

# Meta-analysis of the Association Between APC Promoter Methylation and Bladder Cancer

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## Research Article

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# Abstract

Bladder cancer (BC) is a worldwide disease that affects a large number of people. This study analyzes the sensitivity and specificity of adenomatous polyposis coli (APC) methylation for BC detection in urine and tissues. Combining search results from PubMed and Embase, 20 studies were included. In tissue subgroups, the OR was 6.88 (95% confidence interval (CI) 2.49–19.03,  $P < 0.001$ ), respectively. From urine studies, the pooled OR was 4.73 at a 5% significance level (95% CI 0.87-25.66,  $P < 0.001$ ). In addition, analysis of the interaction between APC methylation and BC showed strong association in the whole data set. From this study, the results suggest that APC promoter methylation may be the potential testing for BC diagnosis and provide a new viewpoint in the treatment of BC.

## Introduction

Bladder cancer (BC) is one of the most prevalent type of urothelial cell carcinoma in industrialized countries, with papillary urothelial carcinoma of transitional cell origin as the predominant histologic type, and its incidence shows a yearly increasing trend<sup>1</sup>. Five-year overall survival rates in patients with invasive BC depend on the tumor infiltration depth. Although superficial bladder cancer generally has a good long-term prognosis, up to 80 % of patients will have local recurrence within 5 years of the primary tumor resection, which necessitates life-long cystoscopic follow-up and frequent transurethral resections (TURs)<sup>2</sup>.

## Methods

### Search strategy

We conducted a literature search using the PubMed (search updated to MAY 2018; <http://www.ncbi.nlm.nih.gov/pubmed/>), Embase, and MEDLINE (search updated to MAY 2018; <http://www.embase.com/>) databases using the following search terms located in either the title or abstract: ((methylation) AND (((((((Bladder Cancer) OR Cancer of the Bladder) OR Malignant Tumor of Urinary Bladder) OR Urinary Bladder Cancer) OR Tumor, Bladder) OR Bladder Tumors) OR Bladder Neoplasm) OR Neoplasms, Bladder) OR Neoplasm, Urinary Bladder)) AND (((apc) OR APC Genes) OR Genes, Adenomatous Polyposis Coli) OR APC Gene [mesh]). The search was limited to human studies. Additionally, we hand-searched the references of the review articles and, as needed, contacted the first author of a given paper to obtain any missing data.

### Study selection

Studies were selected for the current meta-analysis according to the following criteria: they determined APC promoter methylation in specimens of bladder tissue, blood, plasma, serum, buffy coat, or urine; and they provided sufficient information to evaluate odds ratios (ORs) and 95% confidence intervals (CIs). In addition, when the same author or group reported results that were obtained from the same patient population in more than one article, only the most recent or most informative report was included. Our

exclusion criteria were: review articles or conference reports; a lack of information about the degree of APC promoter methylation in patient cases and controls; and when screening for methylation of the APC promoter was conducted in cell lines.

## Quality assessment

The assessment of study quality was conducted independently by two reviewers using the Newcastle-Ottawa Scale (NOS). The NOS evaluation system consists of three parameters (selection, comparability, and outcome) and assigns a maximum of four points for selection, two points for comparability, and three points for outcome. A NOS score  $> 6$  indicates a higher quality study, whereas a score  $\leq 6$  indicates a lower quality study. Any discrepancies between the two reviewers were settled by a third reviewer.

## Data extraction

The data were extracted independently by two authors, and discrepancies were resolved by consensus including a third author. The data were collected using a pilot-tested data extraction form that included the following items: the first author's name; the year of publication; the number of participants that exhibited APC promoter methylation both among specific cases and controls; the screening methods used; and the demographic and clinical characteristics of the patients. All procedures conformed to the established guidelines for the meta-analysis of observational studies in epidemiology.

## Statistical analysis

The meta-analysis was performed using Stata software (version 12; StataCorp LP, College Station, TX, USA). The association between APC promoter methylation and the risk of developing BC or its clinical characteristics (such as in tissue, in urine, as well as differences, recurrence or primary, based on patient sex) was measured either by weighted OR by taking into account the 95% CI. We tested for heterogeneity among the studies using the chi-square-based Q-test and the I<sup>2</sup> statistic of inconsistency. Significant heterogeneity was defined as a chi-square test P-value  $< 0.10$  or as an I<sup>2</sup> statistic  $> 50\%$ . We used a random-effects model when significant heterogeneity was observed among the studies; otherwise, we used a fixed-effects model. For a two-tailed significance level of 5%, the probability of rejecting the null hypothesis when it was false was termed the power, which was defined as  $1 - \beta$ . Funnel plots and Egger's test were used to assess publication bias. Additionally, prespecified subgroup analyses, which included the patients' ethnicities, as well as the test samples, testing methods, and sample sizes, were conducted to evaluate potential sources of heterogeneity within the studies investigating the association between APC promoter methylation and the risk of BC. Moreover, sensitivity analyses were performed to examine the influence of each study on the pooled OR by serially omitting each individual study and pooling the remaining studies.

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## Results

# Results of the literature search

Illustrates the detailed process used for study selection. In summary, a total of 225 articles were initially identified; of these, 200 articles were excluded, either based on duplicate results or because they were deemed to be irrelevant to this meta-analysis after careful review of the titles and abstracts. Among the 25 studies that remained, an additional 5 articles were excluded for various reasons: 1 were review articles; 2 were discarded because screening of APC promoter methylation was performed using cell lines; and 2 were excluded because of a lack of data on the association between APC promoter methylation and the development of BC. Thus, 20 articles were ultimately selected for inclusion in the meta-analysis.

## Study characteristics

The characteristics of the studies that met all of the established inclusion criteria for the meta-analysis are presented in Table 1. 9 studies used bladder tissue specimens to screen for APC promoter methylation, while 8 studies used urine. The numbers of articles that reported the associations between APC promoter methylation and sex of the BC patients was 2. For the detection of APC promoter methylation, 18 studies used methylation-specific polymerase chain reaction, 2 used quantitative real-time methylation-specific polymerase chain reaction (Q-MSP).

## Data quality

We estimated the quality of the studies using the NOS evaluation system, and the results showed that 15 of the 19 studies were classified as high-quality (NOS score > 6) and the remaining four trials were classified as lower-quality. The mean NOS score of the studies was 7. Most of the studies did not use community controls when we conducted an assessment of comparability.

## Methylation of the APC promoter and BC

As shown in Fig. 2, 9 studies comprising 511 patients evaluated the association between APC promoter methylation and BC in tissue, (pooled OR 6.88; 95% CI 2.49–19.03,  $P < 0.001$ ). 8 studies assessed the association between APC promoter methylation and BC in urine, and the pooled OR was 4.73 at a 5% significance level (95% CI 0.87–25.66,  $P < 0.001$ ; Fig. 3). 2 studies assessed the association between APC promoter methylation and recurrence or primary BC, and the pooled OR was 1.44 at a 5% significance level (95% CI 0.25–8.17,  $P < 0.05$ ). We also evaluated the association between APC promoter methylation and patient sex, and no significant association was identified between APC promoter methylation and patient sex (Table 2).

## Discussion

Currently, the gold standard for bladder cancer detection remains to be cystoscopy followed by histological examination for making the initial diagnosis and monitoring progression of bladder tumors. Although this approach provides valuable prognostic information regarding tumor status, it involves an invasive procedure that causes considerable discomfort to the patients which shows insufficient power to

predict precisely the patient outcome and 10–40% of malignancies may be undetected. Because of the risk of recurrence, bladder cancer is a disease that requires life-long surveillance with periodic cystoscopy. Alternatively, urine cytology which has been used for decades is the standard noninvasive method for cancer detection. But the sensitivity of cytology is specifically insufficient for the detection of low stage and grade bladder tumors, whereby its accuracy is influenced by the pathologist's experience.

An array of urine markers have been identified to improve the diagnostic ability of urine cytology and, perhaps, reduce the number of cystoscopies, consequently improving patient quality of life and patient care. In view of this, there is a dire need to identify prognostic biomarkers that can serve as reliable indicators of disease and predict recurrence more accurately. Over the past two decades a number of biomarkers have been identified and approved by FDA for screening of bladder cancers but initial enthusiasm for their clinical utility waned quickly because of lacked specificity, reproducibility as well as sensitivity, especially for low grade and stage of bladder cancer<sup>3</sup>. Hence the clinical applicability of these markers remains limited. Therefore, a more sensitive and noninvasive method is imperative for efficient BC detection.

Epigenetic changes are defined as changes in gene expression that are heritable through cell division, without associated DNA sequence alterations. DNA methylation occurs in distinct regions of promoters where cytosine residues located at the 5' position of guanines in CpG dinucleotides which is not randomly distributed but is especially important in CpG-rich areas found in over 60% of the human genes, also called CpG islands. Hypermethylation of promoter CpG islands and somatic mutations are among the most common and specific types of genome alterations in human cancer and represent causative events in tumor development. Promoter hypermethylation negatively influences transcription and mutations may lead to activation of proto-oncogenes or inactivation of tumor suppressor genes.

Some epigenetic alterations occur early during tumorigenesis has been intensively investigated over the last ten years<sup>4,5</sup>, and could be used as targets for the molecular diagnosis of neoplastic cells in clinical specimens such as biological fluids that are readily accessible. Aberrant DNA promoter hypermethylation has been described in bladder cancer and have shown promising results. It seems to be an early event in the development of a number of solid tumors including bladder cancer<sup>6,7</sup> and can thus be regarded as an early sign of cancer before the disease becomes muscle-invasive. The CpG methylation in BC has been reported to occur frequently in bladder cancer and to be associated with age, smoking status, gender, tumor location, stage, recurrence rate and progression<sup>8–12</sup>. In addition to detection of methylation in tumor tissue, analysis of gene methylation has been shown to be feasible from body fluids, including voided urine of patients with BC and appears to be more sensitive than urine cytology.

In bladder cancer, a large number of genes have been shown to harbor promoter hypermethylation, including Adenomatous Polyposis Coli (*APC*) as one of the most consistent and frequent targets. Expression and function of *APC* is known to be impacted either by physical changes in the sequence of DNA or by unprogrammed DNA methylation<sup>13,14</sup>. It is isolated and mapped at chromosomal band 5q21<sup>15,16</sup>. Loss of *APC* function results in nuclear accumulation of  $\beta$ -catenin, which acts as a

transcriptional activator, ultimately leading to loss of cellular growth control<sup>17,18</sup>. Many studies have reported a high methylation frequency of *APC* promoter region in bladder cancer<sup>19–21</sup>. And DNA hypermethylation at the *APC* promoter correlated with cancer specific mortality following radical cystectomy.

Meta-analysis was used to evaluate the association between *APC* methylation and malignant tumors of bladder cancer, and to provide clues for further exploring its pathogenic effects and pathways in these diseases.

## Declarations

## Data availability

All data generated or analyzed during the present study are included in this published article.

## Contribution

Xuedong Chen and Peng Li conceived of the presented idea. Qi Wu developed the theory and performed the computations. Xuedong Chen and Qi Wu verified the analytical methods. Fuchen Xie encouraged Xuedong Chen to investigate and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

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## Conflict of interest

No conflicts exist for any specified author.

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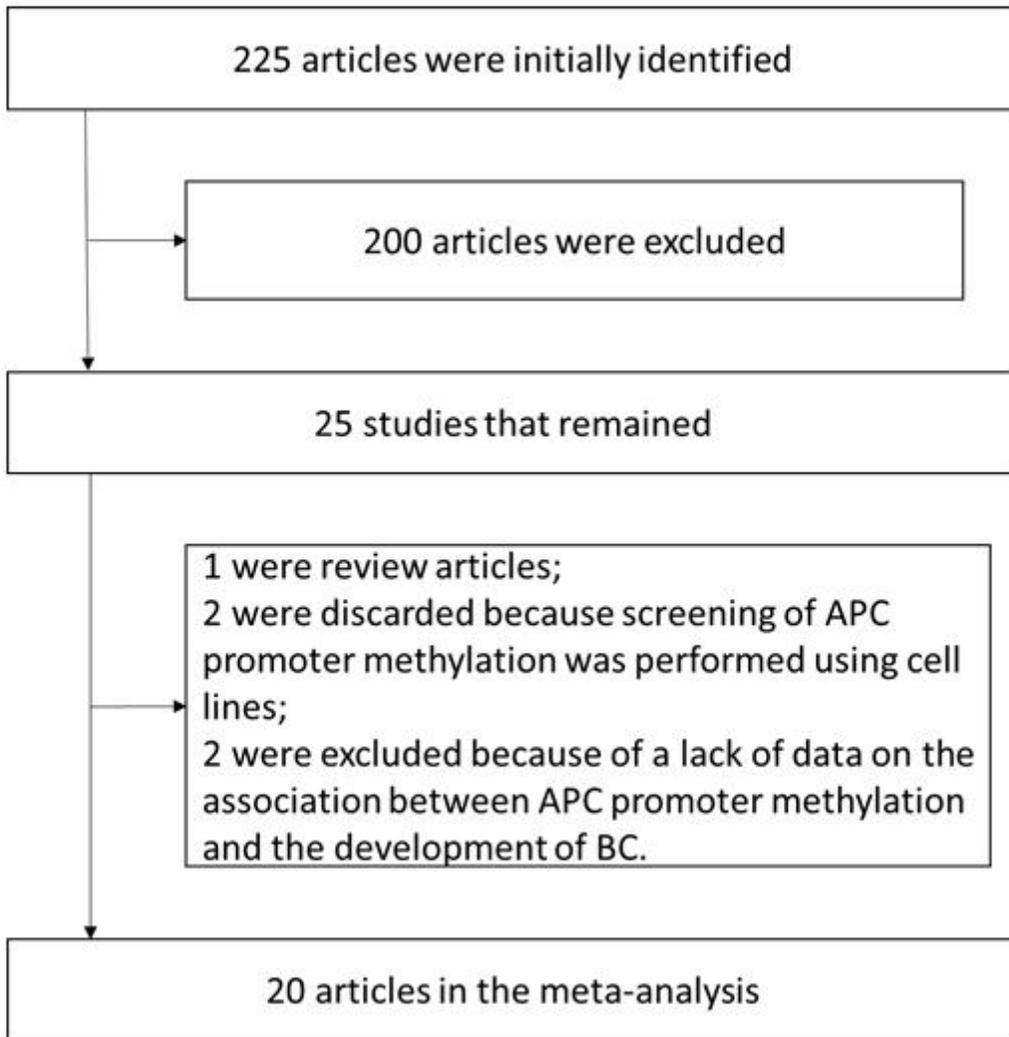
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## Tables

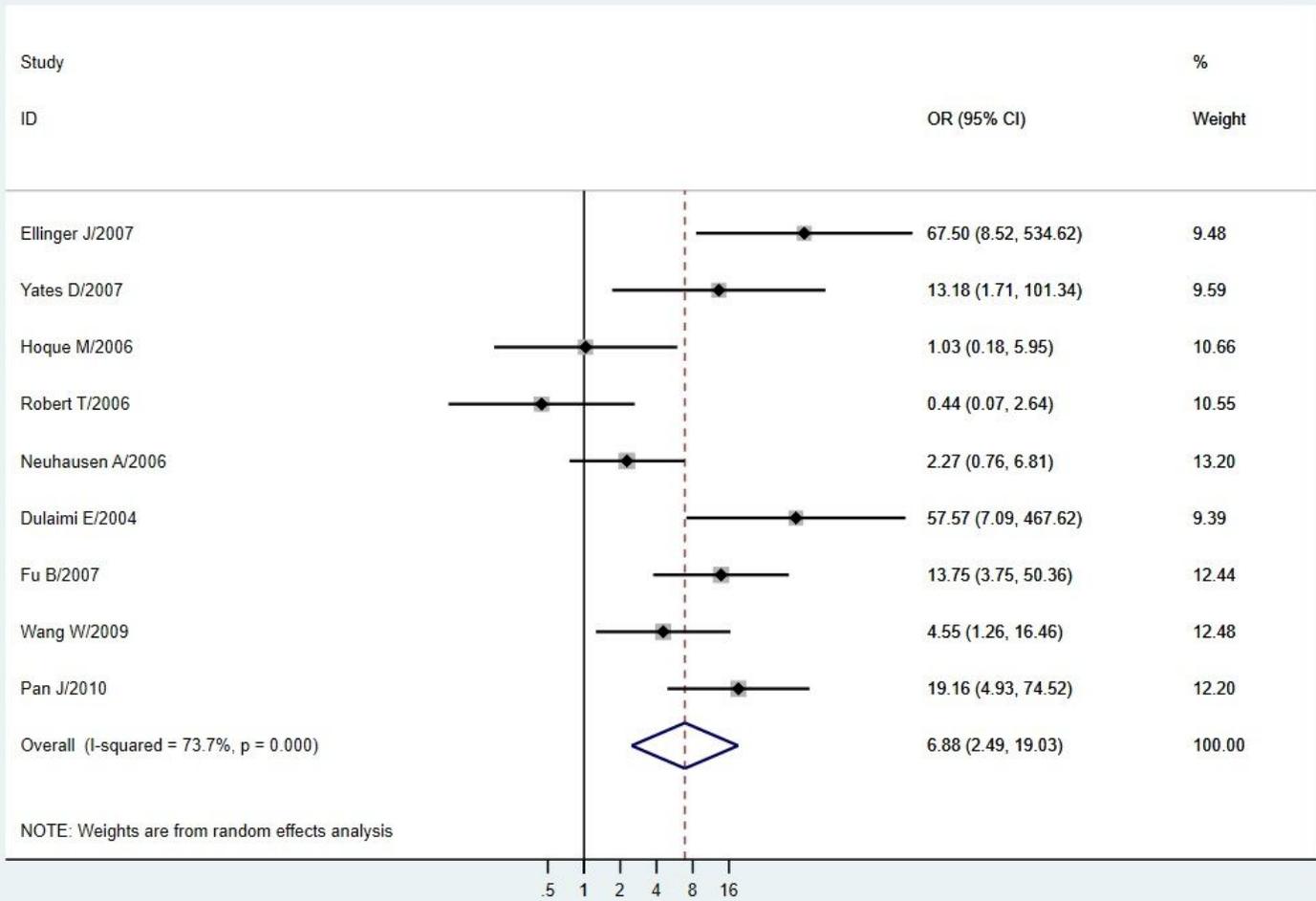
Due to technical limitations, table 1 and 2 tif are only available as a download in the Supplemental Files section.

## Figures



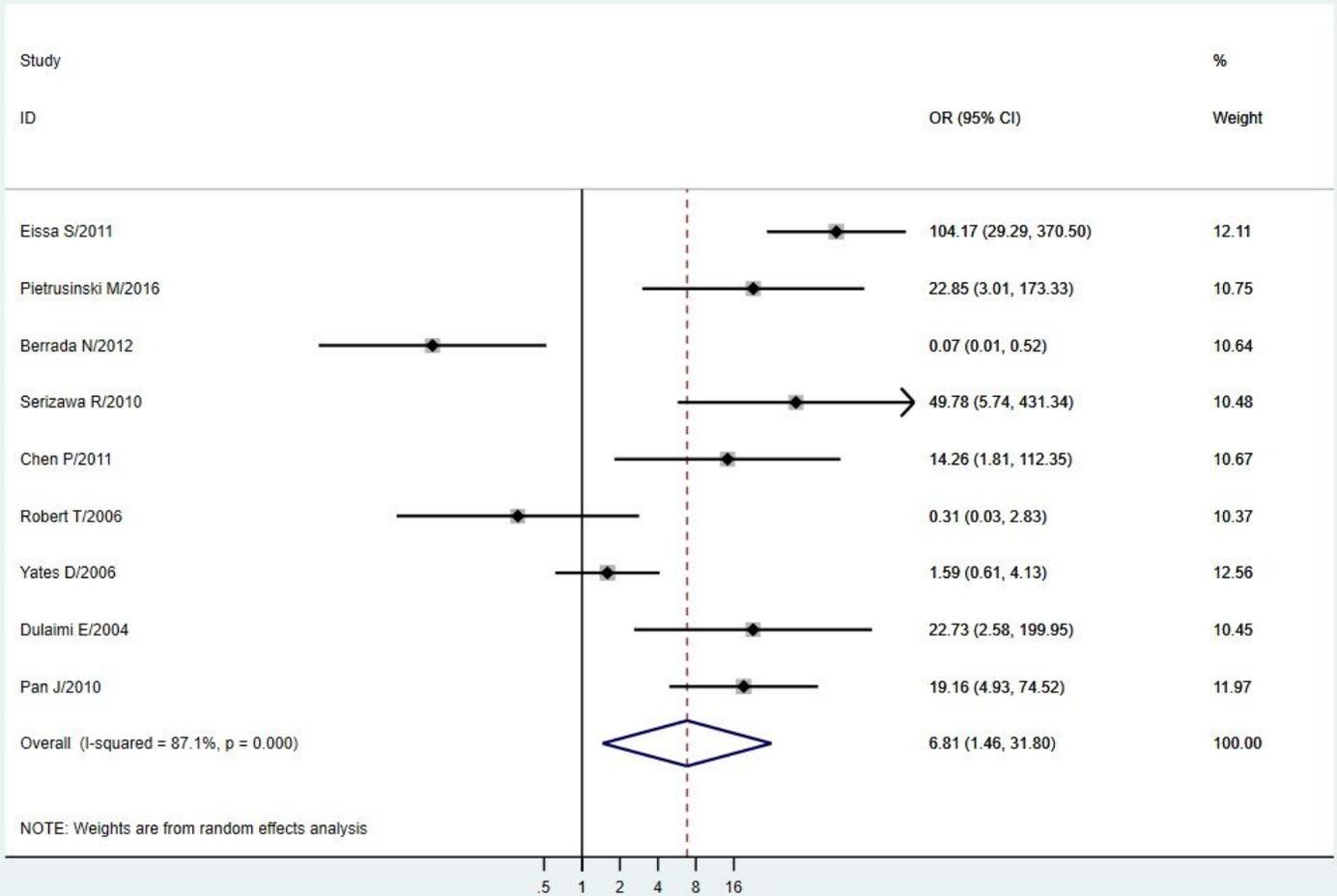
**Figure 1**

Flow diagram of the study selection process showing the number of eligible articles included in this meta-analysis.



**Figure 2**

Meta-analysis of the association between APC promoter methylation and the risk of bladder cancer in tissue. The circles and horizontal lines correspond to the study specific OR and 95% CI. The sizes of the data markers indicate the weight of each study in the analysis.



**Figure 3**

Meta-analysis of the association between APC promoter methylation and the risk of bladder cancer in urine. The circles and horizontal lines correspond to the study specific OR and 95% CI. The sizes of the data markers indicate the weight of each study in the analysis.

## Supplementary Files

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- [Table1.tif](#)
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