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META-ANALYSIS

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 Journals 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. A quality assessment of diagnostic accuracy studies tool (QADAS) was used to evaluate the quality of the included articles, and the Meta-disc 1.4 and SPSS 13.0 software programs 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 colorectal cancer (CRC) were 73% (95%CI: 71%-75%) and 92% (95%CI: 90%-93%), respectively. For adenoma, the sensitivity and specificity were 51% (95%CI: 47%-54%) and 92% (95%CI: 90%-93%), respectively. Pooled diagnostic performance of *SFRP2* methylation for 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 that *SFRP2* methylation serves as a promising marker with great potential in early colorectal cancer diagnosis.

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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, the 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 a methylation marker test in stool samples is its higher accuracy and sensitivity for 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 Journals 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 analysis 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, and true-negative and false-positive values.

Inclusion and exclusion criteria

Eligible studies were required to meet all of the following criteria: (1) the data were independent; (2) the CRC was diagnosed using DNA methylation analysis in stool sample; (3) the patients were diagnosed with colorectal cancer or colorectal adenomas by pathology; and (4) the colonoscopy result of the control individuals was normal.

Exclusion criteria for this meta-analysis were as follows: (1) studies on secondary CRC or primary CRC with other organ metastases; and (2) 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 (QUADAS)[9] 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%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 χ^2 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

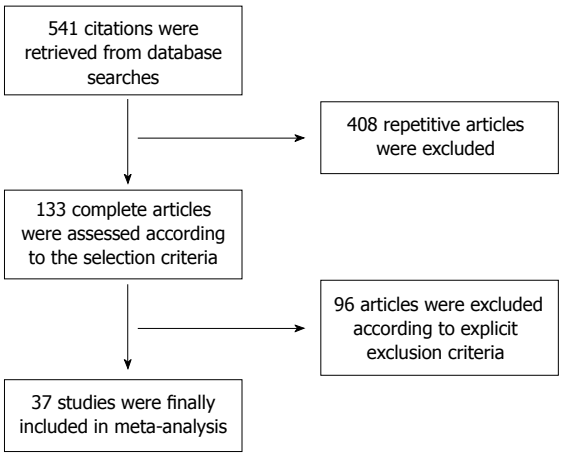


Figure 1 Flowchart of the study selection.

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).

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 United States, 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 were 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 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 ISPG20*), *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 Methyl-BEAMing; quantitative MSP (qMSP); MethyLight; combined bisulfite restriction analysis (COBRA); pyrosequencing; and quantitative, allele-specific, real-time target and signal amplification (QuARTS).

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 those of 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 an area under the curve of 0.9565 (Figure 2C).

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 group. The sensitivity, specificity, DOR and AUC of the different methylated genes in the three types of pathways were calculated (Table 3), and

Table 1 Characteristics of the included studies in the meta-analysis and quality assessment of studies of diagnostic accuracy scores

Ref.	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	The 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 al*[12] 2011	Ireland	*PHACTR3*	639	214	38	51	43	29	264	Yes	QuARTS	11
Azuara *et al*[13] 2010	Spain	*RARB2/P16/MGMT/APC*	98	25	13	20	20	0	20	Yes	MS-MCA	10
		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			
Tang *et al*[14] 2011	China	*SFRP2*	262	142	27	29	34	2	28	Yes	MSP	9
Baek *et al*[15] 2009	South Korea	*Vimentin/MGMT/MLH1*	149	45	15	31	21	5	32	Yes	MSP	9
		MLH1	149	18	42	6	46	0	37			
		Vimentin	149	23	37	8	44	0	37			
		MGMT	149	31	29	19	33	5	32			
Li *et al*[16] 2009	United States	*Vimentin*	80	9	13	9	11	2	36	Unclear	Methyl-BEAMing	5
Melotte *et al*[17] 2009	The Netherlands	*NDRG4*	150	42	33	NR	NR	3	72	Yes	qMSP	11
Ausch *et al*[18] 2009	United States	*IGTA4*	37	NR	NR	7	2	6	22	Unclear	qMSP	4
Hellebrekers *et al*[19] 2009	The Netherlands	*GATA4*	150	44	31	NR	NR	9	66	Yes	qMSP	10
Mayor *et al*[20] 2009	Spain	*EN1*	60	8	22	NR	NR	1	29	Unclear	MS-MCA	7
Kim *et al*[21] 2009	United States	*OSMR/SFRP1*	42	12	8	6	11	0	5	Yes	qMSP	9
		OSMR	201	35	54	2	14	4	92			
		SFRP1	52	11	9	5	12	0	15			
Nagasaka *et al*[22] 2009	Japan	*SFRP2*	253	53	31	18	38	9	104	Unclear	COBRA	10
		RASSF2	253	38	46	7	49	6	107			
Glöckner *et al*[23] 2009	United States	*TFPI2*	129	44	14	7	19	2	43	Yes	qMSP	12
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	United States	*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	United States	*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			
Abbaszadegan *et al*[29] 2007	Hong kong	*p16*	45	5	20	NR	NR	0	20	Unclear	MSP	8
Zhang *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			
Petko *et al*[32] 2005	United States	*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			
Lenhard *et al*[33] 2005	Germany	*HIC1*	71	11	15	4	9	0	32	Yes	MSP	11
Chen *et al*[34] 2005	United States	*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
Kang *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			
Zhang *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			

Fu *et al*[39] 2010	China	*Vimentin*	22	5	9	NR	NR	0	8	Unclear	MSP	5
Ling *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
Zhao *et al*[42] 2009	China	*NDRG4*	114	64	20	NR	NR	3	27	Unclear	MSP	6
Chang *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			
		9-Sep	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

+: Represents the number of individuals when the DNA methylation test was positive; -: Represents the number of individuals when the DNA methylation test was negative; MSP: Methylation-specific PCR; NR: Not reported; *n*: Total number.

Table 2 Methylation of pooled genes for the diagnosis of colorectal cancer

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)	0.928
Wnt pathway	-	-	72% (68%-75%)	93% (90%-96%)	33.99 (17.99-60.50)	0.931
-	DNA damage repair pathway	-	42% (36%-47%)	97% (94%-99%)	12.87 (5.98-27.72)	0.730
-	-	Other pathways	57% (55%-59%)	94% (93%-95%)	20.17 (15.18-26.80)	0.921
SFRP2	-	-	79% (75%-82%)	93% (90%-96%)	47.57 (20.08-112.72)	0.957
-	MGMT	-	47% (40%-53%)	95% (90%-98%)	11.67 (5.10-26.67)	0.709
-	MLH	-	28% (18%-39%)	100% (95%-100%)	23.68 (3.02-185.44)	0.500
-	-	Vimentin	49% (43%-54%)	93% (90%-95%)	13.81 (8.57-22.27)	0.847
-	-	OSMR	47% (40%-54%)	95% (91%-98%)	14.66 (5.06-42.47)	0.225
-	-	P16	50% (42%-58%)	98% (92%-100%)	24.39 (7.26-81.96)	0.975
SFRP2	MGMT	-	69% (66%-72%)	94% (91%-96%)	33.24 (16.76-65.93)	0.946
SFRP2	MLH	-	72% (68%-75%)	94% (92%-96%)	43.03 (20.15-91.87)	0.953
SFRP2	MLH	Vimentin	64% (60%-67%)	93% (92%-95%)	24.93 (15.34-40.50)	0.928
SFRP2	MLH	OSMR	65% (62%-69%)	95% (93%-96%)	33.10 (17.12-63.98)	0.951
SFRP2	MLH	P16	68% (64%-71%)	95% (93%-97%)	38.86 (20.11-67.54)	0.952

SE: Sensitivity; SP: Specificity; DOR: Diagnostic odds ratios; AUC: The area under the curve; CI: Confidence interval; MLH: MutL Homologue; MGMT: O-6-Methylguanine-DNA Methyltransferase.

the results indicated that the values of DOR and AUC of *P16* and *SFRP2* were higher than those of other genes, but the accuracy of faecal *SFRP2* methylation for the diagnosis of colorectal adenoma was higher than that of *P16* according to sensitivity (Figure 3A-C).

Meta-regression

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

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 (Figures 4A and B).

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 gene 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

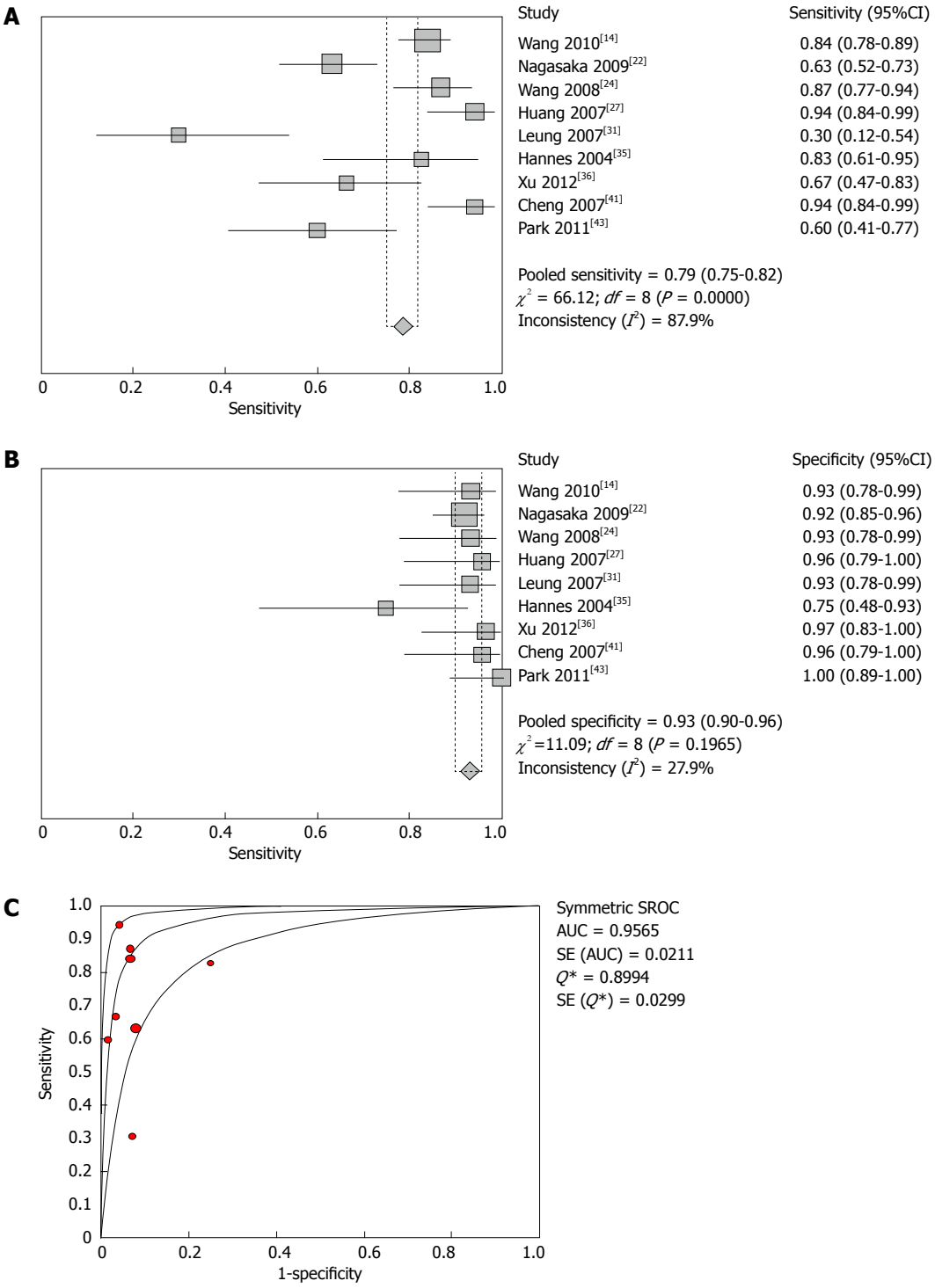


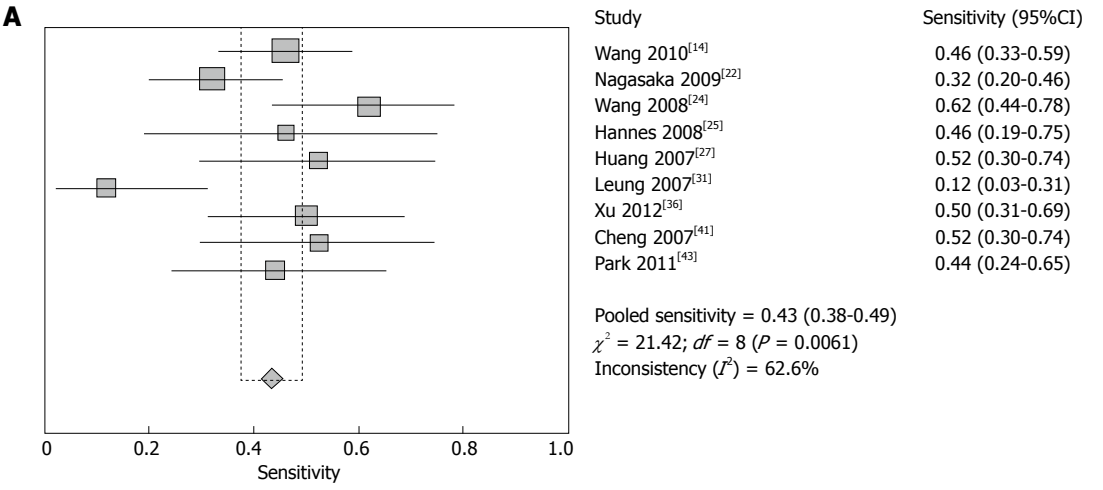
Figure 2 Forest plot of *SFRP2* methylation in the diagnosis of colorectal cancer. A: Shows the sensitivity of *SFRP2* methylation in stool samples used for colorectal carcinoma diagnosis. The point estimates of specificity from each study are shown as red squares; B: Shows the specificity of *SFRP2* methylation in stool samples used for colorectal cancer diagnosis. The point estimates of specificity from each study are shown as blue squares; C: 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. SROC curves summarize the overall diagnostic accuracy. Error bars indicate the 95%CI, and df indicates the degrees of freedom.

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) values of the SROC curve for faecal

Table 3 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)	0.883
Wnt pathway	-	-	40% (35%-46%)	95% (92%-97%)	10.81 (6.43-18.16)	0.932
-	DNA damage repair pathway	-	21% (17%-27%)	95% (91%-97%)	4.23 (2.01-8.88)	0.672
-	-	Other pathways	32% (28%-35%)	94% (93%-95%)	7.78 (5.48-11.05)	0.873
SFRP2	-	-	43% (38%-49%)	94% (91%-97%)	11.06 (5.77-21.18)	0.956
-	MGMT	-	29% (22%-36%)	93% (87%-96%)	4.42 (2.18-8.95)	0.614
-	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)	0.898
-	-	OSMR	25% (14%-39%)	95% (91%-98%)	5.20 (1.44-18.82)	0.817
-	-	P16	33% (23%-44%)	97% (89%-100%)	13.27 (3.40-51.83)	0.97
SFRP2	MLH	-	34% (29%-39%)	95% (92%-97%)	9.62 (4.64-19.93)	0.947
SFRP2	MGMT	-	38% (33%-42%)	94% (91%-96%)	7.85 (4.79-12.87)	0.753
SFRP2	-	OSMR	41% (35%-46%)	95% (92%-96%)	9.25 (5.13-16.69)	0.948
SFRP2	-	Vimentin	36% (32%-41%)	95% (93%-96%)	9.88 (5.55-17.57)	0.946
SFRP2	-	P16	41% (36%-46%)	95% (92%-97%)	10.37 (6.21-17.31)	0.948
SFRP2	MGMT	Vimentin	34% (30%-38%)	94% (92%-96%)	7.81 (4.96-12.29)	0.804
SFRP2	MGMT	OSMR	36% (32%-41%)	94% (92%-96%)	7.25 (4.61-11.39)	0.775
SFRP2	MGMT	P16	37% (33%-41%)	94% (92%-96%)	7.92 (5.14-12.21)	0.772
SFRP2	MLH	Vimentin	31% (27%-35%)	95% (93%-97%)	8.99 (4.95-16.31)	0.944
SFRP2	MLH	OSMR	33% (29%-38%)	95% (93%-97%)	8.37 (4.50-15.59)	0.941
SFRP2	MLH	P16	34% (30%-38%)	95% (93%-97%)	9.98 (5.45-18.27)	0.947

SE: Sensitivity; SP: Specificity; DOR: Diagnostic odds ratios; AUC: The area under the curve; MLH: MutL Homologue; MGMT: O-6-Methylguanine-DNA Methyltransferase.



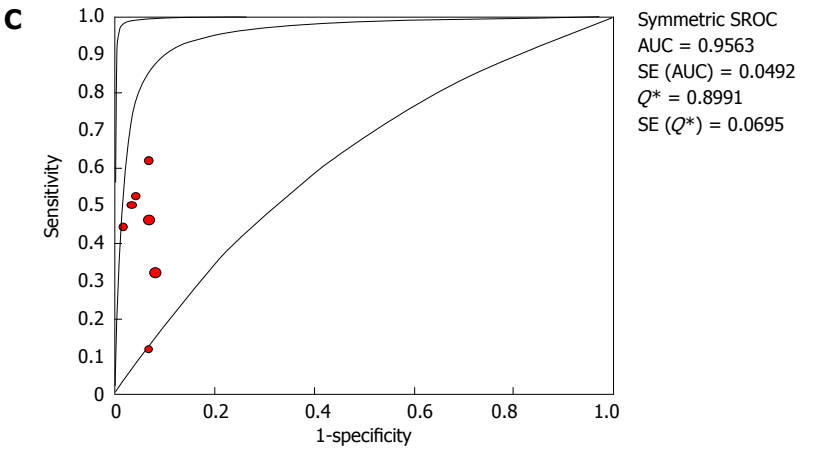


Figure 3 Forest plot of *SFRP2* methylation in the diagnosis of colorectal adenomas. A: Shows the sensitivity of *SFRP2* methylation in stool samples for colorectal adenoma diagnosis; B: Shows the specificity of *SFRP2* methylation in stool samples for colorectal adenoma diagnosis; C: Shows the summary receiver operating characteristic curves (SROC) of *SFRP2* methylation assays for the diagnosis of colorectal adenomas.

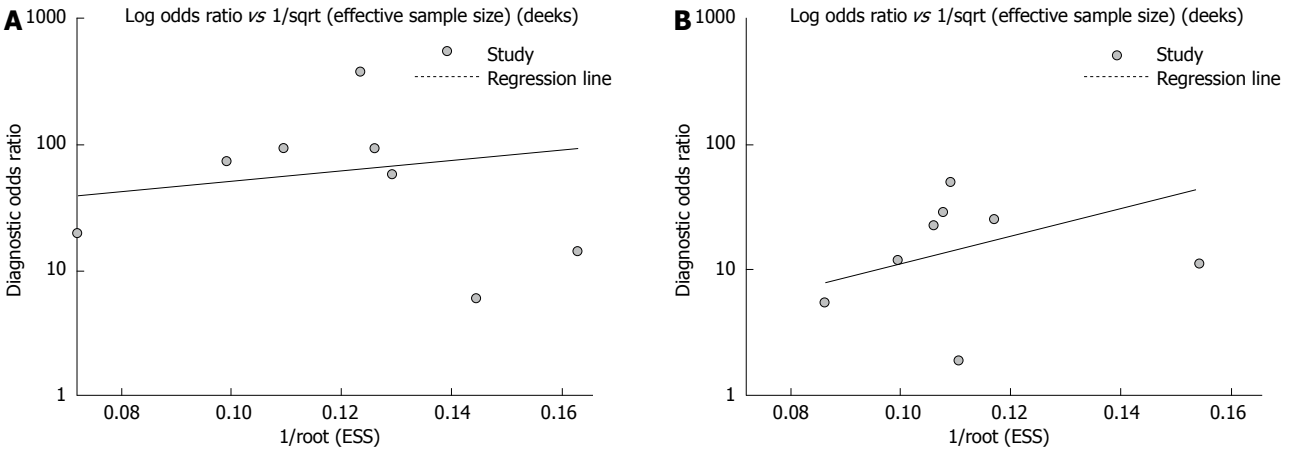


Figure 4 Assessment of the publication bias in faecal *SFPR2* methylation for the diagnosis of colorectal cancer (A) and adenomas (B). No significant publication biases were found in any of these studies (all $P > 0.05$).

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 rather 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 tumourigenesis. These epigenetic events are involved in the early steps of colon carcinogenesis, and changes in the status of DNA methylation are associated with early stages 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

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

Covariates	Coefficient	SE	*P* value	RDOR	95%CI
QUADAS score[1]	0.062	0.413	0.881	1.06	(0.46-2.47)
Detection method[2]	-0.146	0.401	0.719	0.86	(0.38-1.96)
Blinded design[3]	-0.166	0.364	0.651	0.85	(0.40-1.78)
Methylation genes[4]	-0.036	0.444	0.936	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.

cannot be generally used as a screening tool because of financial limitations, 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; and (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-invasive 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 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. The authors 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.

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