

## Association between Ras association domain family 1A promoter methylation and hepatocellular carcinoma: A meta-analysis

Ze-Hua Zhao, Yu-Chen Fan, Yang Yang, Kai Wang

Ze-Hua Zhao, Yu-Chen Fan, Yang Yang, Kai Wang, Department of Hepatology, Qilu Hospital of Shandong University, Jinan 250012, Shandong Province, China

Yu-Chen Fan, Kai Wang, Institute of Hepatology, Shandong University, Jinan 250012, Shandong Province, China

**Author contributions:** Zhao ZH and Wang K designed research; Zhao ZH and Fan YC performed the research and analyzed the data; Zhao ZH, Fan YC and Yang Y wrote the paper; Wang K supervised the whole study.

**Supported by** Key Project of Chinese Ministry of Science and Technology, No. 2012ZX10002007 and No. 2013ZX10002001; National Natural Science Foundation of China, No. 81171579 and No. 81201287; Natural Science Foundation of Shandong Province, China, No. ZR2010HM070 and No. ZR2010HQ040

**Correspondence to:** Kai Wang, MD, PhD, Department of Hepatology, Qilu Hospital of Shandong University, Wenhua Road 107, Jinan 250012, China. wangdoc876@126.com

Telephone: +86-531-86630809 Fax: +86-531-86927544

Received: July 14, 2013 Revised: August 15, 2013

Accepted: August 20, 2013

Published online: November 7, 2013

### Abstract

**AIM:** To assess diagnostic accuracy of Ras association domain family 1A (RASSF1A) promoter methylation in body fluids (serum, plasma and whole blood) for hepatocellular carcinoma (HCC).

**METHODS:** Relative information about study characteristics and incidence of RASSF1A methylation was collected. Quality of all included studies was evaluated by Quality Assessment of Diagnostic Accuracy Studies-2. Sensitivity and specificity were pooled using a random-effect model, and a summary receiver operating characteristic curve was used to demonstrate the overall diagnostic performance. Positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) with 95%CI were also calculated. Meta-regression was applied to analyze observed heterogeneity, and

Deeks' test was performed to detect publication bias.

**RESULTS:** After a systematic literature review, seven studies with a total of 302 cases of HCC and 250 cases of chronic liver diseases were included in the analysis. The pooled sensitivity and specificity were 0.70 (95%CI: 0.49-0.85) and 0.72 (95%CI: 0.54-0.85), respectively. The PLR was 2.51 (95%CI: 1.64-3.86), NLR was 0.41 (95%CI: 0.25-0.68), and DOR was 6.13 (95%CI: 3.17-11.84). The  $\chi^2$  values of sensitivity, specificity, PLR, NLR and DOR were 59.41 ( $P < 0.001$ ), 50.50 ( $P < 0.001$ ), 17.40 ( $P = 0.010$ ), 31.24 ( $P < 0.001$ ) and 80.51 ( $P < 0.001$ ), respectively. The area under the curve was 0.77 (95%CI: 0.73-0.81). Three factors were analyzed by univariate meta-regression and none was significant to interpret the observed heterogeneity ( $P > 0.05$ ). No significant publication bias was detected by Deeks' test ( $P = 0.346$ ).

**CONCLUSION:** We showed the potential diagnostic value of RASSF1A methylation in body fluids in HCC patients and it may improve diagnostic accuracy combined with the  $\alpha$ -fetoprotein test.

© 2013 Baishideng Publishing Group Co., Limited. All rights reserved.

**Key words:** Methylation; Ras association domain family 1A; Hepatocellular carcinoma; Biomarker; Diagnostic sensitivity; Diagnostic specificity

**Core tip:** The published results on the diagnostic potential of Ras association domain family 1A (RASSF1A) promoter methylation for detection of hepatocellular carcinoma (HCC) are not consistent. We performed a comprehensive literature search to assess the diagnostic accuracy of RASSF1A promoter methylation. We rigorously selected patients with chronic liver diseases as controls to mimic clinical practice, and we only included studies that used body fluids as samples for detection

because such an approach is non-invasive and promising for clinical application. Our meta-analysis demonstrated good sensitivity and specificity of RASSF1A methylation and may complement the  $\alpha$ -fetoprotein test to improve HCC detection.

Zhao ZH, Fan YC, Yang Y, Wang K. Association between Ras association domain family 1A promoter methylation and hepatocellular carcinoma: A meta-analysis. *World J Gastroenterol* 2013; 19(41): 7189-7196 Available from: URL: <http://www.wjg-net.com/1007-9327/full/v19/i41/7189.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i41.7189>

## INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most prevalent cancer and the third most frequent cause of cancer-related death worldwide<sup>[1]</sup>. With a dramatic increase in incidence, HCC has become a major health challenge and has aroused growing concern. Due to the fact that most HCC patients at the advanced stage are prone to present a poor prognosis, early HCC detection is urgently needed. Currently, the serum  $\alpha$ -fetoprotein (AFP) test is widely used, however, with a relatively low sensitivity, the application is barely satisfactory and its function is limited<sup>[2]</sup>.

Recent studies have revealed that inactivation of multiple tumor suppressor genes underlain by hypermethylation of promoter CpG islands can be a critical event in hepatocarcinogenesis<sup>[3,4]</sup>. Thus, DNA methylation may provide an ideal route for screening because methylated DNA can be detected with a high sensitivity and specificity<sup>[5]</sup>. Furthermore, the circulating DNA in cell-free serum/plasma is becoming a new focus. Increasing studies have attempted to identify abnormal methylation pattern in cfDNA of patients with human cancers<sup>[6-10]</sup>. Several tumor-associated alterations including plasma/serum DNA methylation have been well demonstrated in liver cancer<sup>[11]</sup> and such cfDNA is thought to be derived from apoptosis and necrosis of cancer cells in tumor microenvironment<sup>[12]</sup>. Thus, approaches have been developed to analyze promoter methylation using DNA isolated from fluid samples, which makes it possible to perform noninvasively and allows patients to avoid physical discomfort and other complications.

Among the biomarkers studied previously, the Ras association domain family 1A (RASSF1A) gene has been extensively investigated. RASSF1A is located at 3p21.3 and is implicated in the Ras signaling pathway, which plays a pivotal role in cell cycle control, microtubule stabilization, cellular adhesion, cell motility, and apoptosis<sup>[13]</sup>. The tumor suppressor function of RASSF1A has been identified by both *in vivo* and *in vitro* observations in which re-expression of the gene in RASSF1A-negative cancer cells results in reduced colony formation in soft agar and reduced tumorigenicity in nude mice<sup>[14]</sup>. Loss of RASSF1A expression is one of the most common events in human cancer, with aberrant promoter methylation

reported in a variety of tumor types, including HCC<sup>[15]</sup>.

During the past decade, there have been an increasing number of investigations focusing on the diagnostic role of RASSF1A promoter methylation in HCC and many of them utilized body fluids as samples, which can be used for clinical application. Although the results of the studies are encouraging, there was some disagreement in relation to diagnostic accuracy. Therefore, we conducted a meta-analysis on the diagnostic sensitivity and specificity of RASSF1A methylation in body fluids for diagnosis of HCC. The results of this study indicate the potential diagnostic value of RASSF1A methylation and provide evidence for a reliable biomarker to discriminate HCC.

## MATERIALS AND METHODS

### Study selection

We performed a comprehensive literature search of articles through the following databases without date limitation: PubMed, Embase, Web of Science, and the Cochrane Library. The search terms used were: "hepatocellular carcinoma/ HCC/liver cancer", "Ras association domain family protein 1A/RASSF1A", "sensitivity", "specificity" and "diagnosis". The search was updated to May 24, 2013. The reference lists of the publications were also manually searched for additional related studies.

### Inclusion and exclusion criteria

We considered studies eligible for inclusion if they met the following criteria: (1) measurement of DNA methylation in one of the following samples: whole blood, plasma, serum, and buffy coat; (2) designed as a cohort study or a case-control study; (3) published in the English language; and (4) conducted in adults.

We excluded studies that tested RASSF1A methylation in liver tissues and cell lines. We also excluded studies in which body fluid samples were not collected before surgery or other treatment which may have reduced RASSF1A methylation dramatically. In clinical practice, patients who are recommended for advanced imaging examination (such as computed tomography and magnetic resonance imaging) and biopsies typically, have elevated AFP levels, aberrant ultrasonic images, and abnormal liver function or other related symptoms, and can differ from healthy controls<sup>[1]</sup>. Therefore, we rigorously classified the controls into two categories: (1) patients who had negative biopsies but suffered from chronic liver diseases such as liver cirrhosis, chronic hepatitis B, and chronic hepatitis C; and (2) healthy controls. In particular, we excluded healthy controls in order to diminish selection bias and avoid exaggerating diagnostic power. The selection process for studies included in this review is shown in Figure 1.

### Data extraction and quality assessment

For each study included, two reviewers (ZH Zhao and YC Fan) independently extracted the following information: authors' names, year of publication, sample type, detection technique, primer sequence, annealing tempera-

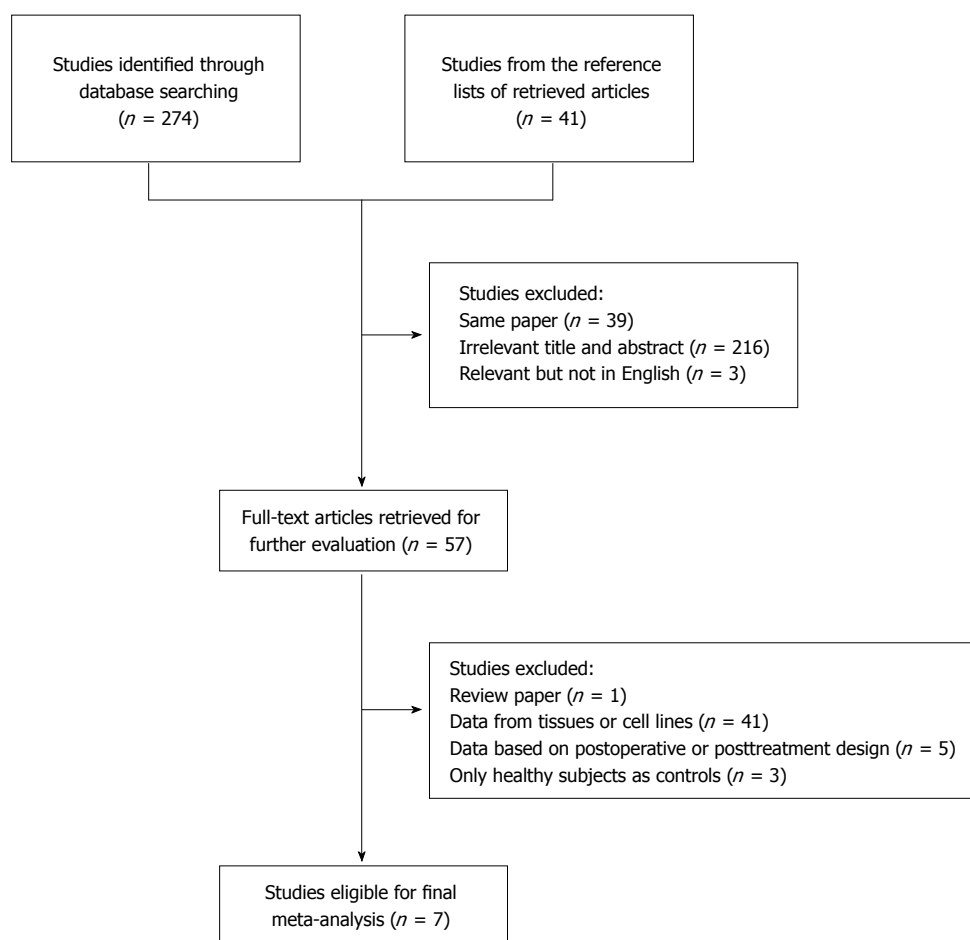


Figure 1 Flowchart of study selection.

ture, country, race, number of positive and negative results among cases and controls, and other characteristics of the study population. All disparities were resolved by discussion.

An updated quality evaluation tool Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) guideline was consulted to assess the methodological quality of each study. The newly revised tool is reported to perform better because it offers additional and improved features, including distinguishing between bias and applicability, identifying four key domains supported by signaling questions to aid judgment on risk of bias, and rating risk of bias and concerns about applicability as “high” and “low”<sup>[16]</sup>. The results were presented in a recommended way.

### Statistical analysis

We referred to a standard procedure recommended for meta-analysis of diagnostic test accuracy (DTA) studies<sup>[17]</sup>. Before the statistical analysis was conducted, we gathered the number of cases and controls with RASSF1A methylation. The true-positive (TP) ones were indicated to have RASSF1A methylation within cases, and false-negative (FN) ones were without RASSF1A methylation. A similar

definition was given to FP and TN controls. The sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) estimates with 95%CI from each study were analyzed using a random-effect model<sup>[17,18]</sup>. The pooled sensitivity and specificity were illustrated with a coupled forest plot. We also created a summary receiver operation characteristic (SROC) curve that displayed the results of individual studies in ROC space and reflected the discriminating ability<sup>[19]</sup>. The area under the curve (AUC) was calculated to present the general performance of the test and could be interpreted as the probability that the test would correctly rank a randomly chosen case/non-case pair with respect to their test values<sup>[20]</sup>. To assess the heterogeneity between studies, the  $\chi^2$ -based Cochrane  $Q$  test of heterogeneity was performed for each analysis. The  $I^2$  statistic, which measured the extent of inconsistency between studies, was also assessed. For detecting publication bias, Deeks’ test was conducted to examine funnel plot asymmetry, which was reported to be more appropriate for reviews of DTA studies<sup>[21]</sup>. The analysis was conducted using Stata version 12.0 (Stata Corporation, College Station, TX, United States). All  $P$  values were two-tailed and  $P < 0.05$  was considered significant.

**Table 1** Characteristics of included studies in meta-analysis

| Author                                | Year | Country/area | Race      | Sample      | Method    | Case type | Cases (n) | Control type                                   | Controls (n) |
|---------------------------------------|------|--------------|-----------|-------------|-----------|-----------|-----------|--|--------------|
| Chan <i>et al</i> <sup>[22]</sup>     | 2008 | Hong Kong    | Asian     | Serum       | MSRE-qPCR | HCC       | 63        | HBV infection                                  | 63           |
| Chang <i>et al</i> <sup>[23]</sup>    | 2008 | China        | Asian     | Plasma      | MSP       | HCC       | 26        | Liver cirrhosis                                | 16           |
| Maurizio <i>et al</i> <sup>[24]</sup> | 2011 | Italy        | Caucasian | Whole blood | MSP       | HCC       | 31        | Liver cirrhosis/<br>chronic hepatitis C        | 33/30        |
| Mohamed <i>et al</i> <sup>[25]</sup>  | 2012 | Egypt        | Caucasian | Plasma      | QMSP      | HCC       | 40        | HCV infection                                  | 40           |
| Azab <i>et al</i> <sup>[26]</sup>     | 2011 | Egypt        | Caucasian | Whole blood | MSP       | HCC       | 20        | Liver cirrhosis                                | 14           |
| Zhang <i>et al</i> <sup>[27]</sup>    | 2007 | Taiwan       | Asian     | Serum       | MSP       | HCC       | 50        | HBV infection/<br>HCV infection/coinfection    | 9/6/2        |
| Huang <i>et al</i> <sup>[28]</sup>    | 2011 | China        | Asian     | Plasma      | MSRE-qPCR | HCC       | 72        | Liver cirrhosis/<br>chronic inactive hepatitis | 25/12        |

MSP: Methylation-specific polymerase chain reaction (PCR); QMSP: Quantitative methylation-specific PCR; MSRE-qPCR: Methylation-sensitive endonuclease-qPCR; HCC: Hepatocellular carcinoma.

**Table 2** Primer sequences used in included studies for detection of Ras association domain family 1A methylation

| Author                                | Year | Method    | Forward  | Reverse  | Annealing T (°C) |
|---------------------------------------|------|-----------|--|--|------------------|
| Chan <i>et al</i> <sup>[22]</sup>     | 2008 | MSRE-qPCR | 5'-AGCCTGAGCTCATTGAGCTG-3'                                       | 5'-ACCAGCTGCCGTGTGG-3'   | 60               |
| Chang <i>et al</i> <sup>[23]</sup>    | 2008 | MSP       | M 5'-GTGTTAACGCGTTGCGTATC-3'<br>U 5'-TTTGGTTGGAGTGTGTTAATGTG-3'  | M 5'-AACCCCGCGAACTAAAAACGA-3'<br>U 5'-CAAACCCACAAACTAAAAACAA-3'  | 60               |
| Maurizio <i>et al</i> <sup>[24]</sup> | 2011 | MSP       | NA   | NA   | NA               |
| Mohamed <i>et al</i> <sup>[25]</sup>  | 2012 | QMSP      | 5'-AGCCTGAGCTCATTGAGCTG-3'                                       | 5'-ACCAGCTGCCGTGTGG-3'   | 60               |
| Azab <i>et al</i> <sup>[26]</sup>     | 2011 | MSP       | M 5'-GTGTTAACGCGTTGCGTATC-3';<br>U 5'-TTTGGTTGGAGTGTGTTAATGTG-3' | M 5'-AACCCCGCGAACTAAAAACGA-3';<br>U 5'-CAAACCCACAAACTAAAAACAA-3' | 54               |
| Zhang <i>et al</i> <sup>[27]</sup>    | 2007 | MSP       | M 5'-GTGTTAACGCGTTGCGTATC-3'<br>U 5'-TTTGGTTGGAGTGTGTTAATGTG-3'  | M 5'-AACCCCGCGAACTAAAAACGA-3'<br>U 5'-CAAACCCACAAACTAAAAACAA-3'  | 60               |
| Huang <i>et al</i> <sup>[28]</sup>    | 2011 | MSRE-qPCR | 5'-AGCCTGAGCTCATTGAGCTG-3'                                       | 5'-ACCAGCTGCCGTGTGG-3'   | 58               |

M: Methylated sequence; NA: Not available; U: Unmethylated sequence; MSP: Methylation-specific polymerase chain reaction (PCR); QMSP: Quantitative methylation-specific PCR; MSRE-qPCR: Methylation-sensitive endonuclease-qPCR.

## RESULTS

### Study characteristics

According to our search strategy and inclusion and exclusion criteria, seven studies with a total of 302 cases and 250 controls were included in the final meta-analysis (Table 1)<sup>[22-28]</sup>. The eligible studies were published between 2007 and 2012. Among the studies, two used sera, three used plasma, and two used whole blood as samples. Three methods were applied to detect the methylation status of RASSF1A promoter: four studies used methylation-specific polymerase chain reaction (MSP); one study used quantitative MSP (QMSP); and two used methylation-sensitive endonuclease-quantitative polymerase chain reaction (qPCR). The primer sequences used in the studies are summarized in Table 2.

### Quality assessment

Quality assessment results based on the updated QUADAS-2 are shown in Table 3. According to the guidelines, if the case-control design was not avoided, the risk of bias should be considered to be high in the patient selection domain. All seven studies included in our analysis were case-control studies and could have introduced selection bias. For other domains, the studies were basically satisfactory.

### Diagnostic accuracy analysis

The coupled forest plot of sensitivity and specificity for RASSF1A methylation assays in the diagnosis of HCC of the seven studies is shown in Figure 2. The sensitivity ranged from 0.27 to 0.94 (pooled: 0.70; 95%CI: 0.49-0.85) and the specificity ranged from 0.38 to 0.95 (pooled: 0.72; 95%CI: 0.54-0.85). The PLR was 2.51 (95%CI: 1.64-3.86), NLR was 0.41 (95%CI: 0.25-0.68), and DOR was 6.13 (95%CI: 3.17-11.84). The  $\chi^2$  values of sensitivity, specificity, PLR, NLR, and DOR were 59.41 ( $P < 0.001$ ), 50.50 ( $P < 0.001$ ), 17.40 ( $P = 0.010$ ), 31.24 ( $P < 0.001$ ), and 80.51 ( $P < 0.001$ ), respectively, which indicated significant heterogeneity between studies. The graph of the SROC curve is shown in Figure 3. We noted that the curve was positioned near the desirable upper left corner. The AUC was 0.77 (95%CI: 0.73-0.81), which represented a relatively high level of overall accuracy.

### Meta-regression and publication bias

Due to the heterogeneity observed in both analyses, we conducted meta-regression to search the sources<sup>[29]</sup>. The accuracy estimate we used was DOR, because it could demonstrate the diagnostic performance combining both sensitivity and specificity. The assay methods used to detect RASSF1A methylation could have affected the diagnostic accuracy directly for discriminating HCC.

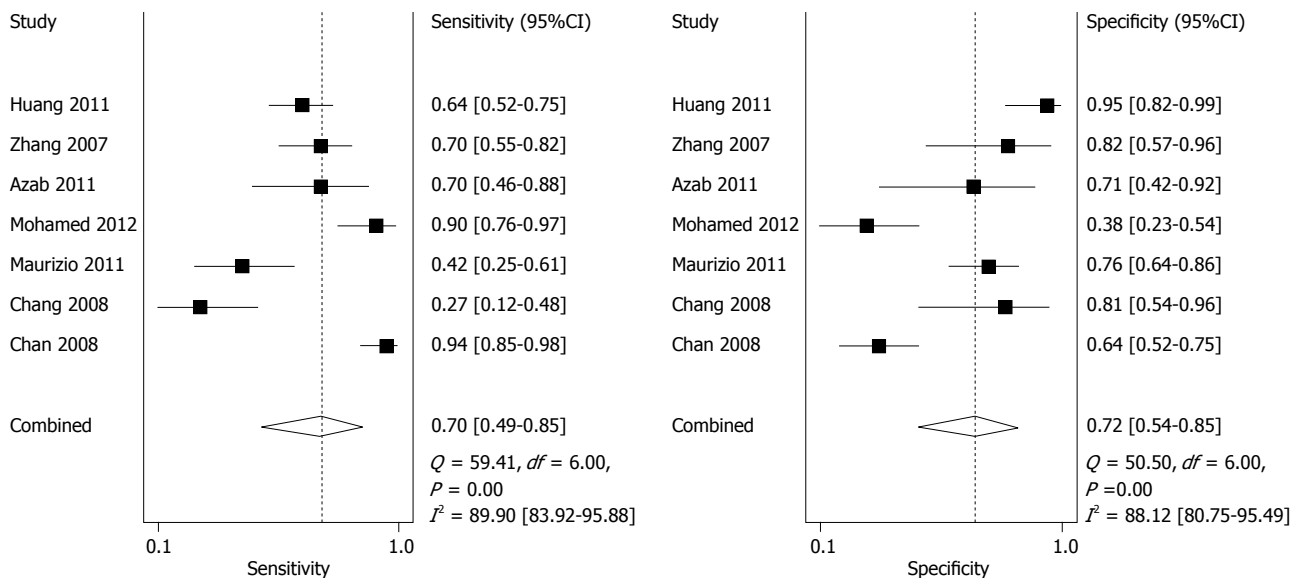
**Table 3** Quality Assessment of Diagnostic Accuracy Studies-2 results of all included studies

| Study                                 | Risk of bias      |            |                    |                 | Applicability concerns |            |                    |
|---------------------------------------|-------------------|------------|--------------------|-----------------|------------------------|------------|--------------------|
|                                       | Patient selection | Index test | Reference standard | Flow and timing | Patient selection      | Index test | Reference standard |
| Chan <i>et al</i> <sup>[22]</sup>     | ↑                 | ?          | ↓                  | ↓               | ↓                      | ?          | ↓                  |
| Chang <i>et al</i> <sup>[23]</sup>    | ↑                 | ?          | ↓                  | ↓               | ↓                      | ?          | ↓                  |
| Maurizio <i>et al</i> <sup>[24]</sup> | ↑                 | ?          | ↓                  | ?               | ↓                      | ?          | ↓                  |
| Mohamed <i>et al</i> <sup>[25]</sup>  | ↑                 | ↓          | ↓                  | ↓               | ↓                      | ↓          | ↓                  |
| Azab <i>et al</i> <sup>[26]</sup>     | ↑                 | ↓          | ↓                  | ↓               | ↓                      | ↓          | ↓                  |
| Zhang <i>et al</i> <sup>[27]</sup>    | ↑                 | ↓          | ↓                  | ↓               | ↓                      | ↓          | ↓                  |
| Huang <i>et al</i> <sup>[28]</sup>    | ↑                 | ?          | ↓                  | ↓               | ↓                      | ?          | ↓                  |

↓: Low risk; ↑: High risk; ?: Unclear risk.

**Table 4** Meta-regression results of meta-analysis

| Sources | Coefficient (95%CI) | SE   | T    | P value | $\tau^2$ | $I^2$ Res (%) | Adjusted $R^2$ (%) |
|---------|---------------------|------|------|---------|----------|---------------|--------------------|
| Method  | 1.09 (-0.55-2.73)   | 0.64 | 1.71 | 0.148   | 0.24     | 37.16         | 50.47              |
| Race    | 0.83 (-1.00-2.66)   | 0.71 | 1.17 | 0.294   | 0.36     | 46.68         | 26.54              |
| Sample  | 0.88 (-1.09-2.85)   | 0.77 | 1.87 | 0.301   | 0.36     | 45.96         | 26.46              |



**Figure 2** Coupled forest plot showing the sensitivity and specificity of Ras association domain family 1A methylation in diagnosis of hepatocellular carcinoma. Forest plots document estimates of sensitivity and specificity for each study together with 95%CI. The point estimates of sensitivity and specificity from each study are shown as solid squares.

Also, race and sample type may have had an influence to a varying degree. Therefore, we considered the three factors above as covariates and performed univariate meta-regression analysis. However, none of the factors was statistically significant ( $P > 0.05$ , Table 4).

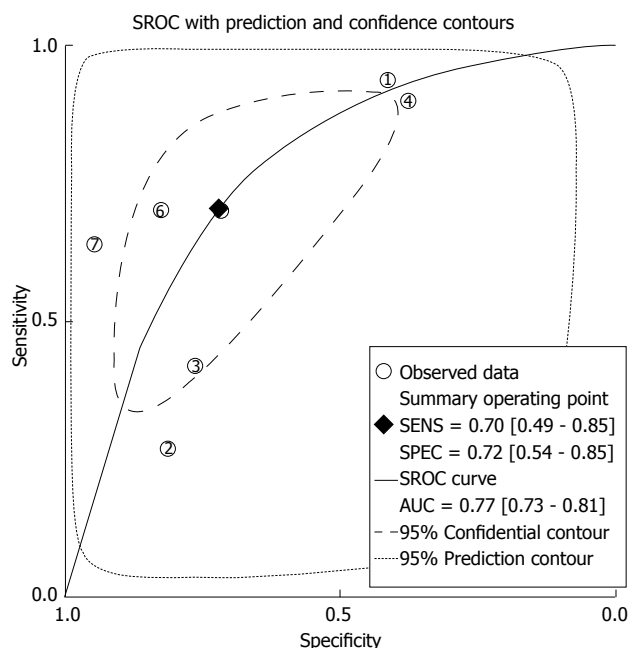
Publication bias was evaluated using Deeks' test (Figure 4). We found no significant publication bias among the studies that detected RASSF1A methylation in body fluids from patients with HCC ( $P = 0.346$ ).

## DISCUSSION

Although there have been many studies about sensitivity and specificity of RASSF1A promoter methylation

in diagnosis of HCC, to the best of our knowledge, no meta-analysis has been reported. For the traditional serum marker AFP, the specificity varied, but the sensitivity was generally low. A large multi-center survey including 1158 patients with HCC reported that the sensitivity for the most used cutoff value (20 ng/mL) was only 0.54<sup>[2]</sup>. Another recent study showed that the sensitivity and specificity of the AFP test was 0.42 and 0.95 when the cutoff value was 20 ng/mL<sup>[30]</sup>. In comparison, our analysis showed a relatively high sensitivity (0.70) of RASSF1A promoter methylation, which, if combined, may complement the AFP test and reduce FNs by performing detection of RASSF1A methylation status and the AFP test simultaneously. Positive results for either of

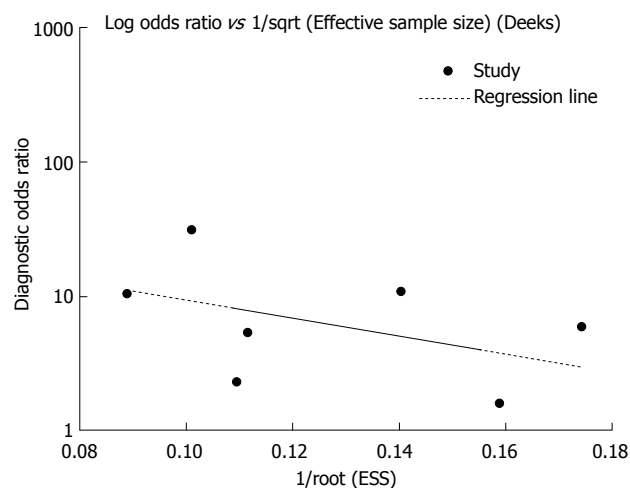




**Figure 3** Summary receiver operation characteristic curve for Ras association domain family 1A methylation assays. Hollow numbered circles represent included studies. Summary receiver operation characteristic (SROC) curve summarizes the overall diagnostic accuracy.

the two tests demonstrate a high likelihood of HCC and patients should be recommended for advanced imaging or percutaneous liver biopsies. Nevertheless, more evidence on the correlation between RASSF1A methylation and AFP level should be collected. In our analysis, we observed FPs in some of included studies. *RASSF1A* gene promoter methylation might occur in controls including chronic liver disease and/or in preneoplastic (cirrhotic) liver to hepatocellular nodules to HCC<sup>[25]</sup>.

The *RASSF1A* gene has been long noted and intensively studied for its role in tumor suppression, and aberrant methylation in the promoter region is suspected as the main mechanism of downregulation and silencing that is widely observed in human malignancies<sup>[15]</sup>. However, in this meta-analysis, we aimed to verify the feasibility of body fluid RASSF1A methylation in identifying HCC from high risk population rather than healthy population. So we selected patients with chronic liver diseases which were in high risk of developing HCC as controls. Under this condition, HCC was of greater possibility than other types of cancer. Thus, methylation of RASSF1A is relatively specific to HCC and the detection was still meaningful in distinguishing HCC from benign liver diseases. The potential clinical use of RASSF1A methylation as a biomarker for HCC diagnosis has not been fully exploited, and studies measuring RASSF1A methylation in body fluids are rare. Nevertheless, the non-invasive test possesses considerable advantages. It avoids the risk of complications and sampling error caused by imaging or percutaneous liver biopsies and is more acceptable to patients<sup>[31]</sup>. Although tumor-specific cfDNA is thought to be mainly derive from apoptosis and necrosis of cancer



**Figure 4** Assessment of the potential publication bias in Ras association domain family 1A methylation detection. We found no significant publication bias ( $P > 0.05$ ). Each spot represents one study and the regression line is shown.

cells in tumor microenvironment, discordance between frequencies of alterations found in DNA extracted from tumor tissue and cfDNA do exist<sup>[12]</sup>. Zhang *et al.*<sup>[32]</sup> showed that the frequency of RASSF1A methylation in 48 HCC tissues was 100%, which was greatly higher than our results.

Moreover, rapidly developed technologies for DNA methylation detection make it easier, faster and cheaper to measure RASSF1A methylation accurately<sup>[5,33-35]</sup>. The technologies applicable to the analysis of the small amounts of DNA present in the body fluids are mainly real-time PCR-based approaches such as MethyLight, HeavyMethyl, methylation-sensitive high-resolution melting analysis, and methylation-sensitive melting analysis after real-time methylation specific PCR<sup>[36]</sup>. However, some methodological issues should be resolved. The primer sequences and PCR procedures are varied in studies applying qualitative MSP. Also, MSP may have difficulties in distinguishing TP from FP due to the methodological limitations. Besides, the different cut-off values chosen could affect judgment of positive or negative results in detection using QMSP. We summarized the primer sequences for RASSF1A methylation detection in all the included studies to facilitate further confirmation. Before the assay can be put into clinical practice, more evidence based on methodological investigations should be collected and a standard protocol developed.

Exploring the sources of heterogeneity is one major purpose of meta-analysis<sup>[37]</sup>. Due to the significant heterogeneity observed in our analysis, we performed meta-regression. However, we failed to figure out the sources as all the covariates we took into consideration could not significantly interpret the heterogeneity. We surmise that the limited number of included studies was the main factor that hampered the analysis of heterogeneity, which also made subgroup analysis not possible.

In addition to the small number of studies, there were

some other limitations. First, the case-control design used in all our included studies was likely to have introduced bias in patient selection<sup>[16]</sup> and more prospective studies are needed. Second, although we classified the controls and excluded healthy controls, the remaining patients still presented a spectrum of diseases. Due to the insufficient detailed information provided by the publications, we failed to subdivide the chronic liver diseases and specific stages of one single disease, which may have introduced considerable heterogeneity. Third, it has been reported that time of sampling influences the level of methylation dramatically<sup>[38,39]</sup>. The studies included in our analysis varied greatly in time of sampling, which may have caused heterogeneity.

In conclusion, RASSF1A promoter methylation is a valuable diagnostic biomarker with a qualified sensitivity, which may complement the AFP test in screening for HCC. More prospective diagnostic trials with strictly defined controls are needed to elucidate further the accuracy of RASSF1A methylation for diagnosis of HCC.

## COMMENTS

### Background

Hepatocellular carcinoma (HCC) is one of the most prevalent human malignancies. HCC patients are prone to present a poor prognosis due to failure of early detection. The currently used serum  $\alpha$ -fetoprotein (AFP) test has a relatively low sensitivity, which limits its application. Ras association domain family 1A (RASSF1A) methylation has been shown to be a diagnostic marker for HCC.

### Research frontiers

Recently, there has been an increasing number of investigations focusing on the diagnostic role of RASSF1A promoter methylation in HCC. However, there is disagreement about the actual diagnostic accuracy. This study was conducted to assess pooled estimates of the diagnostic accuracy of RASSF1A methylation in HCC.

### Innovations and breakthroughs

The authors assessed RASSF1A methylation for HCC diagnosis by meta-analysis. The controls were rigorously defined as patients with chronic liver diseases to mimic clinical practice and only studies that used body fluids as samples for detection were included because they were non-invasive. The results showed a better sensitivity compared to the AFP test. The findings may improve HCC diagnostic accuracy.

### Applications

RASSF1A methylation may have diagnostic potential for HCC and could be a new candidate marker. Furthermore, detection in body fluids makes it possible to be non-invasive and more acceptable to patients.

### Terminology

DNA methylation is a conversion of the cytosine to 5-methylcytosine which typically occurs at CpG islands in the promoter region with the help of DNA methyltransferases. This process may result in gene silencing without changing its coding sequence.

### Peer review

The authors showed that RASSF1A methylation in body fluids in HCC patients can improve HCC diagnostic accuracy using meta-analysis. The idea of this study is novel and important as they continue to evaluate novel potential biomarkers for the early diagnosis of HCC.

## REFERENCES

- 1 **Forner A**, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379**: 1245-1255 [PMID: 22353262 DOI: 10.1016/S0140-6736(11)61347-0]
- 2 **Farinati F**, Marino D, De Giorgio M, Baldan A, Cantarini

- M, Cursaro C, Rapaccini G, Del Poggio P, Di Nolfo MA, Benvegnù L, Zoli M, Borzio F, Bernardi M, Trevisani F. Diagnostic and prognostic role of alpha-fetoprotein in hepatocellular carcinoma: both or neither? *Am J Gastroenterol* 2006; **101**: 524-532 [PMID: 16542289 DOI: 10.1111/j.1572-0241.2006.00443.x]
- 3 **Tischhoff I**, Tannapfe A. DNA methylation in hepatocellular carcinoma. *World J Gastroenterol* 2008; **14**: 1741-1748 [PMID: 18350605 DOI: 10.3748/wjg.14.1741]
- 4 **Herath NI**, Leggett BA, MacDonald GA. Review of genetic and epigenetic alterations in hepatocarcinogenesis. *J Gastroenterol Hepatol* 2006; **21**: 15-21 [PMID: 16706806 DOI: 10.1111/j.1440-1746.2005.04043.x]
- 5 **Eads CA**, Danenberg KD, Kawakami K, Saltz LB, Blake C, Shibata D, Danenberg PV, Laird PW. MethyLight: a high-throughput assay to measure DNA methylation. *Nucleic Acids Res* 2000; **28**: E32 [PMID: 10734209 DOI: 10.1093/nar/28.8.e32]
- 6 **Shivapurkar N**, Gazdar AF. DNA methylation based biomarkers in non-invasive cancer screening. *Curr Mol Med* 2010; **10**: 123-132 [PMID: 20196733 DOI: 10.2174/156652410790963303]
- 7 **Summers T**, Langan RC, Nissan A, Brücher BL, Bilchik AJ, Protic M, Daumer M, Avital I, Stojadinovic A. Serum-based DNA methylation biomarkers in colorectal cancer: potential for screening and early detection. *J Cancer* 2013; **4**: 210-216 [PMID: 23459561 DOI: 10.7150/jca.5839]
- 8 **Ramzy II**, Omran DA, Hamad O, Shaker O, Abboud A. Evaluation of serum LINE-1 hypomethylation as a prognostic marker for hepatocellular carcinoma. *Arab J Gastroenterol* 2011; **12**: 139-142 [PMID: 22055592 DOI: 10.1016/j.ajg.2011.07.002]
- 9 **Pike BL**, Guerry P, Poly F. Global Distribution of Campylobacter jejuni Penner Serotypes: A Systematic Review. *PLoS One* 2013; **8**: e67375 [PMID: 23826280 DOI: 10.1371/journal.pone.0067195]
- 10 **Majchrzak-Celińska A**, Paluszczak J, Kleszcz R, Magiera M, Barciszewska AM, Nowak S, Baer-Dubowska W. Detection of MGMT, RASSF1A, p15INK4B, and p14ARF promoter methylation in circulating tumor-derived DNA of central nervous system cancer patients. *J Appl Genet* 2013; **54**: 335-344 [PMID: 23661397 DOI: 10.1007/s13353-013-0149-x]
- 11 **Fleischhacker M**, Schmidt B. Circulating nucleic acids (CNAs) and cancer—a survey. *Biochim Biophys Acta* 2007; **1775**: 181-232 [PMID: 17137717 DOI: 10.1016/j.bbcan.2006.10.001]
- 12 **Danese E**, Minicozzi AM, Benati M, Montagnana M, Paviati E, Salvagno GL, Gusella M, Pasini F, Guidi GC, Lippi G. Epigenetic alteration: new insights moving from tissue to plasma - the example of PCDH10 promoter methylation in colorectal cancer. *Br J Cancer* 2013; **109**: 807-813 [PMID: 23839493 DOI: 10.1038/bjc.2013.351]
- 13 **Agathangelou A**, Cooper WN, Latif F. Role of the Ras-association domain family 1 tumor suppressor gene in human cancers. *Cancer Res* 2005; **65**: 3497-3508 [PMID: 15867337 DOI: 10.1158/0008-5472.CAN-04-4088]
- 14 **Donninger H**, Vos MD, Clark GJ. The RASSF1A tumor suppressor. *J Cell Sci* 2007; **120**: 3163-3172 [PMID: 17878233 DOI: 10.1242/jcs.010389]
- 15 **van der Weyden L**, Adams DJ. The Ras-association domain family (RASSF) members and their role in human tumorigenesis. *Biochim Biophys Acta* 2007; **1776**: 58-85 [PMID: 17692468 DOI: 10.1016/j.bbcan.2007.06.003]
- 16 **Whiting PF**, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MM, Sterne JA, Bossuyt PM. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* 2011; **155**: 529-536 [PMID: 22007046 DOI: 10.7326/0003-4819-155-8-201110180-00009]
- 17 **Deville WL**, Buntinx F, Bouter LM, Montori VM, de Vet HC, van der Windt DA, Bezemer PD. Conducting sys-

- tematic reviews of diagnostic studies: didactic guidelines. *BMC Med Res Methodol* 2002; **2**: 9 [PMID: 12097142 DOI: 10.1186/1471-2288-2-9]
- 18 **DerSimonian R**, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; **7**: 177-188 [PMID: 3802833 DOI: 10.1016/0197-2456(86)90046-2]
  - 19 **Rosman AS**, Korsten MA. Application of summary receiver operating characteristics (sROC) analysis to diagnostic clinical testing. *Adv Med Sci* 2007; **52**: 76-82 [PMID: 18217394]
  - 20 **Walter SD**. Properties of the summary receiver operating characteristic (SROC) curve for diagnostic test data. *Stat Med* 2002; **21**: 1237-1256 [PMID: 12111876 DOI: 10.1002/sim.1099]
  - 21 **Deeks JJ**, Macaskill P, Irwig L. The performance of tests of publication bias and other sample size effects in systematic reviews of diagnostic test accuracy was assessed. *J Clin Epidemiol* 2005; **58**: 882-893 [PMID: 16085191 DOI: 10.1016/j.jclinepi.2005.01.016]
  - 22 **Chan KC**, Lai PB, Mok TS, Chan HL, Ding C, Yeung SW, Lo YM. Quantitative analysis of circulating methylated DNA as a biomarker for hepatocellular carcinoma. *Clin Chem* 2008; **54**: 1528-1536 [PMID: 18653827 DOI: 10.1373/clinchem.2008.104653]
  - 23 **Chang H**, Yi B, Li L, Zhang HY, Sun F, Dong SQ, Cao Y. Methylation of tumor associated genes in tissue and plasma samples from liver disease patients. *Exp Mol Pathol* 2008; **85**: 96-100 [PMID: 18691570 DOI: 10.1016/j.yexmp.2008.07.001]
  - 24 **Maurizio MS**, Fracanzani AL, Tavazzi D, Cespiati A, Bertelli C, Bignamini D, Valenti L, Fargion SR. Aberrant methylation of oncosuppressor genes detected in the peripheral blood of patients with chronic hepatitis C and hepatocellular carcinoma. *J Hepatol* 2011; **54** Suppl 1: S107 [DOI: 10.1016/S0168-8278(11)60262-6]
  - 25 **Mohamed NA**, Swify EM, Amin NF, Soliman MM, Tag-Eldin LM, Elsherbiny NM. Is serum level of methylated RASSF1A valuable in diagnosing hepatocellular carcinoma in patients with chronic viral hepatitis C? *Arab J Gastroenterol* 2012; **13**: 111-115 [PMID: 23122451 DOI: 10.1016/j.ajg.2012.06.009]
  - 26 **Azab NI**, Abd El Kariem HM, Mowafi T, Fouad HF, El Abd AM. Blood Ras-association domain family 1A gene methylation status in some liver diseases. *Life Sci* 2011; **8**: 531-539
  - 27 **Zhang YJ**, Wu HC, Shen J, Ahsan H, Tsai WY, Yang HI, Wang LY, Chen SY, Chen CJ, Santella RM. Predicting hepatocellular carcinoma by detection of aberrant promoter methylation in serum DNA. *Clin Cancer Res* 2007; **13**: 2378-2384 [PMID: 17438096 DOI: 10.1158/1078-0432.CCR-06-1900]
  - 28 **Huang ZH**, Hu Y, Hua D, Wu YY, Song MX, Cheng ZH. Quantitative analysis of multiple methylated genes in plasma for the diagnosis and prognosis of hepatocellular carcinoma. *Exp Mol Pathol* 2011; **91**: 702-707 [PMID: 21884695 DOI: 10.1016/j.yexmp.2011.08.004]
  - 29 **Thompson SG**, Higgins JP. How should meta-regression analyses be undertaken and interpreted? *Stat Med* 2002; **21**: 1559-1573 [PMID: 12111920 DOI: 10.1002/sim.1187]
  - 30 **Choi JY**, Jung SW, Kim HY, Kim M, Kim Y, Kim DG, Oh EJ. Diagnostic value of AFP-L3 and PIVKA-II in hepatocellular carcinoma according to total-AFP. *World J Gastroenterol* 2013; **19**: 339-346 [PMID: 23372355 DOI: 10.3748/wjg.v19.i3.339]
  - 31 **Carey E**, Carey WD. Noninvasive tests for liver disease, fibrosis, and cirrhosis: Is liver biopsy obsolete? *Cleve Clin J Med* 2010; **77**: 519-527 [PMID: 20682514 DOI: 10.3949/ccjm.77a.09138]
  - 32 **Zhang X**, Li HM, Liu Z, Zhou G, Zhang Q, Zhang T, Zhang J, Zhang C. Loss of heterozygosity and methylation of multiple tumor suppressor genes on chromosome 3 in hepatocellular carcinoma. *J Gastroenterol* 2013; **48**: 132-143 [PMID: 22766745 DOI: 10.1007/s00535-012-0621-0]
  - 33 **Herman JG**, Graff JR, Myöhänen S, Nelkin BD, Baylin SB. Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. *Proc Natl Acad Sci USA* 1996; **93**: 9821-9826 [PMID: 8790415 DOI: 10.1073/pnas.93.18.9821]
  - 34 **Harrison A**, Parle-McDermott A. DNA methylation: a timeline of methods and applications. *Front Genet* 2011; **2**: 74 [PMID: 22303369 DOI: 10.3389/fgene.2011.00074]
  - 35 **Heyn H**, Esteller M. DNA methylation profiling in the clinic: applications and challenges. *Nat Rev Genet* 2012; **13**: 679-692 [PMID: 22945394 DOI: 10.1038/nrg3270]
  - 36 **How Kit A**, Nielsen HM, Tost J. DNA methylation based biomarkers: practical considerations and applications. *Biochimie* 2012; **94**: 2314-2337 [PMID: 22847185 DOI: 10.1016/j.biochi.2012.07.014]
  - 37 **Petitti DB**. Approaches to heterogeneity in meta-analysis. *Stat Med* 2001; **20**: 3625-3633 [PMID: 11746342 DOI: 10.1002/sim.1091]
  - 38 **Ushijima T**. Epigenetic field for cancerization. *J Biochem Mol Biol* 2007; **40**: 142-150 [PMID: 17394762 DOI: 10.5483/BMBRep.2007.40.2.142]
  - 39 **Ushijima T**, Nakajima T, Maekita T. DNA methylation as a marker for the past and future. *J Gastroenterol* 2006; **41**: 401-407 [PMID: 16799880 DOI: 10.1007/s00535-006-1846-6]

**P- Reviewers:** Tomizawa M, Tsuchiya A, Wong RJ

**S- Editor:** Gou SX **L- Editor:** A **E- Editor:** Ma S







Published by **Baishideng Publishing Group Co., Limited**

Flat C, 23/F., Lucky Plaza,  
315-321 Lockhart Road, Wan Chai, Hong Kong, China

Fax: +852-65557188

Telephone: +852-31779906

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

<http://www.wjgnet.com>



ISSN 1007-9327

