Systematic Review and Meta-Analysis

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PITX2 methylation: a novel and effective biomarker for monitoring biochemical recurrence risk of prostate cancer

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Abstract

Aims: Prostate cancer is one of the most common malignancies in men. Biochemical recurrence (BCR) and progression following curative treatment pose a significant public health challenge. Thus, it is essential to explore effective biomarkers for disease progression monitoring and risk stratification. The promoter region of the paired-like homeodomain transcription factor 2 (PITX2) gene has been found to be frequently methylated in prostate cancer. However, the prognostic role of PITX2 methylation in prostate cancer and which patients most likely to be recommended for PITX2 methylation tests to assess BCR risk remain controversial. Therefore, a systematic review was performed to explore the relationship of PITX2 methylation with the BCR risk of prostate cancer.

Methods: The PubMed, EMBASE, and Cochrane Library databases were systematically searched for eligible studies. Seven studies with a total of 2185 patients were included. Pooled hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) were calculated.

Results: The overall HR was 2.71 (95% CI, 2.21–3.31), suggesting that PITX2 methylation has an adverse impact on BCR of prostate cancer. The pooled estimate of 5-year BCR-free survival for patients with a high methylation status was significantly lower than that for patients with a low methylation status (71% vs 90%; odds ratio [OR] = 3.50; 95% CI, 2.67–4.60, $P = .000$). A subgroup analysis was conducted according to detection method; the combined HRs were 2.68 (95% CI, 2.02–3.55) for quantitative methylation-specific PCR (qMSP) and 3.29 (95% CI, 2.31–4.68) for microarray EpiChip. In subgroups defined by region, Gleason score, pathological stage, surgical margin status and ethnicity, high methylation status was also associated with BCR of prostate cancer.

Conclusions: As an effective biomarker, PITX2 methylation is feasible for individualized BCR risk assessment of prostate cancer following radical prostatectomy.

Abbreviations: APC = adenomatous polyposis coli, BCR = biochemicalrecurrence, CIs = confidence intervals, GS = Gleason score, HRs = hazard ratios, PITX2 = paired-like homeodomain transcription factor 2, PSA = prostatic specific antigen, qMSP = quantitative methylation-specific PCR.

Keywords: biochemical recurrence, meta-analysis, methylation, PITX2, prostate cancer

Editor: Guanyi Zhang.

All procedures performed in the included studies involving human participants were approved by the ethics committee and were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

QJ and MX contributed equally to this work and share the first authorship.

This work was supported by grants from Department of Health of Zhejiang Province [grant numbers 2017KY330]; and Joint Fund of Zhejiang Natural Science Foundation-Zhejiang Mathematical and Physical Medical Association [grant numbers SY19H310001].

The authors declare no conflict of interest.

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Medicine (2019) 98:1(e13820)

Received: 2 September 2018 / Received in final form: 8 November 2018 / Accepted: 1 December 2018

http://dx.doi.org/10.1097/MD.0000000000013820

1. Introduction

Prostate cancer imposes a great health burden on men, while its incidence has significantly increased in recent years. According to the latest estimates of global cancer incidence, prostate cancer is a third most common malignancy among men and the ranks sixth in the world.[1] Radical prostatectomy has been used as the main primary treatment for prostate cancer for many years with excellent oncologic results. However, recurrence of prostate cancer following radical prostatectomy is an important public health challenge. Up to 20% of patients develop biochemical recurrence (BCR) within 5 years of radical prostatectomy, and many subsequently develop metastatic diseases.[2] Prostatic specific antigen (PSA) has been the pivotal tool for recurrence diagnosis and is introduced as a BCR marker. A rising serum PSA level after achieving undetectable value is the first sign of recurrent disease and defined as BCR in year 2003.[3] Over time, it was possible to realize that PSA relapse has different meanings accordingly to clinicopathological features as Gleason score (GS), PSA doubling time (PSA-DT), clinical stage and surgical margins status. In addition, there is no consensus about the best PSA threshold ($\geq$0.05, 0.2 or 0.4 ng/mL) to define BCR until this moment.[4–7] Several prognostic markers, such as GS, clinical stage, and pretreatment prostate-specific antigen (PSA) levels,

have been reported, but they have limited prognostic value for individual patients.[8] Therefore, it is necessary to find other effective biomarkers to predict the BCR of prostate cancer.

Some genes, such as ras association domain-containing protein 1 (RASSF1A), adenomatous polyposis coli (APC) and encode glutathione S-transferase pi 1 (GSTP1) have been shown to be hypermethylated in prostate cancer but not in normal tissue,[9–11] further improving the diagnostic sensitivity of prostate cancer. Epigenetic mechanisms such as DNA methylation were also found to be involved in the regulation of metastasis development.[12,13] Therefore, it is reasonable to believe that the hypermethylation of certain genes may predict the biological behavior of tumors and may serve as an effective biomarker of tumor progression. Paired-like homeodomain transcription factor 2 (PITX2) is a bicoid-related transcription factor induced by the Wnt/Dvl/β-catenin pathway and is required for cell type-specific proliferation.[14] Several studies have shown that hypermethylation of PITX2 is closely related to BCR in patients with prostate cancer after radical prostatectomy.[15–21] However, largely owing to the relatively small sample sizes of the individual studies, the prognostic role of PITX2 methylation in prostate cancer and the patients most likely to be recommended for PITX2 methylation tests to assess individual risk remain controversial. Therefore, we performed this systematic meta-analysis by combining data from published research to evaluate the association of PITX2 methylation with BCR in prostate cancer patients.

2. Materials and methods

2.1. Data sources

The PubMed, EMBASE and Cochrane Library databases were systematically searched for eligible studies. The search time was from database inception to April 1, 2018. A combination of free-text words and MeSH terms was used as follows: (prostate cancer/prostate neoplasms) AND (paired-like homeodomain transcription factor 2 [PITX2]) AND (methylation/hypermethylation) AND (recurrence/relapse/biochemical recurrence). Reference lists from eligible studies were also thoroughly searched for potential relevant studies.

2.2. Study selection, meta-analysis inclusion criteria, and data extraction

The identified publications were carefully screened. Two reviewers (J Q and XM) screened all publications identified based on our inclusion criteria. In the event of disagreement between the 2 reviewers, we obtained and inspected the full-text article independently. In total, 7 studies were included in the final analysis. The inclusion criteria were as follows:

(1) clinical trial or research, not letters or reviews;
(2) trials/research focusing on patients with prostate cancer;
(3) study exploring the relationship between PITX2 methylation and BCR;
(4) analysis using Cox proportional hazards modeling; and
(5) published in English.

When extracting time-to-event data, the authors attempted to use the measure reported within the text of the report. When relevant data were not reported in the text, Engauge Digitizer software (http://digitizer.sourceforge.net) was used to extract the data directly from the Kaplan–Meier survival curve reported in the article.

2.3. Statistical analysis

BCR was analyzed as a hazard ratio (HR) and pooled effect size with the 95% confidence interval (CI). A pooled estimate of 5-year BCR-free survival was also computed. The odds ratio (OR) was used for comparing the 5-year BCR-free survival rates. We assessed heterogeneity in the results of the studies using the $\chi 2$ test of heterogeneity and the I^2 measure of inconsistency. Heterogeneity was considered to be present when the P value of the Cochran Q test was <0.05 and the I^2 statistic was $>50\%$. The random effects model was used for meta-analysis if there was significant heterogeneity; otherwise, the fixed effects model was used. Publication bias was evaluated visually by Deeks funnel plot and analytically by Begg or Egger test.[22,23] A statistical test with $P<.05$ was considered significant. All statistical analyses were performed using the meta-analysis command in STATA (version 12.0 for Windows; Stata Corp LP, College Station, TX).

3. Results

3.1. Study selection and characteristics

The search strategy identified 160 records that were screened for inclusion. 37 studies were excluded on ground of duplicated or overlapping reporting. Based on title and abstract review, a total of 20 studies were determined to be inapplicable to BCR risk of prostate cancer, and were excluded. Additionally, we excluded 12 studies based on lack of sufficient data. In total, 7 studies[15–21] with a total of 2185 cases were collected in the meta-analysis (Fig. 1). All the patients were diagnosed with prostate cancer following radical prostatectomy. According to GSs, patients in 1 trial[16] were considered to have high-risk prostate cancer (GS 8–10 and/or a PSA level >20 ng/mL), whereas in another 4 trials,[17–19,21] more than 50% of the patients had intermediate- or high-risk prostate cancer (GS >7). The methods used to detect PITX2 methylation included quantitative methylation-specific PCR (qMSP) and microarray EpiChip: 4 studies[15–17,21] used qMSP, 1 study[19] used microarray EpiChip, and 2 studies[18,20] used both qMSP and microarray EpiChip. The selected patients were from North America and Europe: 3 studies[18,20–21] were from North America, 3 studies[15–17] were from Europe, and 1 study[19] included patients from both North America and Europe. The other general information on these studies is presented in Table 1. BCR was defined as 2 consecutive increased total PSA measurements, which was defined as total serum PSA 0.2 ng/mL or greater.

3.2. Publication bias

Begg or Egger tests revealed no evidence of publication bias across the included studies regarding BCR (Begg test, $P=.488$; Egger test, $P=.588$; Fig. 2).

3.3. Association of PITX2 methylation and biochemical recurrence

The combined analysis of the 7 studies[15–21] showed that high methylation status of PITX2 was associated with BCR (HR = 2.71, 95% CI, 2.21–3.31; $P=.000$; the fixed effects model; Fig. 3). Because of the high proportion of high-risk patients in 1 trial,[16] the other 6 trials[15,17–21] were included for a pooled estimate of 5-year BCR-free survival to guarantee study homogeneity. The pooled estimate of 5-year BCR-free survival of patients with high methylation status (71%; 95% CI, 59%-

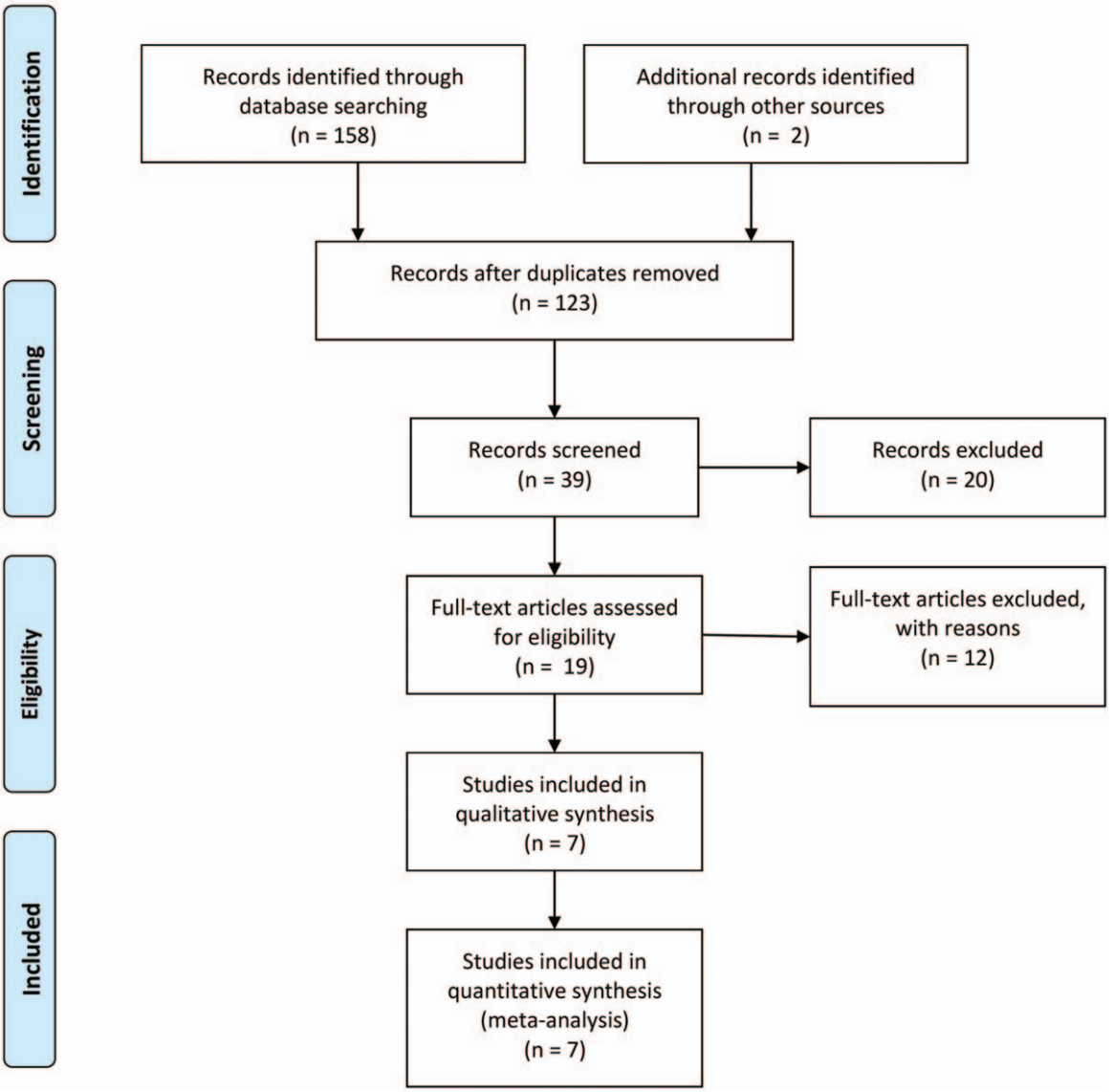

Figure 1. Flow diagram of the study selection process.

83%; Fig. 4) was significantly lower than that of patients with low methylation status (90%; 95% CI, 86%-95%; Fig. 4) (OR = 3.50; 95% CI, 2.67–4.60, $P = .000$, fixed effects model). A subgroup analysis was conducted according to patient region; the combined HR was 2.14 (95% CI, 1.49–3.08, $P = .000$) for Europe and 3.00 (95% CI, 2.22–4.04, $P = .000$) for North America (Fig. 5). Another subgroup analysis was performed with detection method; the combined HR was 2.68 (95% CI, 2.02–3.55, $P = .000$) for qMSP and 3.29 (95% CI, 2.31–4.68, $P = .000$) for microarray EpiChip (Fig. 5). In the subgroup analysis of GSs,

Table 1

General parameters of the 7 studies included.

Author	Year	Country	Sample size	Method	Cut-off of methylation
Uhl[10]	2017	Germany	260	qMSP	6.43%***
Litovkin[11]	2014	Belgium	71	qMSP	24%**
Vinarskaja[12]	2013	Germany	93	qMSP	median (23.3%)
Dietrich[13]	2013	USA	523	qMSP/microarray EpiChip	median (6.43%)/CMS = 0
Bañez[14]	2010	USA and Europe	476	microarray Epichip	NR
Schatz[15]	2010	USA	157	qMSP/microarray EpiChip	NR*
Weiss[16]	2009	USA	605	qMSP	median (NR)

* cutoff values were selected based on a model fit (likelihood).
** the cut-off maximizing this measure defined the decision point.
*** cutoff values according to Dietrich et al; CMS = calibrated methylation score according to Schatz et al NR = not reported.

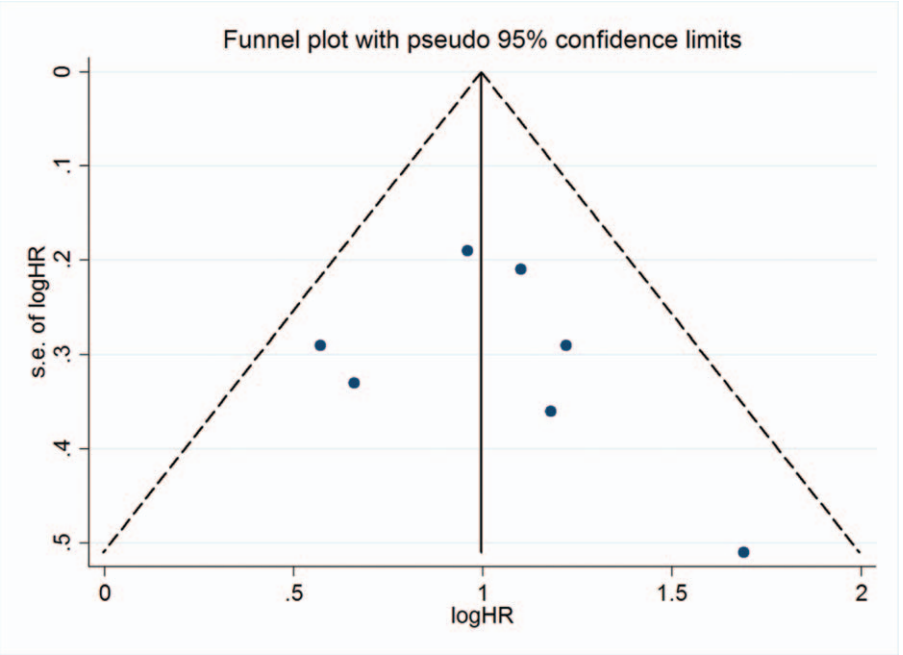

Figure 2. Deek funnel plot with 95% confidence intervals for publication bias testing.

high methylation status of PITX2 was associated with BCR, irrespective of whether GS was <7 (HR = 3.7, 95% CI, 1.52–9.01, $P = .004$), = 7 (HR = 2.08, 95% CI, 1.39–3.11, $P = .000$), >7 (HR = 3.9, 95% CI, 1.21–12.59, $P = .023$), or >8 (HR = 3.15, 95% CI, 1.77–5.61, $P = .000$) (Fig. 5). In addition, for subgroups defined by tumor cell contents, pathological stage, surgical margin status and ethnicity, the HRs in the analyses of BCR favoured low methylation status in the following subgroups:

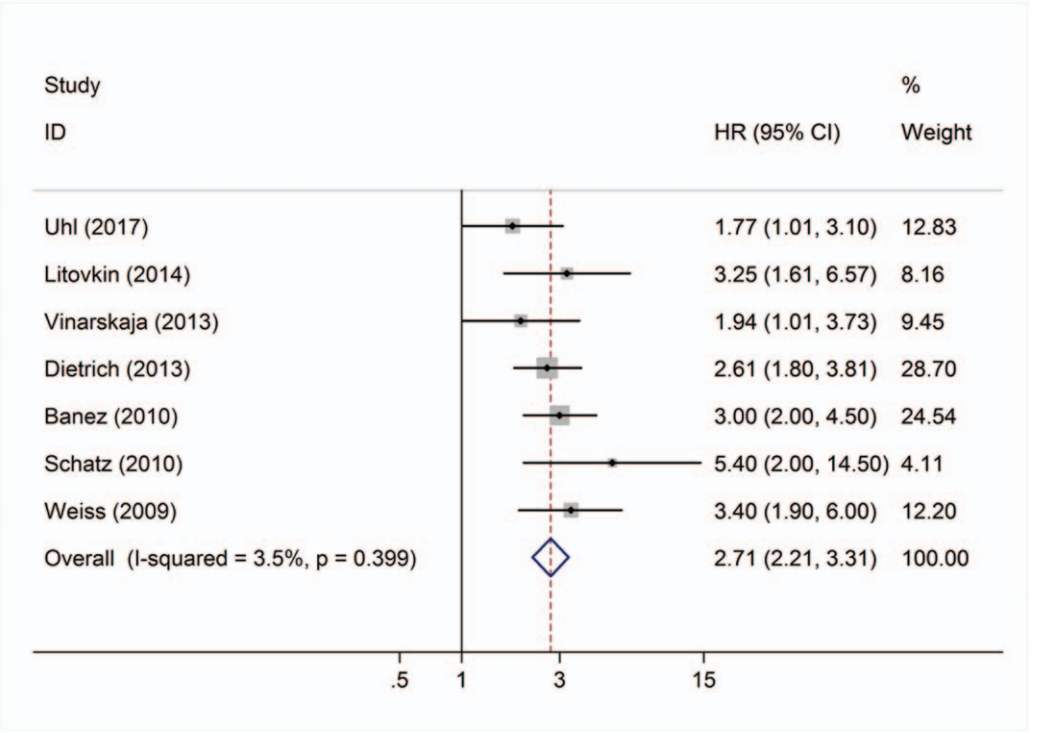

Figure 3. Meta-analysis (Forest plot) showing hazard ratios of the PITX2 methylation on BCR risk. Hazard ratios for each trial are represented by the squares, the size of the square represents the weight of the trial in the meta-analysis, and the horizontal line crossing the square represents the 95% confidence interval. The diamonds represent the estimated pooled effect using the fixed-effect model. All *P* values are 2-sided. BCR = biochemical recurrence, PITX2 = paired-like homeodomain transcription factor 2.

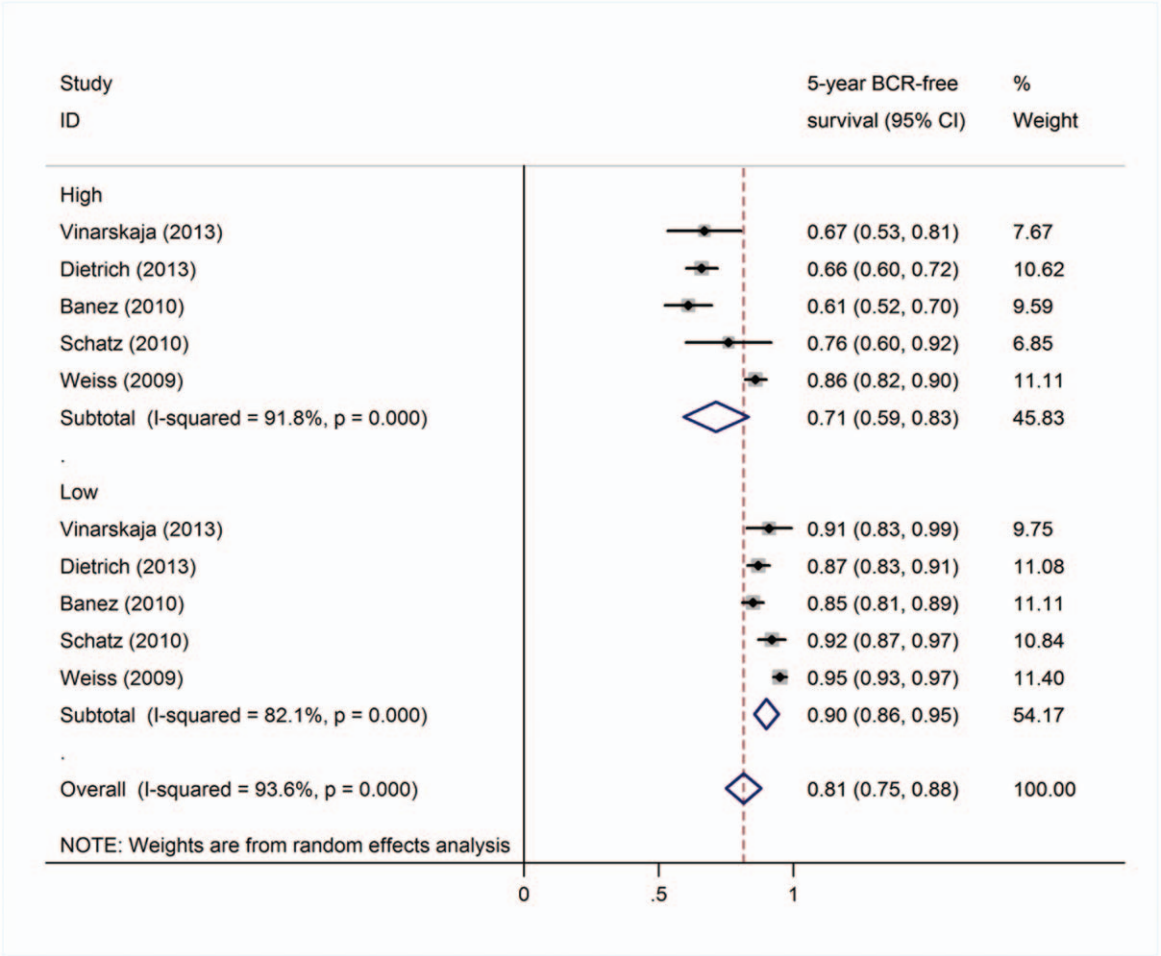

Figure 4. The pooled estimate of 5-year BCR-free survival for patients with high and low PITX2 methylation status. The 5-year BCR-free survival rate for each trial are represented by the squares, the size of the square represents the weight of the trial in the meta-analysis, and the horizontal line crossing the square represents the 95% confidence interval. The diamonds represent the estimated pooled effect using the Mantel–Haenszel random-effect model. BCR= biochemical recurrence, PITX2=paired-like homeodomain transcription factor 2.

patients with tumor cell content >75%, pathological stage of pT2 (HR=3.35, 95% CI, 1.60–6.98) or pT3 (HR=1.82, 95% CI, 1.04–3.20), patients with (HR=2.14, 95% CI, 1.25–3.68) or without (HR=5.86, 95% CI, 2.70–12.68) tumor involvement of surgical margins, and patients who were white (HR=3.46, 95% CI, 1.86–6.44) or black (HR=5.29, 95% CI, 1.72–16.24).

4. Discussion

According to the latest statistics in 2016, prostate cancer has become the most common incident cancer for men (1.4 million cases).[24] Due to high heterogeneity in the clinical course of prostate cancer, it is essential to determine the best methods to monitor disease progression through prognostic biomarkers, risk stratify individual patients and implement personalized treatment strategies. Aberrant DNA hypermethylation is considered an early landmark event in carcinogenesis.[25] Hypermethylation of certain genes, such as APC,[26–28] ABHD9,[29] and Chr3-EST 29], has been reported to affect the biochemical recurrence of prostate cancer.

The human PITX2 gene encoded protein has been proved to be a transcription factor that regulates the expression of procollagen lysyl hydroxylase. It is reported that PITX2 hypermethylation is closely related to the prognosis of several tumor types, including acute myeloid leukemia,[30] lung cancer,[31] and breast cancer.[32] Here, we focused on PITX2 methylation in prostate cancer and collected complete articles to infer potential prognostic value. Patients with high methylation status of PITX2 were more likely to experience BCR and to associated have a lower 5-year BCR-free survival, suggesting that PITX2 hypermethylation is an effective predictor of prostate cancer progression. Another study has confirmed PITX2 hypermethylation leads to PITX2 silencing and patients with decreased PITX2 mRNA level experienced significantly earlier BCR.[17] Notably, it revealed an excellent correlation between PITX2 methylation and ERG overexpression, a common oncogenic ETS family transcription factor acting to promote prostate cancer invasion and progression.[33] Then, we conducted subgroup analyses defined by patient region, detection method, GS, tumor cell content, pathological stage, surgical margin status and ethnicity. In the subgroup analysis according to GS, PITX2 methylation status was also shown to be a prognostic marker to predict BCR in patients, irrespective of low, intermediate or high risk. In recent years, a new and reliable diagnostic microarray, EpiChip, for detecting the methylation status of PITX2 has been developed to improve the ability to predict the prognosis of prostate cancer after radical prostatectomy.[20] In the subgroup analysis of detection method, estimates

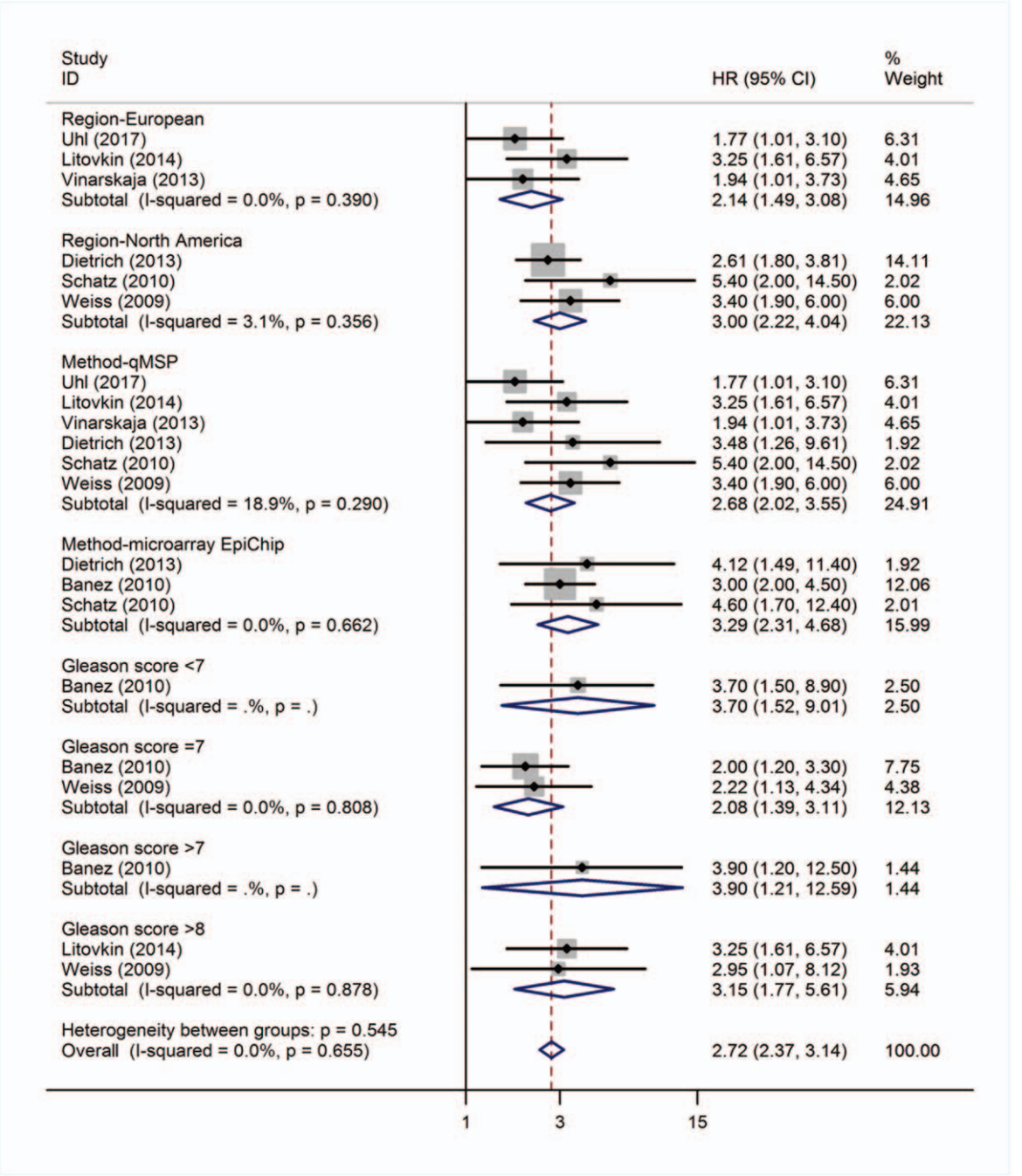

Figure 5. Meta-analysis (Forest plot) showing hazard ratios of the PITX2 methylation on BCR risk. Subgroup analysis of region: European/North America; Method: qMSP/microarray EpiChip; and Gleason score <7/=7/>7/=8. Hazard ratios for each trial are represented by the squares, the size of the square represents the weight of the trial in the meta-analysis, and the horizontal line crossing the square represents the 95% confidence interval. The diamonds represent the estimated pooled effect using the fixed-effect model. All *P* values are 2-sided. BCR= biochemical recurrence, PITX2=paired-like homeodomain transcription factor 2, qMSP=quantitative methylation-specific PCR.

of the HR to quantify the prognostic value of PITX2 methylation detected by qMSP or microarray EpiChip were highly similar and significantly larger than 1, suggesting that qMSP was as sensitive as microarray EpiChip at detecting PITX2 methylation in patients with prostate cancer. The application of microarray technology requires a validated diagnostic platform, the Affymetrix GeneChip System (Affymetrix, Santa Clara, CA). Since the platform is not regular laboratory equipment, the utility of the microarray test is limited. In addition, microarray-based detection methods can be applied to only complete formalin-fixed, paraffin-embedded sections, not to other biopsy samples. Based on DNA quantitative technology, qMSP is capable of analyzing a variety of biological samples, even samples that contain only minute amounts of DNA. With simple technical procedures and highly concordant results with microarray EpiChip, qMSP may be more recommended for use in clinical applications. Patients with positive tumor margins following radical prostatectomy are reported to be more likely to develop

biological, local and systemic progression.[34] About 50% of these patients experience recurrence; so it is an urgent need to explore predictive biomarkers in this subgroup.[35] In the subgroup analysis defined by surgical margin status, PITX2 methylation status was also shown to be a robust predictor for BCR risk in patients with tumor involvement of surgical margins, and this finding may help develop a risk-adjusted approach to adjuvant therapy after radical prostatectomy for those patients. In future studies, it is necessary to confirm the clinical significance of PITX2 methylation in a suited patient cohort (such as an adjuvant therapy cohort).

This study had several limitations. First, we excluded studies that lacked survival data (e.g., HR, CI or survival curves). Second, our endpoint was BCR. Although early BCR is closely related to the risk of disease metastasis and cancer-related death, more studies in the future are needed to assess the prognostic value of PITX2 methylation regarding these clinical end points. Last, some statistical results have heterogeneity, which may be derived from the differences in the patient clinical characteristics, detection methods, cut-off values or any other technical issues.

In conclusion, PITX2 hypermethylation status is an effective molecular predictor of BCR risk in patients with prostate cancer after radical prostatectomy. Adding PITX2 methylation status measurements to routine prostate cancer management may help assess individual prognostic risk and define patients who may benefit from further therapeutic intervention. Larger-scale and more standard investigations are required to better understand the role of PITX2 methylation in disease progression (e.g., metastasis and overall survival) and its utility in clinical applications (involving different therapeutic modalities).

Author contributions

Qi Jiang and Mixue Xie: conception and design of the study, protocol development, searching for studies, acquisition, analysis and interpretation of data, drafting the article; Mengye He, Feifei Yan, Ming Chen and Suzhen Xu: searching for studies, acquisition, analysis and interpretation of data; Xiaochen Zhang and Peng Shen: conception and design of the study, protocol development, revision of the article.

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Formal analysis: Qi Jiang, Mixue Xie, Mengye He, Feifei Yan.
Funding acquisition: Mixue Xie, Xiaochen Zhang.
Project administration: Xiaochen Zhang.
Resources: Qi Jiang, Ming Chen, Suzhen Xu.
Software: Suzhen Xu.
Supervision: Peng Shen.
Writing – original draft: Qi Jiang, Mixue Xie.
Writing – review & editing: Xiaochen Zhang, Peng Shen.

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