ANSWERING REVIEWERS

April 24, 2014

Dear Editor,

Title: Accuracy of early detection of colorectal tumours by stool methylation markers: A Meta-Analysis

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The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1)In most of the studies considered the number of samples from healthy individuals is low. Could this unbalance situation have introduced statistical biases?

ANSWER:

No bias. Because the result of meta-regression indicates there exists no bias between higher quality studies and lower quality studies in table 4. In the higher quality studies, the healthy individuals have balanced with people with illnesses.

(2)Biases possibly due to the different methods used for methylation detection should be also considered.

ANSWER:

We had analyzed the biases between the different methods used for methylation detection in table 4. There was no bias among the different methods.

(3)In the Abstract it should be more clearly reported that several data concerning the methylation status of several genes have been analyzed and not only SFRP2.

ANSWER:

Add the following into the Abstract: The sensitivity and specificity for the detection of CRC were 73% (95%CI: 71%-75%) and 92% (95%CI: 90%-93%), respectively. And for adenoma, the sensitivity and specificity were 51% (95%CI: 47%-54%) and 92% (95%CI: 90%-93%), respectively.

(4)Check table 1 for accuracy of gene symbols used (frequently SFRPs are misspells ad SRRP)

ANSWER:

Yes, We think your opinion is correct.

(5)Footnotes to table 1: + and – appear to reflect the number of individuals with positive and negative test result, respectively, both presenting the disease and normal. Legend

appears to restrict this annotation only to individuals “with the disease”

ANSWER：

Yes, We think your opinion is correct. We have deleted “with the disease”.

(6)Legend to figure 3: change 2a, 2b, 2c to 3a, 3b, 3c.

ANSWER:

Yes, We think your opinion is correct.

(7)Authors compared the accuracy of fecal SFRP2 methylation to FOBT. However, they found that fecal SFRP2 methylation is an optical marker for detection of CRCs but not adenomas. They should separately discuss the availability and potential of fecal SFRP2 methylation and FOBT in the screening for CRCs and colorectal adenomas.

ANSWER:

Stool DNA testing has emerged as a biologically rational and user-friendly strategy for the non-invasive detection of both CRC and critical precursor lesions. Our results indicate that the fecal SFRP2 methylation is high, with the sensitivity of 51% (95%CI: 47%-54%) and the specificity of 92% (95%CI: 90%-93%). FOBT is a normal CRC-screening method, and is confirmed to reduce the mortality of CRC, but the test has little or no impact on the incidence of CRC because of the low level of sensitivity to precursor lesions. The reason may contribution to the cells exfoliated from colorectal neoplasms appear to be a continuous process, while occult bleeding is intermittent.

(8)As stated in the text, previous studies suggested that SFRP can be associated with an early event of colorectal carcinogenesis. However, fecal SFRP2 methylation was not enough for adenoma detection. They should discuss why this happened.

ANSWER:

Research conducted during the past 30 years has increased our understanding of the mechanisms involved in colorectal cancer initiation and development. The findings have demonstrated the existence of at least three pathways: chromosomal instability, microsatellite instability and CpG island methylator phenotype [1]. The CpG island methylator phenotype is one of the pathways through which CRC progresses. Historically, colorectal adenoma has been recognized as the most important precancerous lesion to CRC. It is estimated that fifty percent of individuals will develop adenomas in their lifetime, but only six percent will convert into CRC [2, 3]. Therefore most adenomas do not progress to cancer. And serrated polyp is increasingly recognized as likely precancerous lesion. It is estimated that 20%–30% of CRC arise from serrated polyp rather than adenoma [4].

[1] Colussi D, Brandi G, Bazzoli F, Ricciardiello L. Molecular pathways involved in colorectal cancer: implications for disease behavior and prevention. Int J Mol Sci 2013; 14: 16365-16385.

[2] Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics, 2008. CA Cancer J Clin 2008; 58: 71-96

[3] He B, Reguart N, You L, Mazieres J, Xu Z, Lee AY, Mikami I, McCormick F, Jablons DM. Blockade of Wnt-1 signaling induces apoptosis in human colorectal cancer cells containing downstream mutations. Oncogene 2005; 24: 3054-3058

[4]Noffsinger AE. Serrated polyps and colorectal cancer: new pathway to malignancy. Annu Rev Pathol 2009; 4:

343-364.

(9)Well written meta-analysis on the Accuracy of early detection of colorectal tumours by stool methylation markers. The authors need to update the literature search by Jan, 2014.

ANSWER:

Yes, We think your opinion is correct. We have added there studies including 333 patients into our paper.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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Accuracy of early detection of colorectal tumours by stool methylation markers: A Meta-Analysis

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Abstract

AIM: To evaluate the accuracy of methylation of genes in stool samples for diagnosing colorectal tumours.

METHODS: Electronic databases including PubMed, Web of Science, Chinese Journal Full Text Database and Wanfang Journals Full-text Database were searched to find relevant original articles about methylated genes to be used in diagnosing colorectal tumours. Quality assessment of diagnostic accuracy studies items were used to evaluate the quality of the included articles, and the Meta-disc 1.4 and SPSS 13.0 software were used for data analysis.

RESULTS: Thirty-seven articles met the inclusion criteria, and 4484 patients were included. The sensitivity and specificity for the detection of CRC were 73% (95%CI: 71%-75%) and 92% (95%CI: 90%-93%), respectively. And for adenoma, the sensitivity and specificity were 51% (95%CI: 47%-54%) and 92% (95%CI: 90%-93%), respectively. Pooled diagnostic performances of SFRP2 methylation for colorectal cancer (CRC) provided the following results: the sensitivity was 79% (95%CI: 75%-82%), the specificity was 93% (95%CI: 90%-96%), the diagnostic OR was 47.57 (95%CI: 20.08-112.72), the area under the curve was 0.9565. Additionally, the results of accuracy of SFRP2 methylation for detecting colorectal adenomas were as follows: sensitivity was 43% (95%CI: 38%-49%), specificity was 94% (95%CI: 91%-97%), the diagnostic OR was 11.06 (95%CI: 5.77-21.18), and the area under the curve was 0.9563.

CONCLUSION: Stool-based DNA testing may be useful for noninvasively diagnosing colorectal tumours, and SFRP2 methylation is a promising marker that has great potential in early CRC diagnosis.

Key words: Colorectal carcinoma; Colorectal adenoma; Stool; Methylation; Meta-analysis

Core tip: The analysis of stool methylation markers as a non-invasive test is important for the early diagnosis of colorectal tumours. However, no consensus has been reached with regard to the role of stool methylation markers in colorectal tumour diagnosis. We performed a meta-analysis of 37 articles, and the pooled results showed that stool methylation markers could be used as a valuable diagnostic and predictive tool for colorectal tumours and SFRP2 methylation serves as a promising marker with great potential in early CRC diagnosis.

Zhang H, Qi J, Wu YQ, Zhang P, Jiang J, Wang QX, Zhu YQ. Accuracy of early detection of colorectal tumours by stool methylation markers: A Meta-Analysis

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INTRODUCTION

Colorectal cancer (CRC) is the third most common malignancy and the second leading cause of cancer-related deaths in western countries[1,2]. A 5-year survival rate for stage I CRC has reached 90%[3], but less than 10% for CRC cases who have distant metastases[4]. However, most CRC patients are diagnosed in the middle or late stages because no typical symptoms for the early stage of CRC exist[5]. Therefore, the diagnosis of CRC in early stages has great importance for reducing CRC mortality.

Early diagnosis of colorectal cancer will help to reduce mortality and the costs for surgery. Currently colonoscopy-screening test is of high efficacy, but the acceptability of this procedure in the general public is rather low. As an available non-invasive method, faecal testing has a unique advantage when compared to other screening modalities. Although faecal occult blood testing (FOBT) has been confirmed to reduce mortality due to CRC, the test has little or no impact on the incidence of CRC because of its low-level sensitivity to adenoma[6], i.e., a sensitivity of only 10%-20%[7]. Compared to FOBT, the most important advantage of methylation markers in stool samples is its higher accuracy and sensitivity of the diagnosis of premalignant lesions of CRC[8].

DNA methylation often occurs in the early stages of CRC, and many studies have been performed on the diagnosis of colorectal tumours by determining the methylation of genes in stool samples. However, the results of these studies are variable although inspiring. Thus, this meta-analysis will be conducted to assess the accuracy of the detection of colorectal tumours by the methylation of genes in stool samples.

MATERIALS AND METHODS

Search strategy

A literature search was performed independently by two investigators (Zhang H and Qi J) using the following databases: Pubmed, Web of Science, Chinese Journal Full Text Database and Wanfang Journals Full-text Database. All references that were cited in these studies and all published reviews were also searched. All English and Chinese references for analyse were published before January 2014. The following keywords were used in the search strategy: “colon/rectal/colorectal”, “cancer/tumours”, “stool,” and “methylation”. In this meta-analysis, 2 × 2 tables were constructed from each study for the true-positive, false-negative, true-negative and false-positive values.

Inclusion and exclusion criteria

Eligible studies were required to meet all of the following criteria: (a) the data were independent; (b) the CRC was diagnosed using DNA methylation analysis in stool sample; (c) the patients were diagnosed with colorectal cancer or colorectal adenomas by pathology; and (d) the colonoscopy result of the control individuals was normal.

Exclusion criteria for this meta-analysis were as follows: (a) studies on secondary CRC or primary CRC with other organs metastases; and (b) studies on CRC patients receiving chemotherapy or curative surgery.

Data extraction and quality assessment

The following data were extracted from each study: author, year of publication, country or region, sample size, the name of genes, the detection method of methylation and the study design. The data were independently extracted by two investigators (Zhang H and Qi J), and discrepancies were solved by a third investigator (Zhu YQ) and collective discussion. Quality Assessment of studies of Diagnostic Accuracy[9] (QUADAS) was used to assess the quality of the primary studies with diagnostic accuracy, and quality scoring was appraised based on the empirical evidence, the experts' opinions and the formal consensus. Score of 1, 0 and -1 were given to the articles that were in compliance with the standards completely, unclear or out of standards, respectively, and the full score was 14.

Statistical analysis

All statistics were calculated and then combined using a random-effects model and 95% confidence intervals (CI) as effect measurements. The diagnostic odds ratio (DOR) reflects the relationship between the result of the diagnostic test and the disease. The summary receiver operation characteristic (SROC) curve displays the trade-off between sensitivity and specificity and represents a global summary of test performance. We used the Q-value, which is the intersection point of the SROC curve with a diagonal line from the left upper corner to the right lower corner of the receiver operation characteristic (ROC) space, which corresponds to the highest value of sensitivity and specificity for the test. The positive likelihood ratio (PLR) represents the value by which the odds of the disease increase when a test is positive, whereas the negative likelihood ratio (NLR) shows the value by which

the odds of the disease decrease when a test is negative. Statistical heterogeneity was assessed using the Chi-square test, and alpha significance testing was performed at the two-tailed 0.05 level. The professional statistical software programs (Meta-DiSc 1.4 and SPSS 13.0) were used for analysis. Publication bias was assessed by Egger analysis.

RESULTS

The literature search retrieved 541 citations, 408 of which were excluded because they were duplicates. Of the 133 potentially eligible studies, 96 publications were excluded because they did not investigate colorectal tumour or human stool studies (n = 21), included no diagnostic value studies (n = 20), were reviews (n = 27) or had overlapping data (n = 28). Finally, 37 studies that focused on the target patient spectrum were included (Figure 1).

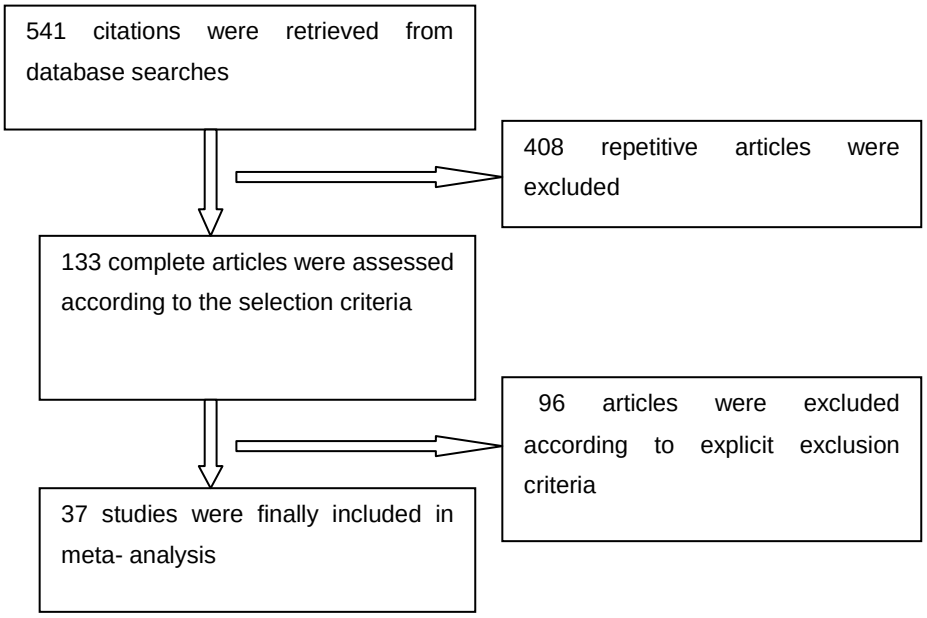


Figure 1 Flowchart of the study selection.

Study characteristics

Of the 37 studies, 7 were Chinese and 30 were English, and they included 4484 patients (Table 1). These studies were performed in 10 countries or regions (including China, the USA, the Netherlands, Spain, Japan, Germany, Iran, Hong Kong, Austria and South Korea). In these studies, 34 evaluated CRC, and 26 evaluated colorectal adenoma. Twenty-four studies focused on the methylation of a single gene, and the other 13 studies involved the methylation of multiple genes.

Genes evaluated in these studies mainly involved in three types of regulation pathways: the Wnt pathway, the DNA damage repair pathway and other pathways. Five genes of the Wnt pathway were involved in 11 studies: *secreted frizzled-related proteins (SFRP1, SFRP2, SFRP5), Adenomatous Polyposis Coli (APC)* and *WNT2*. Two genes of the DNA damage repair pathway were involved in 7 of the studies: *O-6-Methylguanine-DNA Methyltransferase (MGMT)* and *MutL Homologue 1(MLH1)*. Twenty-nine (Twenty-seven) studies involved 22 genes of Other pathways: *Vimentin, Oncostain M Receptor-β(OSMR), Phosphatase and Actin Regulator 3(PHACTR3), Cyclin-Dependent Kinase Inhibitor 2A(CDKN2A), Tissue Factor Pathway Inhibitor (TFPI2), Hyperplastic Polyposis Protein Gene(HPP1), GATA4, Human Lactoferrin (HLTF), ATM, Ras Association Domain Family2(RASSF2), RARB2, Hypermethylated In Cancer 1(HIC), Engrailed gene(EN1), N-Myc Downstream- Regulated Gene family(NDRG4), IGTA4, T-cell differentiation protein(MAL), Spastic Paraplegia-20(SPG20), Fibrillin-1(FBN1), AGTR1, SLIT2, SEPT9* and *Angiotensin II type 1 receptor gene (AGTR1).*

Qualitative and quantitative methods were the two main types of methods used for methylation detection. The qualitative method included methylation-specific PCR (MSP) and methylation-specific melting curve analysis (MS-MCA). The quantitative method included Methl-BEAMing; quantitative MSP (qMSP); MethyLight; combined with bisulfite restrictive inscribed enzymatic (COBRA); Pyrosequencing; and quantitative, allele-specific, real-time target and signal amplification (QuARTS).

Table1 The characteristics of the included studies in the meta-analysis and QUADAS scores

Study/year	Country/ region	Methylation of genes	N	CRC		Adenoma		Normal		Blind design	Detection method	QUADAS score
				+	-	+	-	+	-			
Ahlquist *et al*[10] 2012	Ireland	Vimentin/ NDRG4/ BMP3/TFPI2	98	26	4	18	4	5	41	Yes	QuARTS	11
Bosch *et al*[11] 2011	Netherlands	PHACTR3	185	40	25	6	13	4	97	Unclear	qMSP	10
		GATA4	160	29	11	3	16	6	95			
		OSMR	185	25	40	4	15	7	94			
Ahlquist *et*	Ireland	PHACTR3	639	214	38	51	43	29	264	Yes	QuARTS	11

al[12] 2011												
Capella *et*	Spain	RARB2/P16/	98	25	13	20	20	0	20	Yes	MS-MCA	10
al[13] 2010		MGMT/										
		APC										
		RARB2	85	11	23	7	31	0	13			
		P16	77	9	21	6	28	0	13			
		MGMT	80	9	19	3	34	0	15			
		APC	77	9	19	9	25	0	15			
Wang *et*	China	SFRP2	262	142	27	29	34	2	28	Yes	MSP	9
al[14] 2011												
Baek *et*	South	Vimentin/	149	45	15	31	21	5	32	Yes	MSP	9
al[15] 2009	Korea	MGMT/										
		MLH1										
		MLH1	149	18	42	6	46	0	37			
		Vimentin	149	23	37	8	44	0	37			
		MGMT	149	31	29	19	33	5	32			
Chen *et*	USA	Vimentin	80	9	13	9	11	2	36	Unclear	Methl-BEA	5
al[16] 2009											Ming	
Engeland	Netherla	NDRG4	150	42	33	nr	nr	3	72	Yes	qMSP	11
et al[17] 2009	nds											
Grady *et*	USA	IGTA4	37	nr	nr	7	2	6	22	Unclear	qMSP	4
al[18] 2009												
van den	Netherla	GATA4	150	44	31	nr	nr	9	66	Yes	qMSP	10
Bosch *et*	nds											
al[19] 2009												
Peinado *et*	Spain	EN1	60	8	22	nr	nr	1	29	Unclear	MS-MCA	7
al[20] 2009												
Sidransky	USA	OSMR/	42	12	8	6	11	0	5	Yes	qMSP	9
et al[21]		SFRP1										
2009		OSMR	201	35	54	2	14	4	92			
		SFRP1	52	11	9	5	12	0	15			
Nagasaka	Japan	SFRP2	253	53	31	18	38	9	104	Unclear	COBRA	10
et al[22] 2009												
		RASSF2	253	38	46	7	49	6	107			
Ahuja *et*	USA	TFPI2	129	44	14	7	19	2	43	Yes	qMSP	12
al[23] 2009												

Wang *et al*[24] 2008	China	SFRP2	133	60	9	21	13	2	28	Yes	MethyLight	8
Oberwalder *et al*[25] 2008	Australia	SFRP2	19	nr	nr	6	7	0	6	Yes	MethyLight	9
Itzkowitz *et al*[26] 2008	USA	Vimentin	80	9	13	9	11	2	36	Yes	MSP	13
Huang *et al*[27] 2007	China	SFRP2/HPP1/ MGMT	97	50	2	15	6	1	23	Yes	MSP	8
		SFRP2	97	49	3	11	10	1	23			
		HPP1	97	37	15	12	9	0	24			
		MGMT	97	25	27	6	15	0	24			
Itzkowitz *et al*[28] 2007	USA	Vimentin/ HLTF	162	31	9	nr	nr	19	103	Yes	MSP	13
		HLTF	162	15	25	nr	nr	9	113			
		Vimentin	162	29	11	nr	nr	16	106			
Tavasoli *et al*[29] 2007	Hong kong	p16	45	5	20	nr	nr	0	20	Unclear	MSP	8
Matzel *et al*[30] 2007	Germany	SFRP1	44	16	4	7	0	2	15	Yes	MSP	9
Leung *et al*[31] 2007	Hong kong	SFRP2/ MGMT/ MLH1/ HLTF/ ATM/ APC	75	16	4	18	7	3	27	Yes	MSP	13
		SFRP2	75	6	14	3	22	2	28			
		MGMT	75	4	16	3	22	0	30			
		MLH1	75	4	16	3	22	0	30			
		HLTF	75	5	15	5	20	1	29			
		ATM	75	5	15	5	20	0	30			
		APC	75	4	16	4	21	0	30			
Grady *et al*[32] 2005	USA	MGMT/ CDKN2A/ MLH1	48	nr	nr	16	13	7	12	Yes	MSP	9
		CDKN2A	48	nr	nr	9	20	3	16			
		MGMT	48	nr	nr	14	15	5	14			
		MLH1	48	nr	nr	0	29	2	17			

Kolligs *et al*[33] 2005	Germany	HIC1	71	11	15	4	9	0	32	Yes	MSP	11
Markowitz *et al*[34] 2005	USA	Vimentin	263	43	51	6	44	8	111	Yes	MSP	11
Müller *et al*[35] 2004	Australia	SFRP2/ SFRP5	39	20	3	nr	nr	8	8	Unclear	MethyLight	5
		SFRP2	39	19	4	nr	nr	4	12			
		SFRP5	39	18	5	nr	nr	5	11			
Xu *et al*[36] 2012	China	SFRP2	90	20	10	15	15	1	29	Unclear	MSP	5
Fu *et al*[37] 2011	China	MGMT/ MAL/ CDKN2A	119	64	5	17	7	2	24	Unclear	MSP	7
		MAL	119	54	15	14	10	1	25			
		CDKN2A	119	36	33	10	14	0	26			
		MGMT	119	38	31	9	15	1	25			
Li *et al*[38] 2011	China	Vimentin/ OSMR/ TFPI2	107	52	8	13	4	4	26	Unclear	MSP	9
		Vimentin	107	32	28	5	12	0	30			
		OSMR	107	41	19	7	10	0	30			
		TFPI2	107	45	15	11	6	4	26			
Sheng *et al*[39] 2010	China	Vimentin	22	5	9	nr	nr	0	8	Unclear	MSP	5
Chen *et al*[40] 2009	China	P16	108	47	14	16	11	1	19	Unclear	MSP	7
Cheng *et al*[41] 2007	China	SFRP2	97	49	3	11	10	1	23	Unclear	MSP	5
Xiao *et al*[42] 2009	China	NDRG4	114	64	20	nr	nr	3	27	Unclear	MSP	6
Park *et al*[43] 2010	South Korea	IGTA4/ SFRP2/ P16	86	21	9	18	7	1	30	Yes	MSP	8
		IGTA4	86	11	19	4	21	0	31			
		SFRP2	86	18	12	11	14	0	31			
		P16	86	12	18	6	19	1	30			
Zhang *et al*[44] 2013	China	SPG20	126	77	19	nr	nr	0	30	Unclear	MSP	7
Carmona *et al*[45] 2013	Spain	AGTR1/WNT2 /SLIT2	102	50	14	nr	nr	4	34	Unclear	Pyrosequencing	10

		AGTR1	107	14	54	nr	nr	2	37		
		WNT2	91	21	31	nr	nr	1	38		
		SLIT2	108	37	34	nr	nr	2	35		
		SEPT9	61	7	28	nr	nr	0	26		
		Vimentin	55	18	15	nr	nr	3	19		
Guo *et al*[46] 2013	China	FBN1	105	54	21	nr	nr	2	28	MSP	6

Abbreviations: +: Represents the number of individuals when the DNA methylation test was positive; -: Represents the number of individuals when the DNA methylation test was negative; nr: Not report; N: Total number.

Colorectal carcinoma meta-analysis

The colorectal carcinoma results were pooled from 34 studies and are shown in Table 2. The meta-analysis showed that the sensitivity and specificity of the detection of colorectal carcinoma by the methylation of genes were 73% (95%CI: 71%-75%) and 92% (95%CI: 90%-93%), respectively. The positive likelihood ratio was 8.07 (95%CI: 6.26-10.41), the negative likelihood ratio was 0.31 (95%CI: 0.25-0.38), the diagnostic odds ratio was 31.49 (95%CI: 23.25-42.64), and the symmetric area under the curve was 0.9281.

Heterogeneity was significant for the sensitivity ($P < 0.001$), specificity ($P = 0.0008$), positive likelihood ratio ($P = 0.0025$), negative likelihood ratio ($P < 0.001$), and diagnostic odds ratios ($P = 0.0340$).

Of the involved regulation mechanisms, we found that DOR and AUC of the methylated genes belonging to the Wnt pathway were higher than the genes of the DNA damage repair pathway and other pathways. The sensitivity, specificity, DOR and AUC of different methylated genes in the three types of pathways were calculated (Table 2), and the results indicated that the accuracy of faecal SFRP2 methylation in the diagnosis of colorectal carcinoma was higher than that of other genes, with a sensitivity of 79% (95%CI: 75%-82%) (Figure 2a), a specificity of 93% (95%CI: 90%-96%) (Figure 2b), a diagnostic OR of 47.57 (95%CI: 20.08-112.72), and the area under the curve of 0.9565 (Figure 2c).

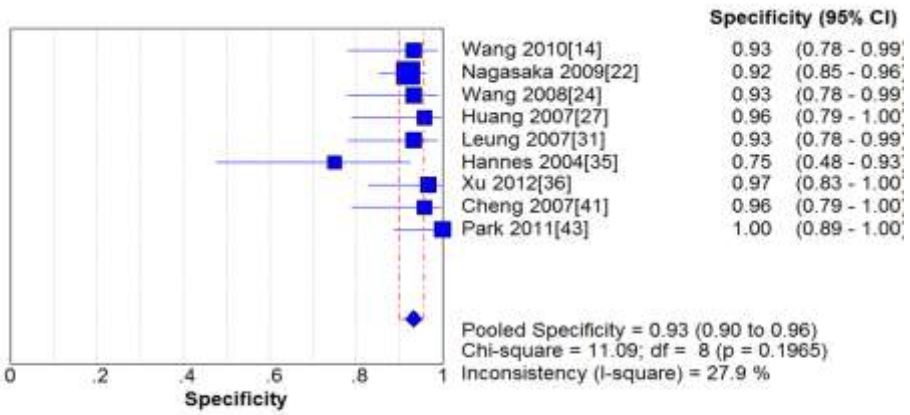
Table 2 Methylation of pooled genes for the diagnosis of CRC

Wnt pathway	DNA damage repair pathway	Other pathways	SE(95%CI)	SP(95%CI)	DOR(95%CI)	AUC

Wnt pathway	DNA damage repair pathway	Other pathways	73% (71%-75%)	92% (90%-93%)	31.49 (23.25-42.64)	0.9281
Wnt pathway	-	-	72% (68%-75%)	93% (90%-96%)	33.99 (17.99-60.50)	0.9305
-	DNA damage repair pathway	-	42% (36%-47%)	97% (94%-99%)	12.87 (5.98-27.72)	0.7296
-	-	Other pathways	57% (55%-59%)	94% (93%-95%)	20.17 (15.18-26.80)	0.9209
SFRP2	-	-	79% (75%-82%)	93% (90%-96%)	47.57 (20.08-112.72)	0.9565
-	MGMT	-	47% (40%-53%)	95% (90%-98%)	11.67 (5.10-26.67)	0.7092
-	MLH	-	28% (18%-39%)	100% (95%-100%)	23.68 (3.02-185.44)	0.5000
-	-	Vimentin	49% (43%-54%)	93% (90%-95%)	13.81 (8.57-22.27)	0.8470
-	-	OSMR	47% (40%-54%)	95% (91%-98%)	14.66 (5.06-42.47)	0.2249
-	-	P16	50% (42%-58%)	98% (92%-100%)	24.39 (7.26-81.96)	0.9751
SFRP2	MGMT	-	69% (66%-72%)	94% (91%-96%)	33.24 (16.76-65.93)	0.9458
SFRP2	MLH	-	72% (68%-75%)	94% (92%-96%)	43.03 (20.15-91.87)	0.9528
SFRP2	MLH	Vimentin	64% (60%-67%)	93% (92%-95%)	24.93 (15.34-40.50)	0.9278
SFRP2	MLH	OSMR	65% (62%-69%)	95% (93%-96%)	33.10 (17.12-63.98)	0.9509
SFRP2	MLH	P16	68% (64%-71%)	95% (93%-97%)	38.86 (20.11-67.54)	0.9523

SE: Sensitivity; SP: Specificity; DOR: Diagnostic odds ratios; AUC: The area under the curve; CI: Confidence interval.

2b



2c

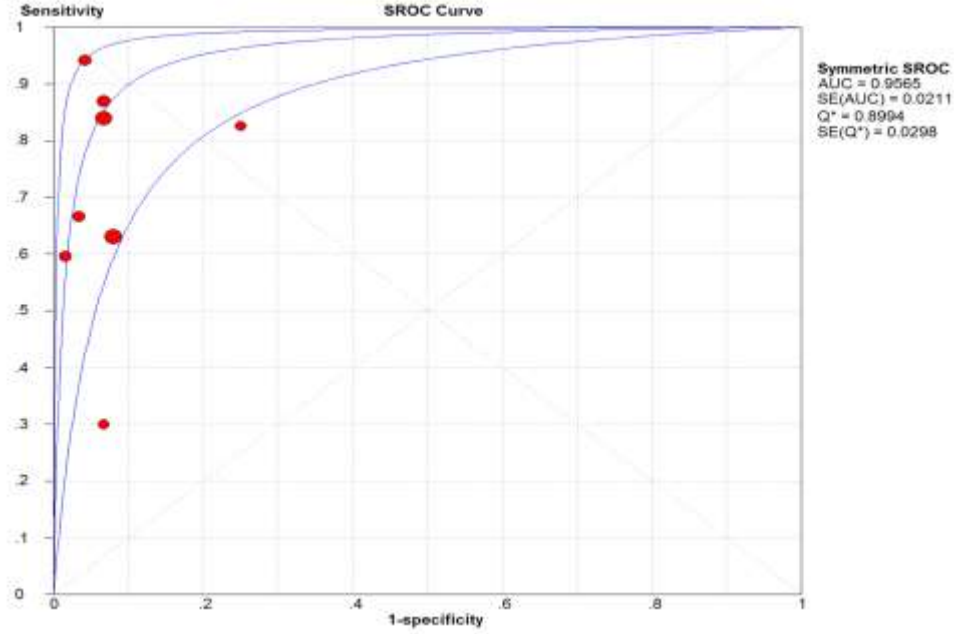


Figure 2 Forest plot of SFRP2 methylation in the diagnosis of CRC. 2a: Shows the sensitivity of SFRP2 methylation in stool samples used for colorectal carcinoma diagnosis. The point estimates of specificity from each study are shown as red squares. 2b: Shows the specificity of SFRP2 methylation in stool samples used for colorectal cancer diagnosis. The point estimates of specificity from each study are shown as blue squares. 2c: Shows the summary receiver operating characteristic curves (SROC) of SFRP2 methylation assays used for diagnosis of colorectal carcinoma. Red circles represent each study that was included in the meta-analysis. The size of each study is indicated by the size of the red circle. Summary receiver operating characteristic curves summarize the overall diagnostic

accuracy. Error bars indicate the 95% confidence interval (CI), and df indicates the degrees of freedom.

Colorectal adenoma meta-analysis

Pooled colorectal adenoma analysis (Table 3), including 26 studies, provided the following results: the sensitivity and specificity of gene methylation for colorectal adenoma diagnosis were 51% (95%CI: 47%-54%) and 92% (95%CI: 90%-93%), respectively. The positive likelihood ratio was 5.52 (95%CI: 4.23-7.19), the negative likelihood ratio was 0.52 (95%CI: 0.44-0.61), and the diagnostic odds ratio and symmetric area under the curve were 12.61 (95%CI: 8.66-18.37) and 0.8830, respectively.

Heterogeneity was also clear regarding sensitivity ($P < 0.001$), specificity ($P = 0.0233$), positive likelihood ratio ($P = 0.1166$), negative likelihood ratio ($P < 0.001$), and diagnostic odds ratios ($P = 0.0565$).

The DOR and AUC of the methylated Wnt pathway genes were higher than those of the genes of the DNA damage repair pathway and other pathways when grouping all of the genes by pathway for analysis. In these regulation mechanisms, we also found that the Wnt pathway was higher than the DNA damage repair pathway and the other pathway. The sensitivity, specificity, DOR and AUC of the different methylated genes in the three types of pathways were calculated (Table 3), and the results indicated that the value of DOR and AUC of P16 and SFRP2 were higher than that of other genes, but the accuracy of faecal SFRP2 methylation for the diagnosis of colorectal adenoma was higher than P16 according to sensitivity (Figure 3a, 3b, 3c).

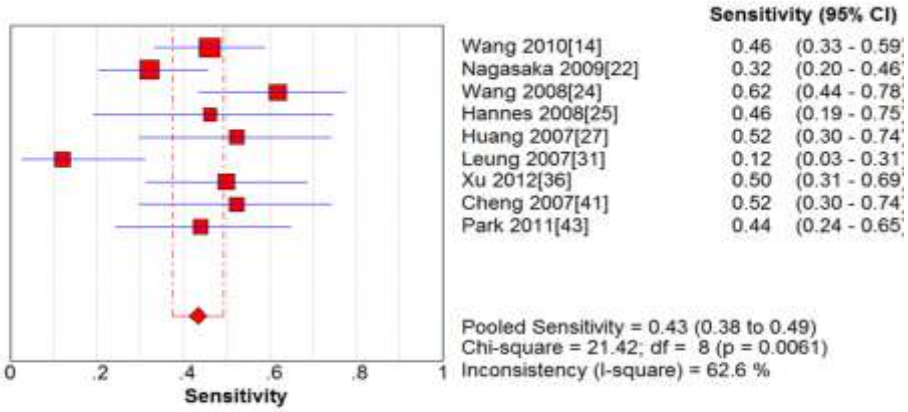
Table3 Methylation of pooled genes for the diagnosis of colorectal adenomas

Wnt pathway	DNA damage repair pathway	Other pathways	SE(95%CI)	SP(95%CI)	DOR(95%CI)	AUC
Wnt pathway	DNA damage repair pathway	Other pathways	51% (47%-54%)	92% (90%-93%)	12.61 (8.66-18.37)	0.8830
Wnt pathwa	-	-	40% (35%-46%)	95% (92%-97%)	10.81 (6.43-18.16)	0.9318

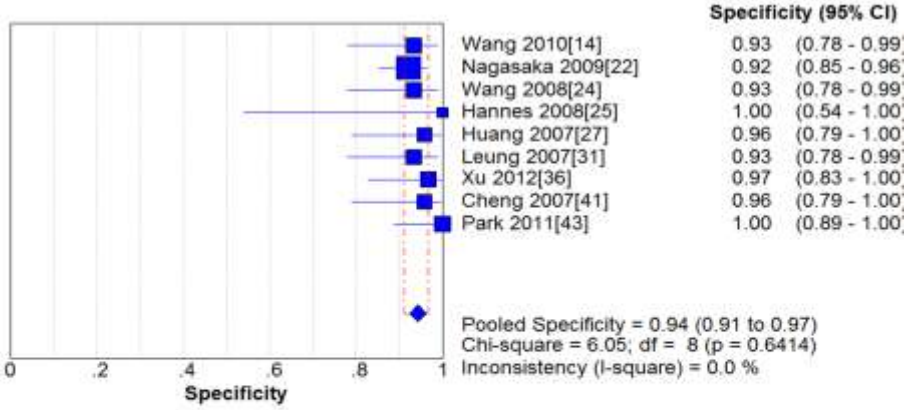
y						
-	DNA damage repair pathway	-	21% (17%-27%)	95% (91%-97%)	4.23 (2.01-8.88)	0.6724
-	-	Other pathways	32% (28%-35%)	94% (93%-95%)	7.78 (5.48-11.05)	0.8730
SFRP2	-	-	43% (38%-49%)	94% (91%-97%)	11.06 (5.77-21.18)	0.9563
-	MGMT	-	29% (22%-36%)	93% (87%-96%)	4.42 (2.18-8.95)	0.6138
-	MLH	-	8% (4%-16%)	98% (92%-100%)	2.35 (0.14-40.83)	-
-	-	Vimentin	23% (17%-31%)	95% (92%-98%)	8.30 (2.60-26.55)	0.8979
-	-	OSMR	25% (14%-39%)	95% (91%-98%)	5.20 (1.44-18.82)	0.8166
-	-	P16	33% (23%-44%)	97% (89%-100%)	13.27 (3.40-51.83)	0.9700
SFRP2	MLH	-	34% (29%-39%)	95% (92%-97%)	9.62 (4.64-19.93)	0.9467
SFRP2	MGMT	-	38% (33%-42%)	94% (91%-96%)	7.85 (4.79-12.87)	0.7531
SFRP2	-	OSMR	41% (35%-46%)	95% (92%-96%)	9.25 (5.13-16.69)	0.9476
SFRP2	-	Vimentin	36% (32%-41%)	95% (93%-96%)	9.88 (5.55-17.57)	0.9461
SFRP2	-	P16	41% (36%-46%)	95% (92%-97%)	10.37 (6.21-17.31)	0.9480
SFRP2	MGMT	Vimentin	34% (30%-38%)	94% (92%-96%)	7.81 (4.96-12.29)	0.8036
SFRP2	MGMT	OSMR	36% (32%-41%)	94% (92%-96%)	7.25 (4.61-11.39)	0.7750
SFRP2	MGMT	P16	37% (33%-41%)	94% (92%-96%)	7.92 (5.14-12.21)	0.7721
SFRP2	MLH	Vimentin	31% (27%-35%)	95% (93%-97%)	8.99 (4.95-16.31)	0.9436
SFRP2	MLH	OSMR	33% (29%-38%)	95% (93%-97%)	8.37 (4.50-15.59)	0.9413
SFRP2	MLH	P16	34% (30%-38%)	95% (93%-97%)	9.98 (5.45-18.27)	0.9470

SE: Sensitivity; SP: Specificity; DOR: Diagnostic odds ratios; AUC: The area under the curve; CI: Confidence interval.

3a


Sensitivity (95% CI)
Wang 2010[14] 0.46 (0.33 - 0.59)
Nagasaka 2009[22] 0.32 (0.20 - 0.46)
Wang 2008[24] 0.62 (0.44 - 0.78)
Hannes 2008[25] 0.46 (0.19 - 0.75)
Huang 2007[27] 0.52 (0.30 - 0.74)
Leung 2007[31] 0.12 (0.03 - 0.31)
Xu 2012[36] 0.50 (0.31 - 0.69)
Cheng 2007[41] 0.52 (0.30 - 0.74)
Park 2011[43] 0.44 (0.24 - 0.65)
Pooled Sensitivity = 0.43 (0.38 to 0.49)
Chi-square = 21.42; df = 8 (p = 0.0061)
Inconsistency (I-square) = 62.6 %
0
.2
.4
.6
.8
1
Sensitivity

3b


Specificity (95% CI)
Wang 2010[14] 0.93 (0.78 - 0.99)
Nagasaka 2009[22] 0.92 (0.85 - 0.96)
Wang 2008[24] 0.93 (0.78 - 0.99)
Hannes 2008[25] 1.00 (0.54 - 1.00)
Huang 2007[27] 0.96 (0.79 - 1.00)
Leung 2007[31] 0.93 (0.78 - 0.99)
Xu 2012[36] 0.97 (0.83 - 1.00)
Cheng 2007[41] 0.96 (0.79 - 1.00)
Park 2011[43] 1.00 (0.89 - 1.00)
Pooled Specificity = 0.94 (0.91 to 0.97)
Chi-square = 6.05; df = 8 (p = 0.6414)
Inconsistency (I-square) = 0.0 %
0
.2
.4
.6
.8
1
Specificity

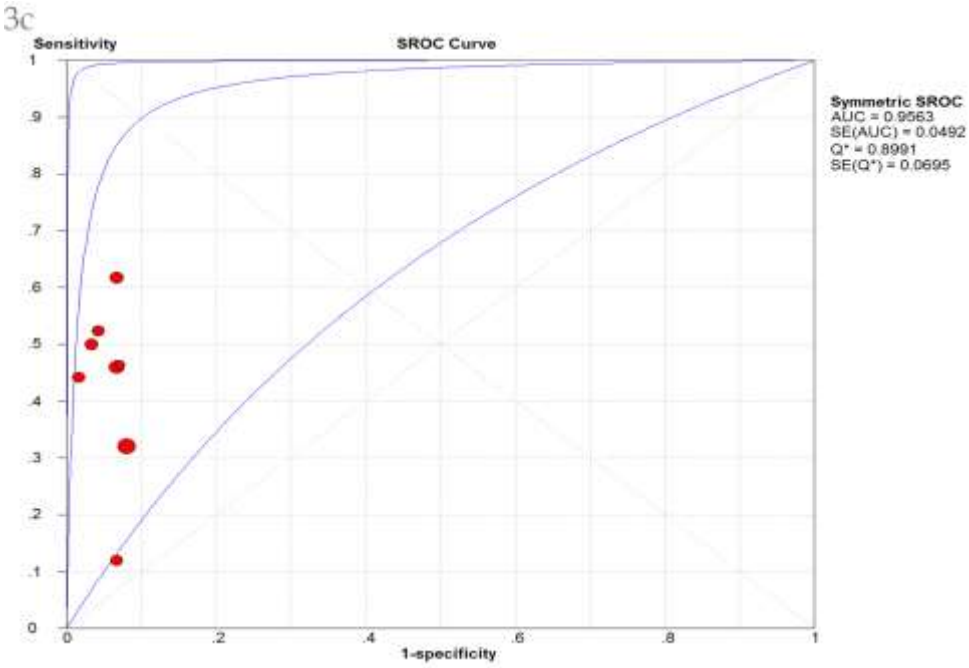


Figure 3 Forest plot of SFRP2 methylation in the diagnosis of colorectal adenomas. 3a: Shows the sensitivity of SFRP2 methylation in stool samples for colorectal adenoma diagnosis. 3b: Shows the specificity of SFRP2 methylation in stool samples for colorectal adenoma diagnosis. 3c: Shows the summary receiver operating characteristic curves (SROC) of SFRP2 methylation assays for the diagnosis of colorectal adenomas.

Meta-regression

In the meta-regression analysis, the difference in relative diagnostic odds ratio (RDOR) values between the higher and lower quality studies was not significant. We also noted that the differences between the blinded and non-blinded methods, qualitative and quantitative methods, single and multiple genes methylation did not reach statistical significance, indicating that these potential factors did not substantially affect the diagnostic accuracy, as shown in Table 4.

Table 4 Weighted meta-regression on the diagnostic accuracy of the methylation of genes assays

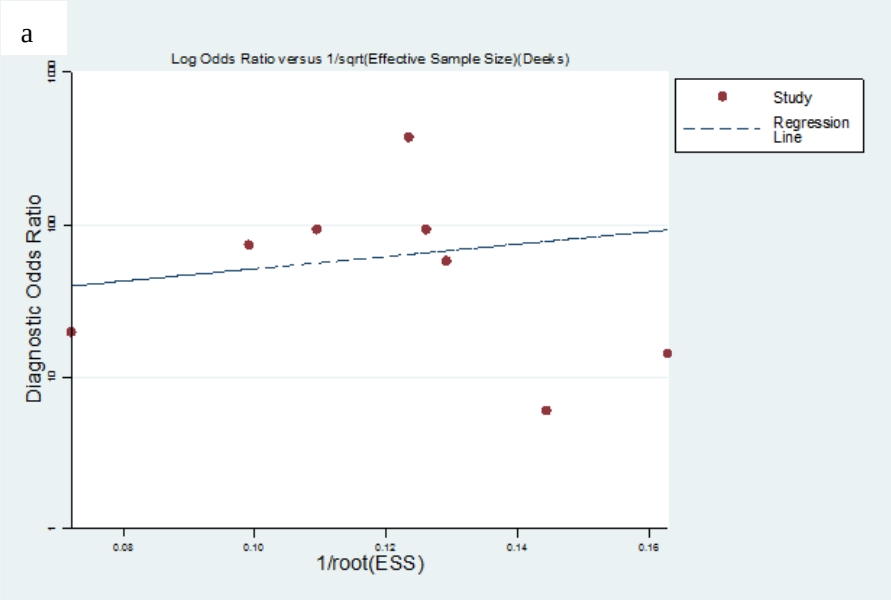
Covariates	Coefficient	SE	*P* value	RDOR	95%CI
QUADAS score1	0.062	0.4130	0.8812	1.06	(0.46;2.47)
Detection method2	-0.146	0.4011	0.7188	0.86	(0.38;1.96)
Blinded design3	-0.166	0.3638	0.6506	0.85	(0.40;1.78)
Methylation genes4	-0.036	0.4442	0.9356	0.96	(0.39;2.39)

[1]QUADAS score, which was divided into studies with higher quality (QUADAS score ≥ 10) and those with lower quality (QUADAS score < 10); [2]Detection method, which was divided into qualitative and quantitative assay methods; [3]Blinded design: the study was included with or without blinded design; [4]Methylation genes, which were divided into single gene and combination genes.

QUADAS: Quality Assessment for Studies of Diagnostic Accuracy was used to assess the quality of primary studies of diagnostic accuracy; SE: Standard error; RDOR: Relative diagnostic odds ratio; 95%CI: 95% confidence interval.

Publication bias

In our meta-analysis, publication bias was evaluated using the Egger test. The results showed no significant publication bias among the studies of SFRP2 methylation in faecal samples from CRC or adenoma patients (Figure 4a, b).



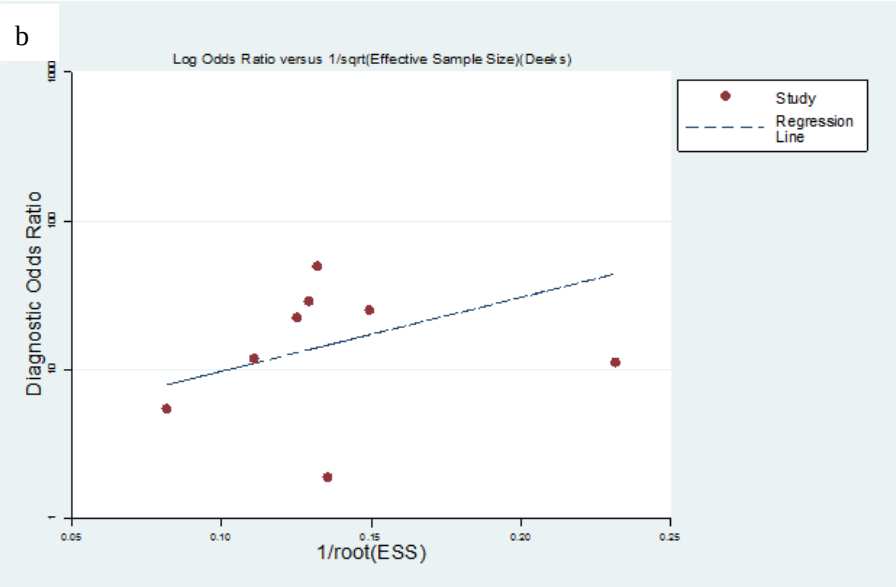


Figure 4 Assessment of the publication bias in the faecal SFPR2 methylation for the diagnosis of CRC (Figure 4a) and adenomas (Figure 4b). No significant publication biases were found in any of these studies (all $P > 0.05$).

DISCUSSION

It is widely accepted that DNA methylation in stool may be valuable for increasing the rate of CRC detection at earlier stages[47]. In the present study, we focused on the detection performance of genes methylation in stool samples for patients with colorectal tumours. Our analysis suggests that the specificity of SFRP2 methylation is high (93% for CRC and 94% for colorectal adenoma) for the detection of colorectal tumours; however, it has moderate (79%) and low sensitivity (43%) for diagnosing CRC and adenoma, respectively. Compared to FOBT, with a sensitivity of 14% for colorectal tumour diagnosis[48], the detection accuracy of faecal methylation biomarkers was higher as a CRC-screening method.

The diagnostic odds ratio (DOR) is an indicator of test accuracy. The value of the DOR ranges from 0 to infinity, and higher values indicate better discriminatory test performance. In this meta-analysis, we found that the DOR of faecal SFRP2 methylation for colorectal carcinoma and adenoma were 47.57 and 11.06, respectively, which indicated a high level of overall accuracy for CRC and a low level for adenoma. The SROC curve represents an overall measure of the discriminatory power of a test. The area under the curve of 1 for any test indicates that the test is excellent. Our data showed that the area under the curve (AUC) of the SROC curve for faecal SFRP2 methylation for the diagnosis

of colorectal carcinoma and adenoma were 0.9565 and 0.9563, respectively, which indicated that faecal SFRP2 methylation is an excellent diagnostic biomarker for colorectal tumours.

Because the DOR and SROC curve are not easy to use in clinical practice, the likelihood ratios are considered to be more clinically meaningful. For a high-quality diagnostic test, a PLR of > 10 or NLR < 0.1 is typically required. However, our meta-analysis showed that neither PLR nor NLR alone was adequate to confirm or exclude the diagnosis of colorectal carcinoma or adenoma. The PLR value was 9.12 in the diagnosis analysis of CRC, which suggested that patients with a positive faecal SFRP2 methylation assay had a nine-fold chance of being diagnosed with CRC than non-CRC. Therefore, a colonoscopy was necessary for patients with a positive faecal SFRP2 methylation assay to confirm the diagnosis of CRC with high probability. On the other hand, a NLR of 0.24 in the diagnosis analysis of CRC suggested that if a faecal SFRP2 methylation assay result was negative, the probability rate of the individual having CRC was 24%. For the diagnosis of colorectal adenoma, a PLR of 5.99 suggested a moderate necessity to consider colonoscopy for patients with a positive faecal SFRP2 methylation assay to confirm the diagnosis of colorectal adenoma. Moreover, the NLR was 0.60 in the diagnosis analysis of colorectal adenoma. These data suggest that a negative faecal SFRP2 methylation assay result should not be used alone as a justification for denying or discontinuing the screening of colorectal adenomas.

An aberrant Wnt signalling pathway is an early event in 90% of colorectal carcinomas. SFRPs are secreted glycoproteins that antagonise Wnt signalling by different direct or indirect mechanisms. Thus, the role of SFRPs as a negative regulator of Wnt signalling may have important significance in tumoursigenesis. These epigenetic events are involved in early steps of colon carcinogenesis, and changes in the status of DNA methylation are associated with early steps of the histologic progression of colon carcinoma. Our previous studies of CRC tissue showed that SFRP1 and SFRP2 were methylated in more than 80.6% of colorectal carcinomas[49]. Therefore, faecal SFRP2 methylation could be expected to be a biomarker for the screening of colorectal tumours. Although it cannot be generally used as a screening tool for the financial limited, the analysis of methylation markers offers a variety of new opportunities for developing biomarkers at the molecular level of colorectal tumours.

Our meta-analysis had several limitations. (1) None of the included studies were multicentre or large-blinded, randomized, controlled trials; (2) conference abstracts and non-English and non-Chinese language studies were excluded, which might have led to publication bias; (3) studies on DNA methylation with statistical significance tend to be published and cited; (4) due to the absence of case-mix difference analysis, smaller trials may show larger treatment effects than larger studies (*e.g.,* patients with only localised *vs* metastatic disease).

To sum up, stool-based DNA methylation has been shown to be highly discriminatory in the detection of colorectal tumours. Our results demonstrate that SFRP2 methylation, as a non-invaded modality, shows promise for the accurate detection of CRC; however, a large number of studies are required to further confirm the role of faecal SFRP2 methylation for the early and accurate CRC diagnosis.

COMMENTS

Background

Colorectal cancer (CRC) is the third-most common malignancy and the second leading cause of cancer-related deaths in western countries. The diagnosis of CRC in early stages has great importance for reducing CRC mortality. Although significant advances have been achieved in diagnostic technologies, the current available modalities for diagnosing CRC remain suboptimal.

Research frontiers

DNA methylation often occurs during the early stages of colon tumours and has played an important role in oncology, especially in the early diagnosis of colorectal tumours. However, no consensus with regard to the role of stool methylation markers in colon tumours exists.

Innovations and breakthroughs

Stool methylation markers as an available non-invasive modality have high accuracy and sensitivity for the diagnosis of premalignant lesions of CRC. A few systematic reviews about the efficacy of stool methylation markers in colorectal tumour diagnosis exist. This article comprehensively assesses the accuracy of methylation genes in stool samples for

diagnosing colorectal tumours.

Applications

Analysis of DNA methylation in stool samples may be used as a non-invasive test for the diagnosis of CRC, and SFRP2 methylation is a promising marker that has great potential in early CRC diagnosis.

Terminology

Diagnostic odds ratio (DOR) reflects the relationship between the result of the diagnostic test and the disease. The summary receiver operation characteristic (SROC) curve displays the trade-off between sensitivity and specificity and represents a global summary of test performance. We used the Q-value, the intersection point of the SROC curve with a diagonal line from the left upper corner to the right lower corner of the receiver operation characteristic (ROC) space, which corresponds to the highest value of sensitivity and specificity for the test. The positive likelihood ratio (PLR) represents the value by which the odds of the disease increase when a test is positive, whereas negative likelihood ratio (NLR) shows the value by which the odds of the disease decrease when a test is negative.

Peer review

This study reviewed 37 trials to evaluate the accuracy of stool methylation genes for diagnosing colorectal tumours. Based on these analyses, the authors conclude that stool SFRP2 methylation is a promising marker that has great potential in early CRC diagnosis. The analysis was carefully performed, and the results were clearly presented and summarized and provided valuable advice for early clinical diagnosis of colorectal tumours.

REFERENCES

1 **Jemal A**, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin* 2009; **59**: 225-249 [PMID: 19474385 DOI: 10.3322/caac.20006]

2 **Schetter AJ**, Harris CC. Alterations of microRNAs contribute to colon carcinogenesis. *Semin Oncol* 2011; **38**: 734-742 [PMID: 22082759 DOI: 10.1053/j.seminoncol.2011.08.009]

3 **Markowitz SD**, Dawson DM, Willis J, Willson JK. Focus on colon cancer. *Cancer Cell* 2002; **1**: 233-236 [PMID: 12086859 DOI: 12086859/S1535610802000533]

4 **Bosch LJ**, Carvalho B, Fijneman RJ, Jimenez CR, Pinedo HM, van Engeland M, Meijer GA. Molecular tests for colorectal cancer screening. *Clin Colorectal Cancer* 2011; **10**: 8-23 [PMID: 21609931 DOI: 10.3816/CCC.2011.n.002]

5 **Jemal A**, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics, 2008. *CA Cancer J Clin* 2008; **58**: 71-96 [PMID: 18287387 DOI: 10.3322/CA.2007.0010]

6 **Heresbach D**, Manfredi S, D'Halluin PN, Bretagne JF, Branger B. Review in depth and meta-analysis of controlled trials on colorectal cancer screening by faecal occult blood test. *Eur J Gastroenterol Hepatol* 2006; **18**: 427-433 [PMID: 16538116 DOI: 00042737-200604000-00018/16538116]

7 **Tagore KS**, Levin TR, Lawson MJ. The evolution to stool DNA testing for colorectal cancer. *Aliment Pharmacol Ther* 2004; **19**: 1225-1233 [PMID: 15191503 DOI: 10.1111/j.1365-2036.2004.02005.x]

8 **Berger BM**, Ahlquist DA. Stool DNA screening for colorectal neoplasia: biological and technical basis for high detection rates. *Pathology* 2012; **44**: 80-88 [PMID: 22198259 DOI: 10.1097/PAT.0b013e3283502fdf]

9 **Whiting P**, Rutjes AW, Reitsma JB, Bossuyt PM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMC Med Res Methodol* 2003; **3**: 25 [PMID: 14606960 DOI: 10.1186/1471-2288-3-25]

10 **Ahlquist DA**, Taylor WR, Mahoney DW, Zou H, Domanico M, Thibodeau SN, Boardman LA, Berger BM, Lidgard GP. The stool DNA test is more accurate than the plasma septin 9 test in detecting colorectal neoplasia. *Clin Gastroenterol Hepatol* 2012; **10**: 272-277 [PMID: 22019796 DOI: 10.1016/j.cgh.2011.10.008]

11 **Bosch LJ**, Oort FA, Neerincx M, Khalid-de BC, Terhaar SDJ, Melotte V, Jonkers DM, Masclee AA, Mongera S, Grooteclaes M, Louwagie J, van Criekinge W, Coupe VM, Mulder CJ, van Engeland M, Carvalho B, Meijer GA. DNA methylation of phosphatase and actin regulator 3 detects colorectal cancer in stool and complements FIT. *Cancer Prev Res (Phila)* 2012; **5**: 464-472 [PMID: 22135045 DOI: 10.1158/1940-6207.CAPR-11-0315]

12 **Ahlquist DA**, Zou H, Domanico M, Mahoney DW, Yab TC, Taylor WR, Butz ML, Thibodeau SN, Rabeneck L, Paszat LF, Kinzler KW, Vogelstein B, Bjerregaard NC, Laurberg S, Sorensen HT, Berger BM, Lidgard GP. Next-generation stool DNA test accurately detects colorectal cancer and large adenomas. *Gastroenterology* 2012; **142**: 248-256, e25-e26 [PMID: 22062357 DOI: 10.1053/j.gastro.2011.10.031]

13 **Azuara D**, Rodriguez-Moranta F, de Oca J, Soriano-Izquierdo A, Mora J, Guardiola J, Biondo S, Blanco I, Peinado MA, Moreno V, Esteller M, Capella G. Novel methylation panel for the early detection of colorectal tumourss in stool DNA. *Clin Colorectal Cancer* 2010; **9**: 168-176 [PMID: 20643622 DOI: 10.3816/CCC.2010.n.023]

14 **Tang D**, Liu J, Wang DR, Yu HF, Li YK, Zhang JQ. Diagnostic and prognostic value of the methylation status of secreted frizzled-related protein 2 in colorectal cancer. *Clin Invest Med* 2011; **34**: E88-E95 [PMID: 21463549]

15 **Baek YH**, Chang E, Kim YJ, Kim BK, Sohn JH, Park DI. Stool methylation-specific polymerase chain reaction assay for the detection of colorectal neoplasia in Korean patients. *Dis Colon Rectum* 2009; **52**: 1452-1459, discussion 1459-1463 [PMID: 19617759 DOI: 10.1007/DCR.0b013e3181a79533]

16 **Li M**, Chen WD, Papadopoulos N, Goodman SN, Bjerregaard NC, Laurberg S, Levin B, Juhl H, Arber N, Moinova H, Durkee K, Schmidt K, He Y, Diehl F, Velculescu VE, Zhou S, Diaz LJ, Kinzler KW, Markowitz SD, Vogelstein B. Sensitive digital quantification of DNA methylation in clinical samples. Nat Biotechnol 2009; **27**: 858-863 [PMID: 19684580 DOI: 10.1038/nbt.1559]

17 **Melotte V**, Lentjes MH, van den Bosch SM, Hellebrekers DM, de Hoon JP, Wouters KA, Daenen KL, Partouns-Hendriks IE, Stessels F, Louwagie J, Smits KM, Weijenberg MP, Sanduleanu S, Khalid-de BC, Oort FA, Meijer GA, Jonkers DM, Herman JG, de Bruine AP, van Engeland M. N-Myc downstream-regulated gene 4 (NDRG4): a candidate tumours suppressor gene and potential biomarker for colorectal cancer. *J Natl Cancer Inst* 2009; **101**: 916-927 [PMID: 19535783 DOI: 10.1093/jnci/djp131]

18 **Ausch C**, Kim YH, Tsuchiya KD, Dzieciatkowski S, Washington MK, Paraskeva C, Radich J, Grady WM. Comparative analysis of PCR-based biomarker assay methods for colorectal polyp detection from faecal DNA. *Clin Chem* 2009; **55**: 1559-1563 [PMID: 19541867 DOI: 10.1373/clinchem.2008.122937]

19 **Hellebrekers DM**, Lentjes MH, van den Bosch SM, Melotte V, Wouters KA, Daenen KL, Smits KM, Akiyama Y, Yuasa Y, Sanduleanu S, Khalid-de BC, Jonkers D, Weijenberg MP, Louwagie J, van Criekinge W, Carvalho B, Meijer GA, Baylin SB, Herman JG, de Bruine AP, van Engeland M. GATA4 and GATA5 are potential tumours suppressors and biomarkers in colorectal cancer. *Clin Cancer Res* 2009; **15**: 3990-3997 [PMID: 19509152 DOI: 10.1158/1078-0432.CCR-09-0055]

20 **Mayor R**, Casadome L, Azuara D, Moreno V, Clark SJ, Capella G, Peinado MA. Long-range epigenetic silencing at 2q14.2 affects most human colorectal cancers and may have application as a non-invasive biomarker of disease. *Br J Cancer* 2009; **100**: 1534-1539 [PMID: 19384295 DOI: 10.1038/sj.bjc.6605045]

21 **Kim MS**, Louwagie J, Carvalho B, Terhaar SDJ, Park HL, Chae YK, Yamashita K, Liu J, Ostrow KL, Ling S, Guerrero-Preston R, Demokan S, Yalniz Z, Dalay N, Meijer GA, Van Criekinge W, Sidransky D. Promoter DNA methylation of oncostatin m receptor-beta as a novel diagnostic and therapeutic marker in colon cancer. *PLoS One* 2009; **4**: e6555 [PMID: 19662090 DOI: 10.1371/journal.pone.0006555]

22 **Nagasaka T**, Tanaka N, Cullings HM, Sun DS, Sasamoto H, Uchida T, Koi M, Nishida N, Naomoto Y, Boland CR, Matsubara N, Goel A. Analysis of faecal DNA methylation to detect gastrointestinal neoplasia. *J Natl Cancer Inst* 2009; **101**: 1244-1258 [PMID: 19700653 DOI: 10.1093/jnci/djp265]

23 **Glöckner SC**, Dhir M, Yi JM, McGarvey KE, Van Neste L, Louwagie J, Chan TA, Kleeberger W, de Bruine AP, Smits KM, Khalid-de BC, Jonkers DM, Stockbrugger RW, Meijer GA, Oort FA, Iacobuzio-Donahue C, Bierau K, Herman JG, Baylin SB, Van Engeland M, Schuebel KE, Ahuja N. Methylation of TFPI2 in stool DNA: a potential novel biomarker for the detection of colorectal cancer. *Cancer Res* 2009; **69**: 4691-4699 [PMID: 19435926 DOI: 10.1158/0008-5472.CAN-08-0142]

24 **Wang DR**, Tang D. Hypermethylated SFRP2 gene in faecal DNA is a high potential biomarker for colorectal cancer noninvasive screening. *World J Gastroenterol* 2008; **14**: 524-531 [PMID: 18203283 DOI: 18203283/PMC2681142]

25 **Oberwalder M**, Zitt M, Wontner C, Fiegl H, Goebel G, Zitt M, Kohle O, Muhlmann G, Ofner D, Margreiter R, Muller HM. SFRP2 methylation in faecal DNA--a marker for

colorectal polyps. *Int J Colorectal Dis* 2008; **23**: 15-19 [PMID: 17639423 DOI: 10.1007/s00384-007-0355-2]

26 **Itzkowitz S**, Brand R, Jandorf L, Durkee K, Millholland J, Rabeneck L, Schroy PR, Sontag S, Johnson D, Markowitz S, Paszat L, Berger BM. A simplified, noninvasive stool DNA test for colorectal cancer detection. *Am J Gastroenterol* 2008; **103**: 2862-2870 [PMID: 18759824 DOI: 10.1111/j.1572-0241.2008.02088.x]

27 **Huang ZH**, Li LH, Yang F, Wang JF. Detection of aberrant methylation in faecal DNA as a molecular screening tool for colorectal cancer and precancerous lesions. *World J Gastroenterol* 2007; **13**: 950-954 [PMID: 17352030]

28 **Itzkowitz SH**, Jandorf L, Brand R, Rabeneck L, Schroy PR, Sontag S, Johnson D, Skoletsky J, Durkee K, Markowitz S, Shuber A. Improved faecal DNA test for colorectal cancer screening. *Clin Gastroenterol Hepatol* 2007; **5**: 111-117 [PMID: 17161655 DOI: 10.1016/j.cgh.2006.10.006]

29 **Abbaszadegan MR**, Tavasoli A, Velayati A, Sima HR, Vosooghinia H, Farzadnia M, Asadzedeh H, Gholamin M, Dadkhah E, Aarabi A. Stool-based DNA testing, a new noninvasive method for colorectal cancer screening, the first report from Iran. *World J Gastroenterol* 2007; **13**: 1528-1533 [PMID: 17461444]

30 **Zhang W**, Bauer M, Croner RS, Pelz JO, Lodygin D, Hermeking H, Sturzl M, Hohenberger W, Matzel KE. DNA stool test for colorectal cancer: hypermethylation of the secreted frizzled-related protein-1 gene. *Dis Colon Rectum* 2007; **50**: 1618-1626, discussion 1626-1627 [PMID: 17762966 DOI: 10.1007/s10350-007-0286-6]

31 **Leung WK**, To KF, Man EP, Chan MW, Hui AJ, Ng SS, Lau JY, Sung JJ. Detection of hypermethylated DNA or cyclooxygenase-2 messenger RNA in faecal samples of patients with colorectal cancer or polyps. *Am J Gastroenterol* 2007; **102**: 1070-1076 [PMID: 17378912 DOI: 10.1111/j.1572-0241.2007.01108.x]

32 **Petko Z**, Ghiassi M, Shuber A, Gorham J, Smalley W, Washington MK, Schultenover S, Gautam S, Markowitz SD, Grady WM. Aberrantly methylated CDKN2A, MGMT, and MLH1 in colon polyps and in faecal DNA from patients with colorectal polyps. *Clin Cancer Res* 2005; **11**: 1203-1209 [PMID: 15709190 DOI: 11/3/1203/15709190]

33 **Lenhard K**, Bommer GT, Asutay S, Schauer R, Brabletz T, Goke B, Lamerz R, Kolligs FT. Analysis of promoter methylation in stool: a novel method for the detection of

colorectal cancer. *Clin Gastroenterol Hepatol* 2005; **3**: 142-149 [PMID: 15704048 DOI: S154235650400624X/15704048]

34 **Chen WD**, Han ZJ, Skoletsky J, Olson J, Sah J, Myeroff L, Platzer P, Lu S, Dawson D, Willis J, Pretlow TP, Lutterbaugh J, Kasturi L, Willson JK, Rao JS, Shuber A, Markowitz SD. Detection in faecal DNA of colon cancer-specific methylation of the nonexpressed vimentin gene. *J Natl Cancer Inst* 2005; **97**: 1124-1132 [PMID: 16077070 DOI: 10.1093/jnci/dji204]

35 **Müller HM**, Oberwalder M, Fiegl H, Morandell M, Goebel G, Zitt M, Muhlthaler M, Ofner D, Margreiter R, Widschwendter M. Methylation changes in faecal DNA: a marker for colorectal cancer screening? *Lancet* 2004; **363**: 1283-1285 [PMID: 15094274 DOI: 10.1016/S0140-6736(04)16002-9]

36 **Xu MH**, Cai KY, Tu Y. Stool DNA methylation analysis in the early diagnosis of colorectal cancer. *Chinese Journal of Clinical Gastroenterology* 2012; **24**: 7-19 [DOI: 10.3870/lcxh. j. issn.1005-541X.2012.01.06]

37 **Kang YP**, Cao FA, Chang WJ, Lou Z, Wang Y, Wu LL, Fu CG, Cao GW. Stool DNA methylation in the screening early colorectal cancer. *Chinese Journal of Gastrointestinal Surgery* 2011; **14**: 52-561 [DOI: 10.3760/cma.j.issn.1671-0274.2011.01.017]

38 **Zhang JP**, Wang J, Gui YL, Zhu QQ, Xu ZW, Li JS. Stool DNA methylation in the diagnosis of colorectal cancer. *Chinese Medical Journal* 2011; **95**: 2482-2484 [DOI: 10.3760/cma.j.issn.0376-2491.2011.35.010]

39 **Fu L**, Sheng JQ, Meng XM, Meng MM, Jin P, Li AQ, Wu ZT, Li SR. Stool Vimentin methylation in the diagnosis of colorectal cancer. *Journal of Gastroenterology and Liver Disease* 2010; **19**: 601-603 [DOI: 10.3969/j.issn.1006-5709.2010.07.007]

40 **Ling ZA**, Chen LS, He CG. Stool P16 methylation in the diagnosis of colorectal cancer. *Colorectal Anus Surgery* 2009; **15**: 144-148 [DOI: 10.3969/j.issn.1674-0491.2009.03.002]

41 **Cheng ZH**. Stool SFRP2 methylation in the diagnosis of colorectal cancer. *Shandong Medical Journal* 2007; **47**: 10-12 [DOI: 10.3969/ j.issn. 1002-266X. 2007. 06.004]

42 **Zhao HX**, Li QW, Dong WW, Duan XH, Zhu JH, Wang RL, Hao YX, Ye M, Xiao WH. Stool NDRG4 methylation in the diagnosis of colorectal cancer. *China Medical Herald* 2012; **9**: 29-31 [DOI: 10.3877/ cma.j.issn. 1674-0785. 2012.10.012]

43 **Chang E**, Park DI, Kim YJ, Kim BK, Park JH, Kim HJ, Cho YK, Sohn CI, Jeon WK, Kim BI, Kim HD, Kim DH, Kim YH. Detection of colorectal neoplasm using promoter methylation of ITGA4, SFRP2, and p16 in stool samples: a preliminary report in Korean patients. *Hepatogastroenterology* 2010; **57**: 720-727 [PMID: 21033217]

44 **Zhang H**, Song YC, Dang CX. Detection of hypermethylated spastic paraplegia-20 in stool samples of patients with colorectal cancer. *Int J Med Sci* 2013; **10**: 230-234 [PMID: 23372428 DOI: 10.7150/ijms.5278]

45 **Carmona FJ**, Azuara D, Berenguer-Llergo A, Fernandez AF, Biondo S, de Oca J, Rodriguez-Moranta F, Salazar R, Villanueva A, Fraga MF, Guardiola J, Capella G, Esteller M, Moreno V. DNA methylation biomarkers for noninvasive diagnosis of colorectal cancer. *Cancer Prev Res (Phila)* 2013; **6**: 656-665 [PMID: 23694962 DOI: 10.1158/1940-6207.CAPR-12-0501]

46 **Guo Q**, Song Y, Zhang H, Wu X, Xia P, Dang C. Detection of hypermethylated fibrillin-1 in the stool samples of colorectal cancer patients. *Med Oncol* 2013; **30**: 695 [PMID: 23963856 DOI: 10.1007/s12032-013-0695-4.]

47 **Kim MS**, Lee J, Sidransky D. DNA methylation markers in colorectal cancer. *Cancer Metastasis Rev* 2010; **29**: 181-206 [PMID: 20135198 DOI: 10.1007/s10555-010-9207-6]

48 **Imperiale TF**, Ransohoff DF, Itzkowitz SH, Turnbull BA, Ross ME. Faecal DNA versus faecal occult blood for colorectal cancer screening in an average risk population. *N Engl J Med* 2004; **351**: 2704-2714 [PMID: 15616205 DOI: 10.1056/NEJMoa033403]

49 **Qi J**, Zhu YQ, Luo J, Tao WH. Hypermethylation and expression regulation of secreted frizzled related protein genes in colorectal tumours. *World J Gastroenterol* 2006; **12**: 7113-7117 [PMID: 17131472]

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