller M, Corn PG, Baylin SB, Herman JG. A gene hypermethylation profile of human cancer. Cancer research. 2001 Apr 15; 61(8):3225–9. PMID: 11309270

15. Friedrich MG, Weisenberger DJ, Cheng JC, Chandrasoma S, Siegmund KD, Gonzalgo ML, et al. Detection of methylated apoptosis-associated genes in urine sediments of bladder cancer patients. Clinical cancer research: an official journal of the American Association for Cancer Research. 2004 Nov 15; 10(22):7457–65. Epub 2004/12/01. eng.

16. Tada Y, Wada M, Taguchi K, Mochida Y, Kinugawa N, Tsuneyoshi M, et al. The association of death-associated protein kinase hypermethylation with early recurrence in superficial bladder cancers. Cancer research. 2002 Jul 15; 62(14):4048–53. Epub 2002/07/19. eng. PMID: 12124340

17. Jablonowski Z, Reszka E, Gromadzinska J, Wasowicz W, Sosnowski M. Hypermethylation of p16 and DAPK promoter gene regions in patients with non-invasive urinary bladder cancer. Archives of medical science: AMS. 2011 Jun; 7(3):512–6. Pubmed Central PMCID: PMC3258754. Epub 2012/02/02. eng. doi: 10.5114/aoms.2011.23421 PMID: 22295037

18. Vinci S, Giannarini G, Selli C, Kuncova J, Villari D, Valent F, et al. Quantitative methylation analysis of BCL2, hTERT, and DAPK promoters in urine sediment for the detection of non-muscle-invasive urothelial carcinoma of the bladder: a prospective, two-center validation study. Urologic oncology. 2011 Mar-Apr; 29(2):150–6. Epub 2009/03/11. eng. doi: 10.1016/j.urolonc.2009.01.003 PMID: 19272801

19. Chen PC, Tsai MH, Yip SK, Jou YC, Ng CF, Chen Y, et al. Distinct DNA methylation epigenotypes in bladder cancer from different Chinese sub-populations and its implication in cancer detection using voided urine. BMC medical genomics. 2011; 4:45. Pubmed Central PMCID: PMC3127971. Epub 2011/05/24. eng. doi: 10.1186/1755-8794-4-45 PMID: 21599969

20. Wolff EM, Liang G, Cortez CC, Tsai YC, Castelao JE, Cortessis VK, et al. RUNX3 methylation reveals that bladder tumors are older in patients with a history of smoking. Cancer research. 2008 Aug 1; 68 (15):6208–14. Pubmed Central PMCID: PMC2536768. Epub 2008/08/05. eng. doi: 10.1158/0008-5472.CAN-07-6616 PMID: 18676844

21. Chan MW, Chan LW, Tang NL, Tong JH, Lo KW, Lee TL, et al. Hypermethylation of multiple genes in tumor tissues and voided urine in urinary bladder cancer patients. Clinical cancer research: an official journal of the American Association for Cancer Research. 2002 Feb; 8(2):464–70. Epub 2002/02/13. eng.

22. Park HJ, Lee EJ, Ha SY, Kwon GY, Oh YL, Kim K-M, et al. Prognostic Significance of Methylation Profiles in Urothelial Carcinomas of the Bladder. Korean Journal of Pathology. 2010 2010; 44(6):623–30.

23. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. European journal of epidemiology. 2010 Sep; 25(9):603–5. Epub 2010/07/24. eng. doi: 10.1007/s10654-010-9491-z PMID: 20652370

24. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986 Sep; 7(3):177–88. Epub 1986/09/01. eng. PMID: 3802833

25. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003 Sep 6; 327(7414):557–60. Pubmed Central PMCID: PMC192859. Epub 2003/09/06. eng. doi: 10.1136/bmj.327.7414.557 PMID: 12958120

26. DerSimonian R. Meta-analysis in the design and monitoring of clinical trials. Statistics in medicine. 1996 Jun 30; 15(12):1237–48; discussion 49–52. Epub 1996/06/30. eng. doi: 10.1002/(SICI)1097-0258 (19960630)15:12<1237::AID-SIM301>3.0.CO;2-N PMID: 8817798

27. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997 Sep 13; 315(7109):629–34. Pubmed Central PMCID: PMC2127453. Epub 1997/10/06. eng. PMID: 9310563

28. Motlagh FZ, Fard NA, Majidizadeh T, Asgari M, Abolhasani M, Shahshahanipour M, et al. Effect of DAPK methylation and Bcl2 over expression on risk of recurrence in Transitional Cell Carcinoma of bladder. Journal of Biological Research-Thessaloniki. 2013 2013; 19:56–64.

29. Nakagawa T, Kanai Y, Ushijima S, Kitamura T, Kakizoe T, Hirohashi S. DNA hypermethylation on multiple CpG islands associated with increased DNA methyltransferase DNMT1 protein expression during multistage urothelial carcinogenesis. The Journal of urology. 2005 May; 173(5):1767–71. doi: 10.1097/01.ju.0000154632.11824.4d PMID: 15821584

30. Christoph F, Weikert S, Kempkensteffen C, Krause H, Schostak M, Miller K, et al. Regularly methylated novel pro-apoptotic genes associated with recurrence in transitional cell carcinoma of the bladder. International journal of cancer Journal international du cancer. 2006 Sep 15; 119(6):1396–402. Epub 2006/04/28. eng. doi: 10.1002/ijc.21971 PMID: 16642478

31. Yates DR, Rehman I, Meuth M, Cross SS, Hamdy FC, Catto JW. Methylational urinalysis: a prospective study of bladder cancer patients and age stratified benign controls. Oncogene. 2006 Mar 23; 25 (13):1984–8. Epub 2005/11/17. eng. doi: 10.1038/sj.onc.1209209 PMID: 16288222

32. Yates DR, Rehman I, Abbod MF, Meuth M, Cross SS, Linkens DA, et al. Promoter hypermethylation identifies progression risk in bladder cancer. Clinical cancer research: an official journal of the American Association for Cancer Research. 2007 Apr 1; 13(7):2046–53. Epub 2007/04/04. eng.

33. Ellinger J, El Kassem N, Heukamp LC, Matthews S, Cubukluoz F, Kahl P, et al. Hypermethylation of cell-free serum DNA indicates worse outcome in patients with bladder cancer. The Journal of urology. 2008 Jan; 179(1):346–52. Epub 2007/11/17. eng. doi: 10.1016/j.juro.2007.08.091 PMID: 18006010

34. Jarmalaite S, Jankevicius F, Kurgonaite K, Suziedelis K, Mutanen P, Husgafvel-Pursiainen K. Promoter hypermethylation in tumour suppressor genes shows association with stage, grade and invasiveness of bladder cancer. Oncology. 2008; 75(3–4):145–51. Epub 2008/10/01. eng. doi: 10.1159/000158665 PMID: 18824877

35. Hellwinkel OJ, Kedia M, Isbarn H, Budaus L, Friedrich MG. Methylation of the TPEF- and PAX6-promoters is increased in early bladder cancer and in normal mucosa adjacent to pTa tumours. BJU international. 2008 Mar; 101(6):753–7. Epub 2007/12/12. eng. doi: 10.1111/j.1464-410X.2007.07322.x PMID: 18070176

36. Brait M, Begum S, Carvalho AL, Dasgupta S, Vettore AL, Czerniak B, et al. Aberrant promoter methylation of multiple genes during pathogenesis of bladder cancer. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2008 Oct; 17(10):2786–94. Pubmed Central PMCID: 2778751. doi: 10.1158/1055-9965.EPI-08-0192 PMID: 18843024

37. Sobti RC, MalekZadeh K, Nikbakht M, Sadeghi IA, Shekari M, Singh SK. Hypermethylation-mediated partial transcriptional silencing of DAP-kinase gene in bladder cancer. Biomarkers: biochemical indicators of exposure, response, and susceptibility to chemicals. 2010 Mar; 15(2):167–74. Epub 2009/12/05. eng.

38. Maruyama R, Toyooka S, Toyooka KO, Harada K, Virmani AK, Zochbauer-Muller S, et al. Aberrant promoter methylation profile of bladder cancer and its relationship to clinicopathological features. Cancer research. 2001 Dec 15; 61(24):8659–63. Epub 2001/12/26. eng. PMID: 11751381

39. Friedrich MG, Chandrasoma S, Siegmund KD, Weisenberger DJ, Cheng JC, Toma MI, et al. Prognostic relevance of methylation markers in patients with non-muscle invasive bladder carcinoma. European journal of cancer (Oxford, England: 1990). 2005 Nov; 41(17):2769–78. Epub 2005/10/26. eng.

40. Neuhausen A, Florl AR, Grimm MO, Schulz WA. DNA methylation alterations in urothelial carcinoma. Cancer biology & therapy. 2006 Aug; 5(8):993–1001. Epub 2006/06/16. eng.

41. Jarmalaite S, Andrekute R, Scesnaite A, Suziedelis K, Husgafvel-Pursiainen K, Jankevicius F. Promoter hypermethylation in tumour suppressor genes and response to interleukin-2 treatment in bladder cancer: a pilot study. Journal of cancer research and clinical oncology. 2010 Jun; 136(6):847–54. Epub 2009/11/20. eng. doi: 10.1007/s00432-009-0725-y PMID: 19924441

42. Guo S, Tan L, Pu W, Wu J, Xu K, Wu J, et al. Quantitative assessment of the diagnostic role of APC promoter methylation in non-small cell lung cancer. Clinical epigenetics. 2014; 6(1):5. Pubmed Central PMCID: 3997934. doi: 10.1186/1868-7083-6-5 PMID: 24661338

43. Esteller M, Herman JG. Cancer as an epigenetic disease: DNA methylation and chromatin alterations in human tumours. The Journal of pathology. 2002 Jan; 196(1):1–7. Epub 2001/12/19. eng. doi: 10.1002/path.1024 PMID: 11748635

44. Chanda S, Dasgupta UB, Guhamazumder D, Gupta M, Chaudhuri U, Lahiri S, et al. DNA hypermethylation of promoter of gene p53 and p16 in arsenic-exposed people with and without malignancy. Toxicological sciences: an official journal of the Society of Toxicology. 2006 Feb; 89(2):431–7. Epub 2005/10/28. eng.

45.  Karpf AR, Jones DA. Reactivating the expression of methylation silenced genes in human cancer. Oncogene. 2002 Aug 12; 21(35):5496–503. doi: 10.1038/sj.onc.1205602 PMID: 12154410

46.  Raveh T, Droguett G, Horwitz MS, DePinho RA, Kimchi A. DAP kinase activates a p19ARF/p53-mediated apoptotic checkpoint to suppress oncogenic transformation. Nature cell biology. 2001 Jan; 3(1):1–7. doi: 10.1038/35050500 PMID: 11146619

47.  Li Y, Zhu M, Zhang XJ, Cheng DJ, Ma XT. Clinical significance of DAPK promoter hypermethylation in lung cancer: a meta-analysis. Drug Des Dev Ther. 2015; 9:1785–96. English.

48.  Christoph F, Kempkensteffen C, Weikert S, Kollermann J, Krause H, Miller K, et al. Methylation of tumour suppressor genes APAF-1 and DAPK-1 and in vitro effects of demethylating agents in bladder and kidney cancer. British journal of cancer. 2006 Dec 18; 95(12):1701–7. Pubmed Central PMCID: PMC2360762. Epub 2006/11/30. eng. doi: 10.1038/sj.bjc.6603482 PMID: 17133271

49.  Xu NR, Liu CX, Zheng SB, Li HL, Xu YW, Xu K. [Reversion transcriptional expression of DAPK in bladder cancer T24 cells 5-aza-2’-deoxycytidine]. Nan fang yi ke da xue xue bao = Journal of Southern Medical University. 2009 Sep; 29(9):1882–6. Epub 2009/09/26. chi. PMID: 19778817

50.  Chen WT, Hung WC, Kang WY, Huang YC, Chai CY. Urothelial carcinomas arising in arsenic-contaminated areas are associated with hypermethylation of the gene promoter of the death-associated protein kinase. Histopathology. 2007 Dec; 51(6):785–92. Epub 2007/10/24. eng. doi: 10.1111/j.1365-2559.2007.02871.x PMID: 17953697

51.  Chai CY, Huang YC, Hung WC, Kang WY, Chen WT. Arsenic salts induced autophagic cell death and hypermethylation of DAPK promoter in SV-40 immortalized human uroepithelial cells. Toxicology letters. 2007 Aug 30; 173(1):48–56. Epub 2007/08/09. eng. doi: 10.1016/j.toxlet.2007.06.006 PMID: 17683884