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ARTICLE

APC gene hypermethylation and prostate cancer: a systematic review and meta-analysis

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Prostate cancer (PCa) is a worldwide disease that affects a large number of males. Although prostate-specific antigen (PSA) screening is used, the specificity is limited. This study analyzes the sensitivity and specificity of adenomatous polyposis coli (APC) methylation for PCa detection in body fluids and tissues. Combining search results from PubMed and Embase, 19 studies were included, 5 involving body fluids and 14 involving prostate tissue, with 2344 subjects. In body fluid subgroups, the pooled sensitivity and specificity was 0.53 (95% confidence interval (CI): 0.28–0.78) and 0.92 (95% CI: 0.86–0.95), respectively. From tissue studies, the results presented as 0.84 (95% CI: 0.70–0.92) and 0.91 (95% CI: 0.77–0.97). To confirm the results, we conducted a further analysis by removing studies which introduced high heterogeneity due to the type of cases and controls. The same degree of sensitivity and specificity was presented in two subgroups (urine: sensitivity 0.46, 95% CI: 0.39–0.53; specificity 0.87, 95% CI: 0.64–0.96; tissue: sensitivity 0.87, 95% CI: 0.72–0.94; specificity 0.89, 95% CI: 0.68–0.97). In addition, analysis of the interaction between APC methylation and PCa showed strong association in the whole data set (odds ratio (OR) = 24.91, 95% CI: 12.86–48.24, $I^2 = 72.5\%$). Pooling the same two main subgroups (tissue/fluid) gave a pooled OR of 33.54 (95% CI: 14.88–75.59; $I^2 = 70.7\%$) and 8.20 (95% CI: 2.84–23.74, $I^2 = 64.2\%$), respectively. From this study, the results suggest that APC promoter methylation may be the potential testing for PCa diagnosis and provide a new viewpoint in the treatment of PCa.

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Keywords: hypermethylation; methylation; prostate cancer; adenomatous polyposis coli

INTRODUCTION

Prostate cancer (PCa) is one of the most common cancers in the western world[1] and is said to be the most frequently detected male cancer and the second most frequent cause of male cancer deaths.[2] In 2009, it was suggested that one in six men would be affected, involving 192 280 new cases of PCa and 27 360 PCa-related deaths in the United States.[3] However, significant symptoms can be found in only about half of all diagnosed patients, which indicate the low diagnosis and high mortality rate.[4]

With an increasing rate of morbidity of 3% per year over several decades,[5] reliable methods of diagnosis are needed urgently. In the 1990s, prostate-specific antigen (PSA) testing became widespread,[6] which provided a new approach in the diagnosis of PCa. Disappointingly, although serum PSA is generally used in PCa screening, the poor baseline values and low specificity limits its functions.[7]

DNA methylation of gene promoters may provide the ideal works. There are important advantages of using DNA methylation as cancer biomarkers. In particular, methylated DNA can be detected with a high degree of specificity and sensitivity,[8] which promotes its application to minimal samples from PCa patients. To date, over 50 hypermethylated loci have been identified in PCa.[9,10] Among these loci, adenomatous polyposis coli (APC) is a well-characterized tumor-suppressor gene. The *APC* gene is located on the long (q) arm of chromosome 5 between positions 21 and 22, between 112 118 468 and 112 209 532 base pairs (bp). Methylation of the genes is associated with PCa.

The purpose of this study was to conduct a meta-analysis of the sensitivity and specificity of APC methylation on PCa detection in body fluid (blood and urine) and prostate tissues. The results of this article will help to provide a reliable biomarker for the diagnosis and discrimination of PCa. We also determined whether APC methylation was correlated with pathological stage, Gleason score and PSA level among the cases.

MATERIALS AND METHODS

Study selection

We conducted a comprehensive literature search of PubMed and Embase databases using the keywords 'prostate cancer', 'PCa', 'prostate adenocarcinoma', 'APC' and 'adenomatous polyposis coli'. Additional studies were found via the reference lists of the identified articles. The last retrieval was conducted in October 2012. Our inclusion criteria were as follows: (1) measurement of DNA methylation in one of the following samples: blood, plasma, serum, buffy coat, urine, ejaculates, or prostate tissues; (2) a case–control study; and (3) published in English language. Our exclusion criteria were: (1) APC methylation conducted in the cell lines; (2) unavailable raw data on the amount of methylation among cases and controls, respectively (some studies reported specificity and sensitivity without the exact counts); (3) review paper. The selection process for studies included in our review is shown in Figure 1. Our

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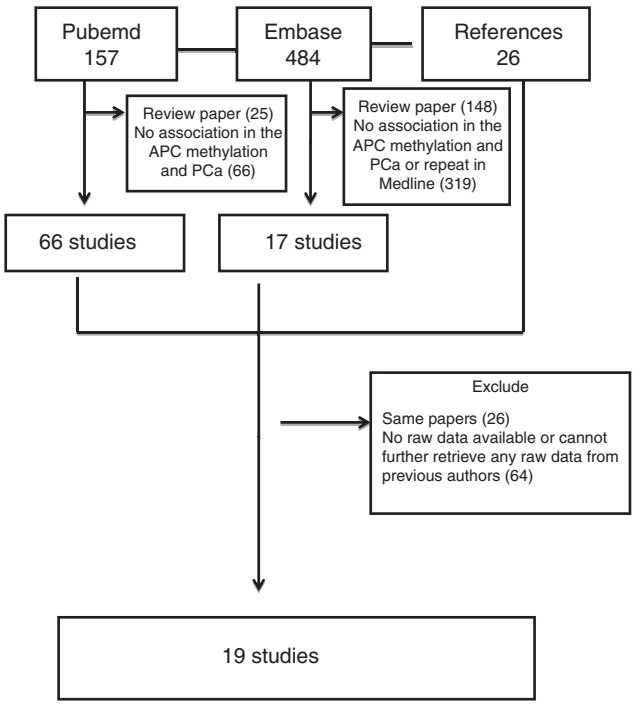

Figure 1 Flow diagram for the selection of studies.

search strategy and application of the inclusion/exclusion criteria resulted in a total of 19 articles that were included in the systematic review.[11–29] A description of the included studies is given in Tables 1 and 2. The following data were recorded for each study: author's name, year of publication, sample forms, method, 5′-3′ primers (forward and reverse, respectively), amplicon size (bp) and annealing temperature (°C), country, race, cancer clinical classification, PSA, Gleason score, type of cases and controls, type of PCR method and other relevant characteristics of the study population.

Sensitivity and specificity analysis

The normalized index of methylation (NIM) and receiver operator characteristic (ROC) curves were applied in most analyses. NIM was a color-scaled figure in which white represented NIM of zero (no methylation detected) and red defined a NIM of 0.99 (99% of input DNA is methylated). NIM was defined in any given sample to be the ratio of the normalized amount of methylated templates at the promoter of interest to the normalized amount of converted MYOD1 templates (NIM = [(GENE sample)/(GENE SssI)]/[(MYODI sample)/(MYOD SssI)]). Here, GENE sample and GENE SssI were said to be the number of entirely methylated copies of the gene of interest in a given sample. Similar definitions were applied to MYOD1 sample and MYOD1 SssI. In addition, the optimal threshold for methylation was determined on the basis of the area under the ROC curve.[26] Combining the NIM and ROC curve; the numbers of the methylation were recorded.

Before we conducted sensitivity and specificity analyses, the amount of case and control methylation were collected. Among the included studies, two categories were assigned as controls: (1) patients who had negative biopsies but had other diseases including benign prostatic hyperplasia (BPH), and (2) healthy controls. Nevertheless, biopsy-confirmed PCa and high-grade prostatic intraepithelial neoplasia (HGPIN) were treated as cases. Thus, the true-positive (TP) samples were limited to those that had methylation within the exact cases. Meanwhile, in the case samples, the false-positive (FP) ones were indicated to have no methylation. The same definitions was given for true negative (TN) and false negative (FN) in controls. All analyses were conducted with the Midas system in Stata.

Owing to the different types of samples in the eligible studies, we conducted a further analysis to present more robust results on APC methylation as the detection marker. In this analysis, the fluid and tissue subgroups were processed. The types of the samples were described in detail. Among the tissue subgroup, we excluded studies that had other cancer diseases samples (such as lung cancer and bladder cancer) because APC methylation might be also expressed in the different cancers. The analysis of the sensitivity and specificity was then conducted as above. The same processes were applied in the fluid subgroup.

Association analysis

Odds ratios (ORs) with corresponding 95% confidence intervals (CIs) were used to describe the effect of the association between APC methylation and PCa, pathological stage, Gleason score, and PSA levels. The pathological stages were categorized into two subgroups: T1/T2 and T3/T4.[27] For the Gleason score, a score of 7 was used as a cutoff. PSA levels were dichotomized as less or greater than 4 ng/ml. On the basis of individual study ORs, pooled OR was estimated. According to the heterogeneity statistic I^2, a fixed effect or a random-effects model was selected: a fixed effect model was used when $I^2 < 50\%$, otherwise a random-effects model was used. In addition, when the results of the constituent studies differed among themselves, the effects incorporated an estimate of the inter-study variance and therefore provided wider 95% CI. At the same time, the I^2-based Q statistic was used, which describes the weighted sum of the squared difference between the overall effect size and the effect size from each study, to assess heterogeneity ($P < 0.10$ as the standard).[30]

ROC analysis

To assess whether variation in the threshold definition of a positive result produced an association between sensitivity and specificity values across studies, we calculated the summary receiving operating characteristic (S-ROC) curve.[31] The logits of the TP and FP rates were used to estimate the linear regression of the log-OR from each study. Independent analysis of pooled sensitivity and specificity using standard methods for binary data were used when the regression between these quantities was null. All data used a log-odds scale (eg, for specificities, the effect size used was log (Spec/(1-Spec)).[32]

Finally, due to the heterogeneity between studies, we performed the Cochrane Q test of heterogeneity for each analysis (based on deviations of observed log-odds from the common log-odds). The analyses were conducted using Stata 9.0 (Stata Corporation, College Station, TX, USA), and all P-values were two-tailed.

RESULTS

Characteristics

After retrieving search results from the PubMed and Embase databases with the associated keywords, there were 157 and 484 articles retrieved from PubMed and Embase, respectively, on PCa and APC methylation/gene. Among these, we identified 95 relevant studies that described PCa and APC methylation. There were 11 and 29 articles that used fluid (urine, blood or others) samples and tissues, respectively; 55 references were conference abstracts, non-experimental studies or otherwise not available, and were removed, resulting in 40 articles. While reading the full texts, 21 articles were removed. Six could not provide data on the methylation among fluid samples (urine and blood), and[33–38] 15 of the 29 articles using tissues had no raw data (the specific numbers of cases and controls with methylation were unclear, which made it difficult to calculate the data required for sensibility and specificity) or studied other factors such as the *TMPRSS2* gene and urothelial carcinomas.[39–53] Finally, 19 studies met the inclusion criteria and were included.[11–29]

Among these 19 studies, 5 involved body fluid (blood, urine and so on)[11–15] and the remaining 14 articles involved sample tissues.[16–29] Ten studies used quantitative real-time methylation-specific PCR (QMSP) to detect APC methylation,[11,13,14,18,19,21–23,25,26] seven were conducted using the method of methylation-specific PCR (MSP),[12,15,17,20,24,27,29] and two studies used pyrosequencing.[16,28] All

Table 1 Primers used to amplify bisulfite converted DNA at APC promoter regions

Study	*Year*	*Method*	*Forward*	*Reverse*	*Amplicon size (bp)*	*Annealing temperature (°C)*
Hoque *et al*	2005	QMSP	5′-GAACCAAAACGCTCCCCAT-3′	5′-TTATATGTCGGTTACGTGCGTTTATAT-3′	NA	NA
Rogers *et al*	2005	MSP	m5′-TATTGCGGAGTGCGGGTC-3′	m5′-TCGACGAACTCCCGACGA-3′	98	60
			u5′-GTGTTTTATTGTGGAGTGTGGGTT-3′	u5′-CCAATCAACAAACTCCCAACAA-3′	108	62
Rouprêt *et al*	2007	QMSP	5′-GAACCAAAACGCTCCCCAT-3′	5′-TTATATGTCGGTTACGTGCGTTTATAT-3′	NA	NA
Rouprêt *et al*	2008	QMSP	5′-GAACCAAAACGCTCCCCAT-3′	5′-TTATATGTCGGTTACGTGCGTTTATAT-3′	NA	NA
Vener *et al*	2008	MSP	NA	NA	NA	NA
Yoon *et al*	2012	Pyrosequencing	First step: 5′-GGTAAGGGGTTAGGGT TAGGTAG-3′	First step: 5′-ACAACACCTCCATTC TATCT-3′	240	59
			Second step: 5′-GGTAAGGGGTTAGGGTTAGG-TAG-3′	Second step: 5′-Biotin-ACTACACCA ATACAA CCACATATC-3′	200	56
Kang *et al*	2004	MSP	m5′-TATTGCGGAGTGCGGGTC-3′	m5′-TCGACGAACTCCCGACGA-3′	98	55
			u5′-GTGTTTTATTGTGGAGTGTGGGTT-3′	u5′-CCAATCAACAAACTCCCAACAA-3′	108	60
Bastian *et al*	2007	QMSP	5′-TTATATGTCGGTTACGTGCGTTTATAT-3′	5′-GAACCAAAACGCTCCCCAT-3′	NA	NA
Ellinger *et al*	2008	QMSP	5′-GAGGGTATATTTTCGAGGGGTAC-3′	5′-CGACTCTACTCAACATTTAAAAACG-3	NA	62
Maruyama *et al*	2002	MSP	m5′-TATTGCGGAGTGCGGGTC-3′	m5′-TCGACGAACTCCCGACGA-3′	NA	64
			u5′-GTGTTTTATTGTGGAGTGTGGGTT-3′	u5′-CCAATCAACAAACTCCCAACAA-3′	NA	62
Jerónimo *et al*	2004	QMSP	5′-GAACCAAAACGCTCCCCAT-3′	5′-TTATATGTCGGTTACGTGCGTTTATAT-3′	NA	NA
Tokumaru *et al*	2004	QMSP	5′-GAACCAAAACGCTCCCCAT-3′	5′-TTATATGTCGGTTACGTGCGTTTATAT-3′	NA	NA
Bastian *et al*	2005	QMSP	m5′-GAGGGTATATTTTCGAGGGGTAC-3′	m5′-AATAAAAAACGCCCTAATCCG-3′	NA	NA
			u5′-GAGGGTATATTTTTGAGGGGTATG-3′	u5′-AATAAAAAACACCCTAATCCACA-3′		
Enokida *et al*	2005	MSP/USP	m5′-TATTGCGGAGTGCGGGTC-3′	m5′-TCGACGAACTCCCGACGA-3′	NA	NA
			u5′-GTGT-TTTATTGTGGAGTGTGGGTT-3′	u5′-CCAATCAACAAAC-TCCCAACAA-3′		
Henrique *et al*	2006	QMSP	5′-GAACCAAAACGCTCCCCAT-3′	5′-TTATATGTCGGTTACGTGCGTTTATAT-3′	NA	NA
Yegnasubramanian *et al*	2004	QMSP	5′-TTATATGTCGGTTACGTGCGTTTATAT-3′	5′-GAACCAAAACGCTCCCCAT-3′	NA	NA
Cho NY *et al*	2007	MSP	5′-TATTGCGGAGTGCGGGTC-3′	5′-TCGACGAACTCCCGACGA-3′	NA	NA
Vasiljević *et al*	2011	Pyrosequencing	NA	NA	NA	NA
Trock *et al*	2011	MSP/USP	Scorpion probe (5′-3′)	Primer (5′-3′)	NA	NA
			GCCGGCGGGTTTTCGACGGGCC GGC-BHQ-HEG-CGAACCAAAACGCTCCCCA	GTCGGTTACGTGCGTTTATATTTAG		

Abbreviations: APC, adenomatous polyposis coli; MSP, methylation-specific PCR; NA, not applicable; QMSP, quantitative real-time methylation-specific PCR.

cases were of PCa or HGPIN and were hospitalized, while controls were limited to be BPH, healthy subjects or those who had genitourinary cancer (bladder carcinoma) with a healthy prostate. In regard to the type of cases, 14 studies were PCa,[11–16,18–20,23,24,27–29] and five were a mixture of PCa, HGPIN and metastasis or other cancer tissues.[17,21,22,25,26] Among the controls, eight studies were normal, biopsy negative, or non-tumor[12–15,17,25,26,29] nine were BPH,[16,18–21,23,24,27,28] and two were a combination of BPH, normal subjects and bladder carcinoma.[11,22] In addition, 14 were Caucasian,[11–15,18–23,25,26,29] four were Asian,[16,17,24,27] and one involved mixed races from different continents[28] who came from United States, United Kingdom, Portugal, Germany, Korea, Japan, France, and China.

Specificity and sensitivity of APC promoter methylation using different types of samples

All the results are shown in Table 3. The pooled specificity for all included studies was 0.91 (95% CI: 0.82–0.95), and the pooled sensitivity was 0.78 (95% CI: 0.63–0.88). For the traditional biomarker, the sensitivity of PSA varied, but the specificity was generally low at about 20%,[32,54] which suggested that the APC methylation test has a much higher specificity than the PSA test. There was no evidence of publication bias ($P = 0.33$). In addition, we classified all samples into two groups according to specimen type (fluid/tissue). Among the fluid studies (urine/blood), the pooled sensitivity and specificity was 0.53 (95% CI: 0.28–0.78) and 0.92 (95% CI: 0.86–0.95), respectively. For the tissue studies, the pooled sensitivity and specificity was 0.84 (95% CI: 0.70–0.92) and 0.91 (95% CI: 0.77–0.97), respectively. Publication bias results are showed in Table 3. The S-ROC curve is showed in Figure 2.

In the extra analysis of the sensitivity and specificity, among the studies, we excluded those that would likely introduce high heterogeneity due to different types of cases and controls. Finally, one study[13] and four articles[19,20,22,26] in the fluid and tissue subgroups, respectively, with different samples. The pooled specificity (0.90, 95% CI: 0.77–0.96) and pooled sensitivity (0.78, 95% CI: 0.59–0.90) were similar to the previous results. In addition, in the urine subgroup, the sensitivity was lower (0.45, 95% CI: 0.39–0.53) and the specificity was 0.92 (95% CI: 0.84–0.96). The similar results were presented in the tissue subgroups (sensitivity 0.87, 95% CI: 0.72–0.94; specificity 0.89, 95% CI: 0.68–0.97), which suggested a high level of sensitivity and specificity.

Table 2 Parameters of the studies we included

Author	*Year*	*Country*	*Race*	*Sample*	*Methods*	*Case type*	*Control type*	*Case character*	*Control character*	*TP*	*FP*	*FN*	*TN*
Hoque *et al*	2005	USA	Caucasian	Urine	QMSP	Pca	BPH/atypical cells/other cancer disease or normal	NA/hospital/ −80 °C	NA/hospital/ −80 °C	25	4	27	87
Rogers *et al*	2005	USA	Caucasian	Urine	MSP	Pca	biopsy negative	NA/hospital/NA	NA/hospital/NA	2	1	10	4
Rouprêt *et al*	2007	France	Caucasian	Urine	QMSP	Pca	Matched males	2005–2006/hospital/ −20 °C DNA	NA/NA/ −20 °C_DNA	48	2	47	36
Rouprêt *et al*	2008	UK	Caucasian	Blood	QMSP	Pca/without or with relapse	Normal	2000/hospital/ −80 °C	2000/NA/ −80 °C	39	2	3	20
Vener *et al*	2008	USA/ European	Caucasian	Urine	MSP	Pca	Biopsy negative	2006–2007/hospital/4 °C	2006–2007/hospital/ 4 °C	48	52	63	71
Yoon *et al*	2012	Korea	Asia	Tissue	Pyrosequencing	Pca	BPH	NA/hospital/ −80 °C	NA/hospital/ −80 °C	86	2	11	50
Kang *et al*	2004	Korea	Asia	Tissue	MSP	Pca/HGPIN	Normal	1999–2001/hospital/NA	1999–2001/hospital/ NA	26	1	25	19
Bastian *et al*	2007	Germany	Caucasian	Tissue	QMSP	Pca	BPH	2000–2003/NA/NA	2000–2003/NA/NA	65	0	13	32
Ellinger *et al*	2008	Germany	Caucasian	Tissue	QMSP	Pca	BPH (after radical cystprostatectomy for bladder cancer or retropubic adenomectomy for BPH)	1995–1999/hospital/NA	1995–1999/hospital/ NA	64	8	16	18
Maruyama *et al*	2002	USA	Caucasian	Tissue	MSP	Pca	BPH/adjacent nonmalignant tissue(PCa7)	1994–2000/hospital/ −70 °C	1994–2000/hospital/ −70 °C	27	2	74	30
Jerónimo *et al*	2004	Portugal	Caucasian	Tissue	QMSP	Pca/HGPIN	BPH	NA/hospital/ −80 °C	NA/hospital/ −80 °C	118	26	0	4
Tokumaru *et al*	2004	USA	Caucasian	Tissue	QMSP	Pca/bladder carcinoma	Nontumor/bladder carcinoma	2001–2002/hospital/ −80 °C	2001–2002/hospital/ −80 °C	48	0	13	11
Bastian *et al*	2005	Germany	Caucasian	Tissue	QMSP	Pca	BPH	2000–2003/hospital/NA	2000–2003/hospital/ NA	44	1	9	13
Enokida *et al*	2005	Japan	Asia	Tissue	MSP/USP	Pca	BPH	1997–2003//hospital/NA	1997–2003//hospital/ NA	109	6	61	63
Henrique *et al*	2006	Portugal	Caucasian	Tissue	QMSP	Pca/HGPIN	Normal	NA/hospital/ −80 °C	NA/hospital/ −80 °C	131	16	27	14
Yegnasubramanian *et al*	2004	USA	Caucasian	Tissue	QMSP	Pca/metastatic	Normal	1988–1995/hospital/ −80 °C	1988–1995/hospital/ −80 °C	66	1	7	24
Cho *et al*	2007	Korea	Asia	Tissue	MSP	Pca	BPH	1999–2004/hospital/NA	1999–2004/hospital/ NA	117	2	62	28
Vasiljević *et al*	2011	London/ China	Mix	Tissue	Pyrosequencing	Pca	BPH	1996–2008/hospital/NA	1996–2008/hospital/ NA	46	0	2	29
Trock *et al*	2011	USA	Caucasian	Tissue	MSP/USP	Pca	Nontumor	NA/hospital/NA	NA/hospital/NA	20	26	1	39

Abbreviations: BPH, benign prostate hyperplasia; BS, bisulphite genomic sequencing; FN, false negative; FP, false positive; HGPIN, high-grade prostatic intraepithelial neoplasia; MSP, methylation-specific PCR; NA, not applicable; Pca, Prostate cancer; QMSP, quantitative real-time methylation-specific PCR; TN, true negative; TP, true positive.
Case and control character: collection years/source of the samples/store temperature.

Table 3 Specificity and sensitivity of APC promoter methylation in all studies and in studies using different types of samples

Number of studies	*Specimen type*	*Methods*	*Pooled sensitivity*	*95% CI*	*Pooled specificity*	*95% CI*	*P-value for heterogeneity*	*P-value for publication bias*
5	Urine/blood	QMSP/MSP	0.53	0.28–0.78	0.92	0.86–0.95	0.00	0.76
14	Tissue	QMSP/MSP/pyrosequencing	0.84	0.70–0.92	0.91	0.77–0.97	0.00	0.56
4[a]	Urine	QMSP/MSP	0.45	0.39–0.53	0.92	0.84–0.96	0.00	0.81
10[a]	Tissue	QMSP/MSP/pyrosequencing	0.87	0.72–0.94	0.89	0.68–0.97	0.00	0.63

Abbreviations: APC, adenomatous polyposis coli; CI, confidence interval; N-MSP, non-quantitative methylation-specific PCR; QMSP, quantitative real-time methylation-specific PCR.
Other methods include quantitative methylation-specific PCR, methylation-sensitive restriction endonuclease-qPCR, and bisulphite genomic sequencing.
[a]Studies were removed on the basis of heterogeneity.[19,24,25,27,31]

Association between APC promoter methylation and pathological stage, Gleason score, and PSA levels in PCa cases

We also conducted an analysis of the relationship between the pathological stage, Gleason score, and PSA levels among PCa cases and APC promoter methylation. Details are shown in Table 4. We found no significant association between groups with the appropriate models except for the pathological stage (OR = 0.42, 95% CI: 0.25–0.70, $I^2 = 0.0\%$). Finally, the association between APC promoter methylation and PCa was conducted and the pooled OR was 24.91 (95% CI: 12.86–48.24, $I^2 = 72.5\%$), with pooled ORs of 33.54 (95% CI: 14.88–75.59, $I^2 = 70.7\%$) and 8.20 (95% CI: 2.84–23.74, $I^2 = 64.2\%$) in the tissue and fluid groups, respectively (Figure 3).

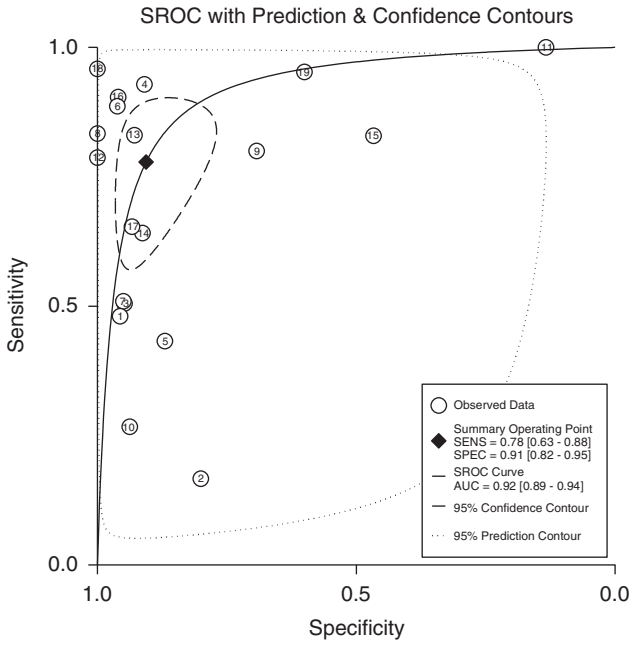

Figure 2 Meta-analysis with the S-ROC curve.

DISCUSSION

Description

Although there were have been many studies about of the sensitivity and specificity of APC promoter methylation in relation to PCa, a summary meta-analysis has not been reported. To confirm the real function of APC promoter methylation in predicting PCa, this study is required. This meta-analysis is based on 19 studies containing a total of 1600 cases and 744 controls. The major finding of this study has demonstrated that APC promoter methylation may be associated with PCa. With the high sensitivity and specificity, it would be an ideal biomarker in diagnosing PCa.

Pooled specificity and sensitivity analysis

With the strong association (OR = 24.91, 95% CI: 12.86 −48.24, $I^2 = 72.5\%$), we conducted the specificity and sensitivity analysis. For all included studies, either in the whole data set (pooled specificity 0.90, 95% CI: 0.80–0.95; pooled sensitivity 0.78, 95% CI: 0.63–0.88) or from subgroup analysis (fluid: specificity 0.92, 95% CI: 0.86–0.95; sensitivity 0.53, 95% CI: 0.28–0.78; tissue: specificity 0.91, 95% CI: 0.77–0.97; sensitivity 0.84, 95% CI: 0.70–0.92), the specificity and sensitivity of the test seemed to be high as an biomarker, which suggests a greater directive function in diagnosis with the biomarker of APC methylation. However, due to differences between cases and controls between studies, we removed some studies to make the data more homogeneous. These results suggested no major changes, and therefore provide support to our conclusion. As we have proposed above, the PSA test had varying high sensitivity and poor specificity (about 20%). Therefore, according to our results, APC methylation with high specificity may increase the veracity of diagnosis when combined with the PSA test. For sensitivity, only the fluid subgroup was lower than the PSA test. Due to the high pooled specificity, we propose the following: (1) because of the high sensitivity of the PSA test, potential patients would be screened out. Likewise, APC promoter methylation would need to be detected with high specificity among those patients who were positive in the PSA test. If the results of both tests were elevated among patients, future biopsies may be warranted. In this way, not only would the diagnosis be elevated, but also the weakness of the PSA test would be remedied. (2) For the tissue subgroup, because of the level of sensitivity (0.84/0.87) and specificity (0.91/0.89) in two analyses, we suggest that the APC methylation test might be a better test to distinguish PCa among the tissue. Although the biopsy was the gold standard in diagnosing PCa, there were still some errors when we did not obtain the cancer tissue well. With the high sensitivity and specificity, the APC methylation test could be used to complement the biopsy, which would decrease the rate of FNs. (3) It has been said that methylation genes might help identify new targets in the individual treatment of some diseases,[55] and our study may provide stronger evidence of the potential function of these genes in finding a cure for PCa.

In addition, although in our analysis we did not find any evidence of publication bias, we should not ignore the potential bias that could affect the results. Nevertheless, regardless of the possible bias, we suggest that studies of the function of methylation in identifying and curing the cancers cannot be neglected.

Limitations

The present study has several limitations. First, the validation assay of the gene promoter methylation used in each study was different (MSP/QMSP/other). In addition, primers selected from different

Table 4 APC promoter methylation in relation to pathological stage, Gleason score, and PSA levels among prostate cases

Clinicopathology	*Category*	*Hypermethylation frequency (%)*	*OR (95% CIs)*	*Heterogeneity test (I^2, P-value)*	*Publication bias test (P-value)*
Gleason score	GS<7	52.2	0.64 (0.26–1.57)	66.4%, 0.33	0.79
	GS≥7	64.6			
PSA levels	PSA≤4	65.5	1.30 (0.53–3.21)	0.0%, 0.57	0.13
	PSA>4	54.8			
Pathological stage	Stage (I,II)	58.2	0.42 (0.25–0.70)	0.0%, 0.00	0.43
	Stage (III,IV)	74.8			

Abbreviations: APC, adenomatous polyposis coli; CI, confidence interval; OR, odds ratio; PSA, prostate-specific antigen.

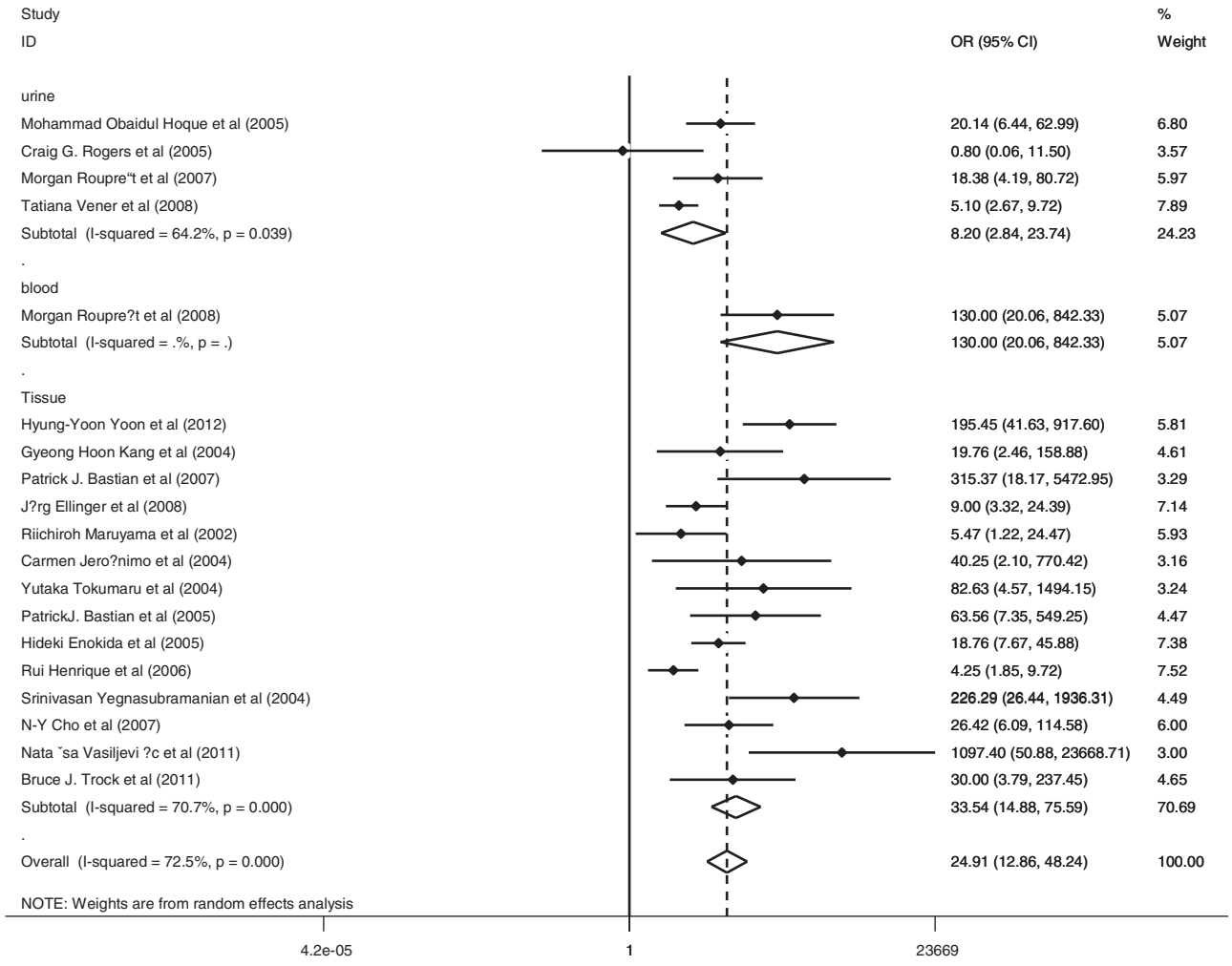

Figure 3 Meta-analysis with random effects for the association between APC methylation and the prostate cancer. The name of the first author and year of publication for each study was shown. For the sample type special analysis, two subgroups were shown: fluid and tissue. For each analysis the OR and accompanying 95% CI are revealed to show the association of the APC methylation and the prostate cancer.

regions of the same CpG Island may have different sensitivities and specificities (Table 1). Second, the thresholds identified by the ROC and INM were determined from individual trials, which may lead to different definitions of methylation. Third, the sample collection time varied widely among the studies. Finally, as mentioned above, we analyzed associations between gene methylation and pathological stage, Gleason score, PSA levels, and other factors, thereby decreasing our statistical power due to multiple testing.

CONCLUSION

PCa is a worldwide disease that affects a large number of men and leads to a serious conclusion. To diagnose and interpose this disease early may indicate a good prognosis. Although the PSA test has been applied in disease diagnosis, its poor specificity limits its function. On the basis of the studies available, this meta-analysis has demonstrated that APC methylation might be an ideal biomarker for screening and identifying PCa when combined with the PSA test to decrease the rate

of unnecessary biopsy. However, given the heterogeneity between the studies and insufficient evidence, the real function of APC methylation in disease diagnosis requires further research.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DISCLAIMER

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