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Original Article

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Effectiveness of single-target fecal DNA methylation test in regional mass screening for colorectal cancer and precancerous lesions in China

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Abstract

Background: Colorectal cancer (CRC) is the third-most-common malignancy and the second-leading cause of cancer-related deaths worldwide and current screening methods such as guaiac-based fecal occult blood test (gFOBT), fecal immunochemical test (FIT), and colonoscopy have their own pros and cons. This study aimed to assess the effectiveness of a fecal DNA methylation test by using methylated *SDC2* (m*SDC2*) as the epigenetic biomarker for detecting CRC in a screening-naïve population.

Methods: Fecal m*SDC2* test and FIT were simultaneously performed on eligible 40- to 74-year-old adults of a regional township in China. Subjects with positive results were recommended for colonoscopy. Data of positivity rates, positive predicted values (PPVs), and detection rates associated with clinical characteristics were analysed.

Results: The positivity rate of m*SDC2* was 7.6% for 10,578 participants with valid results from both fecal m*SDC2* test and FIT. With an adherence rate of 63.8% to colonoscopy referral, 25 CRCs, 189 advanced adenomas (AAs), and 165 non-advanced adenomas (NAAs) and polyps were detected. The PPVs of m*SDC2* were 4.93%, 37.28%, and 32.54% for CRC, AA, and non-advanced lesions, respectively. When the CRCs and AAs were counted as positive findings, the fecal m*SDC2* test showed a higher detective rate than FIT (relative risk [RR], 1.313 [1.129–1.528], $P < 0.001$). When NAAs and polyps were also specified as treatable lesions, the m*SDC2* test was more effective in detecting these benign growths (RR, 1.872 [1.419–2.410]; $P < 0.001$). A combination of m*SDC2* and FIT detected 29 CRCs, 298 AAs, and 234 NAAs and polyps. Overall, the fecal m*SDC2* test had a higher detection rate for both advanced and non-advanced colonic lesions. The false-positive rate of the fecal m*SDC2* test was comparable to that of FIT (RR, 1.169 [0.974–1.403]; $P = 0.113$).

Conclusions: The single-target stool-based m*SDC2* test can effectively and accurately detect CRC and precancerous lesions in a large-scale CRC-screening program.

Trial registration number: NCT05374369.

Keywords: colorectal cancer; stool-based DNA methylation test; m*SDC2*

Introduction

Currently, colorectal cancer (CRC) ranks third in terms of incidence and second in terms of mortality of all malignancies worldwide [1]. In addition, the incidence rate of CRC has been predicted to surpass those of lung cancer and breast cancer by 2030 and 2040, respectively [2]. In China, CRC has the second-highest incidence rate and the fifth-highest mortality rate, both of which have recorded a continuous increase over recent decades [1, 3]. Although early detection of CRC significantly reduces its incidence and mortality rates [4], >50% of new cases in China are diagnosed at an advanced stage, leading to poor prognosis and survival [5]. Therefore, it is necessary to develop a carefully orchestrated program with effective screening modalities for CRC to reverse the trend.

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Fecal immunochemical test (FIT) and colonoscopy are currently the most commonly employed screening methods for CRC. FIT is a non-invasive, sensitive, and specific test for hemoglobin (Hb). It is used as an inexpensive tool for large-scale population screening and has been widely adopted in both developed and developing countries [6–11]. Colonoscopy, along with tissue biopsy, remains a gold standard in diagnosing CRC. However, it has several drawbacks, such as a high cost, invasiveness, and inconvenience. Hence, the participation rate in colonoscopy screenings remains low, especially outside the USA [4, 12, 13]. To address this issue, multiple tests that involve stool and serum samples based on DNA methylation markers have been developed in recent years. These tests have shown robust performance in various randomized, case-controlled, and observational trials in which hospital-based cohorts or average-risk populations were employed [14–29].

Methylated *SDC2* (m*SDC2*) is attracting increasing interest in China and Korea as a biomarker for the early detection of advanced colorectal neoplasms [18, 22–30] because of the following advantages. First, promoter hypermethylation of *SDC2* is a frequent and early event in advanced neoplastic tissue specimens, which is readily detectable in stool and serum samples, and a desirable feature as a biomarker to detect early-stage CRC and its precancerous lesions [18, 22, 23, 31]. Second, m*SDC2* exhibits a comparable or higher sensitivity and specificity for CRC detection than those shown by Hb and other methylation biomarkers in both single-target and multi-target tests [32–35]. Third, m*SDC2* can be applied to varied ethnicities, which makes it an attractive testing target globally [18, 23, 31, 36, 37]. However, despite these advantages, its effectiveness in the mass screening of asymptomatic populations for CRC has rarely been demonstrated.

By using both the m*SDC2* test and FIT, we here aim to develop a screening protocol for application in a screening-naïve population with a low prevalence of CRC. In the current study, >10,000 individuals of eligible age were administered two tests, including the m*SDC2* test and FIT. FIT—a non-invasive method for detecting occult blood in stool as recommended by CRC-screening guidelines—is used as a control to examine the performance of m*SDC2*. Participants with positive results were recommended to undertake further colonoscopy examinations. The test data were analysed separately for each biomarker and when they were combined. This work represents a further extension and analysis of our earlier published research [38].

Materials and methods

Study design

We launched a primary screening program for CRC based on both m*SDC2* testing and FIT in the Town of Shipai (City of Dongguan, Guangdong Province, China) between June 2021 and December 2021. This was the first-ever CRC-screening program to be conducted in Shipai. More than 10,000 participants were enrolled in this program, which was registered at https://clinicaltrials.gov/(NCT05374369). This study was approved by the Institutional Review Board of the Shipai Hospital of Dongguan (IRB approval number: 2022008). Voluntary written informed consent was obtained from all participants.

Screening population

Asymptomatic individuals aged between 40 and 74 years who were residing in the town of Shipai, covering 18 villages, voluntarily enrolled in the CRC-screening program. There was no gender restriction on the target screening-naïve population. Participants who were under routine medications of berberine, which would interfere with the stool test as previously reported [23], were allowed to take part in the test as long as they stopped taking the medicine 1 day before the testing date. We employed the following exclusion criteria: all individuals with a recognition disability; those with a history of cardiac disease or chronic kidney illness; those who had had or were receiving chemotherapy or immunotherapy; those who were pregnant; those who had hypertension; those who had heart diseases; and those who were unable or unwilling to provide voluntary written informed consent.

Sample collection and processing

Each participant was required to simultaneously provide fecal samples for both the m*SDC2* testing (Colosafe®) and FIT (OC-Sensor®) on a single sausage-shaped stool immediately after a bowel movement. A unique product code on a stool-collection device (Colosafe®, Creative Biosciences [Co.], Ltd, Guangzhou, China, or OC-Sensor®, Eiken, Japan) with no other identifiable information was provided to laboratory technicians for blind testing. The Colosafe® syringe-like device was used to collect a full chamber of 4.5 g of stool and transfer it to a 50-mL conical tube with 16.0 mL of preservation solution [23]. An OC-Sensor® was used to collect 10 mg of feces on scrapes with a serrated probe and transfer it into 2.0 mL of a preservation buffer. No specific restrictions on diet or medication use were required. All samples were collected at home and delivered locally by SF Express (Guangzhou, China) within 3 days of collection to the designated medical testing laboratory according to the standard procedure. Upon receipt, all stool specimens that were kept in a storage buffer for m*SDC2* testing were immediately registered, weighed, homogenized, and centrifuged. The supernatants were aliquoted and frozen at −80°C until further use. Frozen aliquots were subsequently assayed in batches by well-trained laboratory technicians from Creative Biosciences (Guangzhou, China). For FIT testing, the samples were either tested on the same day as they were received or stored in a refrigerator at –20°C for testing later within a week.

Target gene capture and bisulfite treatment

For each sample, 3.2 mL of the stool supernatant was first allowed to thaw to room temperature and centrifuged in a solid-phase extraction column that was embedded with polyvinylpolypyrrolidone. The marker genes were enriched and purified from the supernatant by using sequence-specific capture technology with some minor modifications [23]. Positive and negative quality controls of the *SDC2* gene were included along with other stool samples, which were subjected to the same methods of analytical processing and testing.

Real-time quantitative methylation-specific polymerase chain reaction

Real-time quantitative methylation-specific polymerase chain reaction (PCR) was employed to detect the *SDC2* and *ACTB* methylation status in stool samples. *ACTB* was amplified as a reference for DNA input. The primers, probes, and PCR set-ups that were employed in this study were identical to those reported in our previous study [23, 26]. The Abs Quant/2nd Derivative Max method in Roche LightCycler 480 II (Roche, Basel, Switzerland) was used to calculate the cycling threshold (Ct value). A prespecified cut-off was applied to dichotomize the test result. Stool samples with a Ct value of ≤38 for *SDC2* were classified as "positive" and those with a Ct value of >38 were classified as "negative," as long as the Ct value of *ACTB* remained at ≤36. Stool samples with a Ct value of *ACTB* of >36 were considered invalid [26].

FIT

FIT tests were performed on an automated analyser, OC-Sensor io (Eiken Chemical, Tokyo, Japan), by using OC-Auto 3 Latex Reagent according to the manufacturer's instructions. The same stool sample that was used for the m*SDC2* test was also collected for FIT by using an Eiken-proprietary sample collection device for each participant who underwent screening. The frozen samples were warmed up to room temperature before they were loaded onto the analyser. Serial dilutions of the Hb standard as provided by the manufacturer were always measured to construct a standard curve for reagent calibration when a new lot was used for the first time. The linear concentration range of the Hb standard was between 25 and 1,000 ng/mL. Samples with an Hb level that was above the upper limit were not reanalysed; instead, they were labeled as >1,000 ng/mL. Two quality controls of different concentrations (150 and 450 ng/mL) were always included with each batch of analytical runs. A concentration of 100 ng Hb/mL of buffer (FIT100), corresponding to 20 mg Hb/g of feces, was considered a regular cut-off level to determine whether a sample could be called FIT-positive or FIT-negative. Samples with readings that were above the upper analytical limit of 1,000 ng/mL were not diluted and re-assayed; they were reported as >1,000 ng/mL and called positive. FIT-positive participants were invited for colonoscopy.

Colonoscopy and pathological biopsies

Participants who were strongly recommended and encouraged for colonoscopy were examined within 3 months of m*SDC2*-positive or FIT-positive results. The endoscopic procedure with general anesthesia was performed after standard bowel preparation to ensure adequate visualization of the colon lining. All examinations were successfully conducted to completion by reaching the cecum. They met the following quality standards: (i) qualified bowel preparation rate of ≥90% (e.g. Boston marks ≥ 6); (ii) percentage of cecal intubations of ≥95%; (iii) time of withdrawal of ≥6 min; and (iv) adenoma detection rate of ≥15% (10% for females and 20% for males). All detected lesions were measured by using open biopsy forceps and annotated according to their size, morphology, and localization. CRCs were staged according to the standards of the American Joint Committee on Cancer, 8th edition [39]. Adenomas that met the following World Health Organization criteria were identified as advanced adenomas (AAs): maximum diameter of ≥1 cm, high-grade dysplasia (HGD), or substantial villous structures (>25%) [40].

Colonic biopsy specimens were obtained if the colonoscopist deemed the examination necessary. The diagnoses of advanced and non-advanced lesions were confirmed following assessment by a gastrointestinal pathologist. Polyps seen during colonoscopy were recorded based on their size and location in the colon and were then endoscopically removed. The polyp size was measured by using either visual estimation or estimation via open biopsy forceps. A centralized review was conducted for most of the cases at the Department of Pathology in Shipai Hospital of Dongguan.

Statistical analysis

The sample size was estimated based on the expected participation rate (60%), dropping rate (10%), positivity rate (PR) (5%), and positive predicted value (PPV) (30%). Bilateral testing at a significance level of 0.05 showed that ~20,000 screening participants could reach 80% statistical power. The PR was defined as the proportion of participants who had a positive test result; the PPV was defined as the proportion of participants who had a positive test result for CRC, AA, non-advanced adenoma (NAA), or polyp; and the dectection rate (DR) was defined as the proportion of participants with a neoplastic lesion. The number needed to screen (NNS) was defined as the number of participants required to detect one CRC by using the m*SDC2* test [41]. The reduction in NNS (NNS-reduction) reflected a reduction in the relative risk (RR) when screening with m*SDC2* compared with that obtained by using FIT.

PPV, DR, NNS, and NNS-reduction were used to assess the effectiveness of the m*SDC2* and FIT tests for detecting CRC, AAs, NAAs, and polyps. Wilcoxon rank-sum tests were performed to compare the methylation levels of different sample groups. A paired *t*-test was conducted for paired samples. χ^2 tests were carried out to evaluate the association of detection results with demographic and clinical characteristics, such as age, gender, tumor–node–metastasis stage, tumor location, tumor size, and dysplasia.

A logistic regression model was used to evaluate the difference in risk factors (age, gender, m*SDC2*, FIT, and tumor location), which is represented as an OR (odds ratio) and 95% CI (confidence interval). The model was then visualized by using a forest plot. In addition, *P*-values were adjusted by using the false discovery rate (FDR) method if the model was used for conducting more than two comparisons. A *P*-value of <0.05 was considered statistically different. Statistical analyses were conducted by using SPSS, version 15.0 (IBM Corporation, Armonk, NY, USA). Data visualizations were conducted via R, version 4.2.1.

Results

Screening population and adherence rates of m*SDC2* test, FIT, and colonoscopy

Our large-scale screening was limited to a regional population in Shipai Township, Guangdong Province, China. Overall, 10,893 eligible individuals between the ages of 40 and 74 years were enrolled and simultaneously provided with both m*SDC2* and FIT stool-collection devices (Supplementary Figure S1). Of these subjects, 185 individuals were excluded from the m*SDC2* test (1.70%) and 142 from FIT (1.30%) because either their stool samples were found to be unqualified or their testing results were declared invalid (Figure 1). Therefore, 10,708 and 10,751 participants had valid m*SDC2* and FIT data, respectively. In addition, individuals with only one of the two valid test results were excluded. Next, the m*SDC2* and FIT test results of 10,578 participants were subjected to further statistical analyses (Figure 1). Our testing population had a higher proportion of females (5,923 females vs 4,655 males). Moreover, the age group of 50–54 years had the highest number of participants (2,062), whereas that of 60–64 years had the lowest number (802) (Supplementary Table S1).

Among the 10,578 participants with valid m*SDC2* test results, 806 received positive results, displaying a PR of 7.6%. Among these 806 participants, 514 underwent colonoscopy examinations, resulting in a compliance rate of 63.8%. For the same group of participants with valid FIT data, 594 received positive results, 377 of whom underwent colonoscopy examinations, with a PR of 5.6% and a compliance rate of 63.5%, similarly to the m*SDC2* group. A total of 9,916 individuals with double-negative results were not recommended for colonoscopy as suggested by the manufacturers. Among the 514 m*SDC2*-positive and 377 FIT-positive individuals, 7 and 16 additional subjects were excluded owing to incomplete pathological information. Consequently, 507 and 361 participants, for whom both testing and colonoscopy data were available, were finally subjected to a further case–control analysis (Figure 1).

Robust performance of m*SDC2* test in detecting colorectal neoplasms

As shown in Figure 1 and Table 1, CRC was detected in 25 subjects (25/29, 86.2%) in the m*SDC2* group and 19 subjects

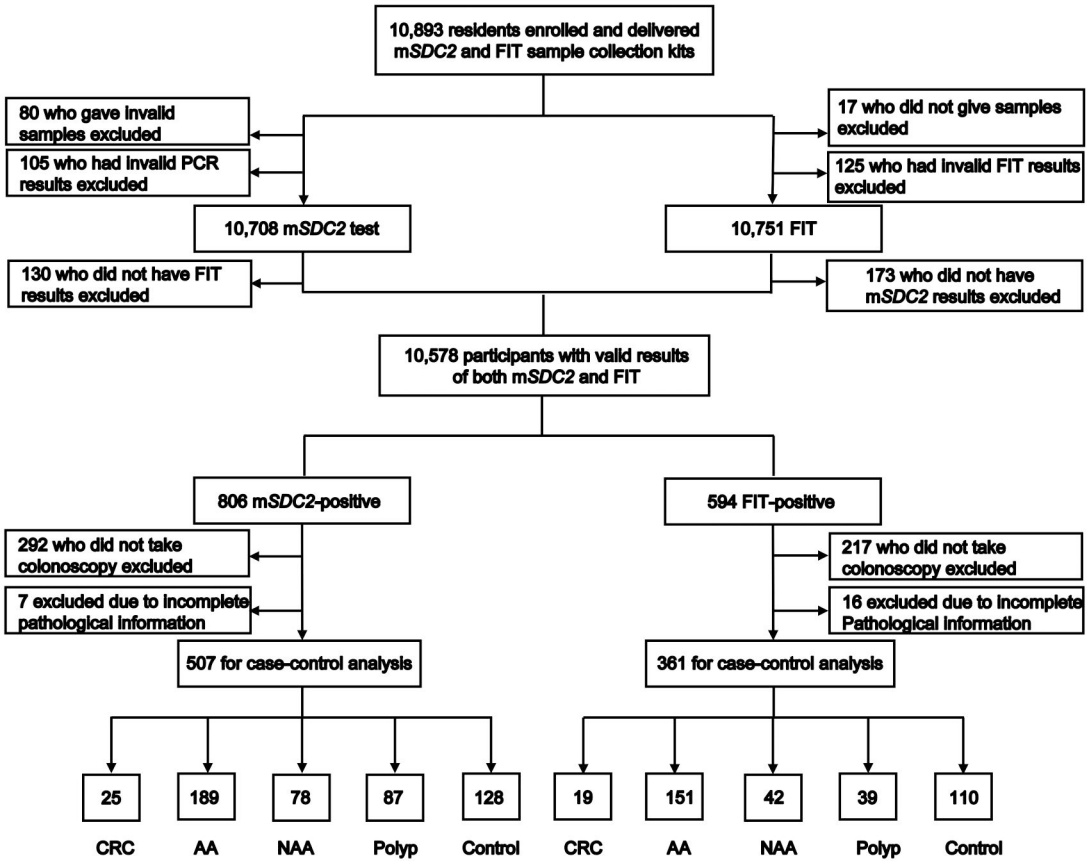

Figure 1. Flowchart of CRC population screening and colonoscopy findings in high-risk groups.

Table 1. mSDC2 vs FIT head-to-head comparison

Colonoscopy	Total ($n = 792$)	mSDC2		FIT		P-value	NNS	NNS-reduction (%)	RR (95% CI)
		+ ($n = 507$)	– ($n = 285$)	+ ($n = 361$)	– ($n = 431$)				
CRC	29	25 (86.2%)	4 (13.8%)	19 (65.5%)	10 (34.5%)	0.123	5	31.6	1.654 (1.030–2.657)
AA	298	189 (63.4%)	109 (36.6%)	151 (50.7%)	147 (49.3%)	0.002	8.3	25.1	1.293 (1.103–1.516)
NAA	118	78 (66.1%)	40 (33.9%)	42 (35.6%)	76 (64.4%)	<0.001	3.3	85.7	1.872 (1.419–2.410)
Polyp	116	87 (75.0%)	29 (25.0%)	39 (33.6%)	77 (66.4%)	<0.001	2.3	123.0	2.347 (1.764–3.723)
Control	231	128 (55.4%)	103 (44.6%)	110 (47.6%)	121 (52.4%)	0.113	14.3	16.3	1.169 (0.974–1.403)

FIT = fecal immunochemical test, mSDC2 = methylated SDC2.
NNS (number needed to scope) = 1/ARR (absolute risk reduction).
NNS-reduction = | NNS by FIT detection – NNS by mSDC2 detection |/NNS by FIT detection = | FIT-positive detection rate – mSDC2-positive detection rate |/FIT-positive detection rate * 100%.
ARR (absolute risk reduction) = FIT-positive detection rate – mSDC2-positive detection rate.
Comparison based on PR of mSDC2 and FIT. RR and 95% CI are used to represent the test effect of mSDC2 compared with FIT.

(19/29, 65.5%) in the FIT group (RR, 1.654; 95% CI, 1.030–2.657; $P = 0.123$). AAs were found in 189 subjects (189/298, 63.4%) in the mSDC2 group and in 151 subjects (151/298, 50.7%) in the FIT group (RR in the mSDC2 group, 1.293; 95% CI, 1.103–1.516; $P = 0.002$). In addition, non-advanced adenomas (NAAs) and polyps were found in 78 (66.1%) and 87 (75.0%) subjects in the mSDC2 group, respectively, and in 42 (35.6%) and 39 (33.6%) subjects in the FIT group, respectively (RR of NAAs in the mSDC2 group, 1.872; 95% CI, 1.419–2.410; $P < 0.001$; RR of polyps in the mSDC2 group, 2.347; 95% CI, 1.764–3.723; $P < 0.001$, Table 1). The methylation test of mSDC2 was superior to FIT in detecting advanced colorectal neoplasms (ACNs, CRCs+AAs) (RR, 1.313; 95% CI, 1.129–1.528; $P < 0.001$) and non-advanced adenomas and polyps (NAPs, NAAs+polyps) (RR, 1.872; 95% CI, 1.419–2.410; $P < 0.001$) (Supplementary Table S2). In addition, a comparison of NNS-reduction values that were obtained by using FIT and mSDC2 screening showed that the NNS-reduction was 31.6% for CRC, 25.1% for AAs, 85.7% NAAs, and 123.0% for polyps (Table 1).

The two groups showed similar test outcomes based on tumor staging. Among the 25 participants in the mSDC2-positive group, 18 (72.0%) had CRC tumors and 7 (28.0%) had stage II tumors (Table 2). Likewise, among 19 participants in the FIT group, 11 (57.9%) were found to have early-stage tumors, 7 (36.8%) had stage II tumors, and 1 (5.3%) had a stage III tumor. Hence, both tests could be employed for detecting early-stage CRCs ($P = 0.385$, Table 2).

When the diagnostic yield was analysed according to gender, no significant difference between males and females was observed for both the mSDC2 and FIT tests ($P = 0.761$, Table 2). Similarly, no significant difference was observed in the DR of CRCs and AAs when

Table 2. mSDC2 vs FIT stratified analysis of CRC according to clinical and pathological characteristics

Characteristic	CRC (*n* = 29)	mSDC2		FIT		P-value
		+ (*n* = 25)	– (*n* = 4)	+ (*n* = 19)	– (*n* = 10)	
Age (years)						0.368
Median (range)	61 (40–74)	64 (40–74)	55 (50–63)	56 (40–74)	67 (44–72)	
Gender, *n* (%)						0.761
Male	18 (62.1)	14 (56.0)	4 (100.0)	10 (52.6)	8 (80.0)	
Female	11 (37.9)	11 (44.0)	0 (0)	9 (47.4)	2 (20.0)	
Stage, *n* (%)						0.385
0/I	20 (69.0)	18 (72.0)	2 (50.0)	11 (57.9)	9 (90.0)	
II	8 (27.6)	7 (38.0)	1 (25.0)	7 (36.8)	1 (10.0)	
III/IV	1 (3.4)	0 (0)	1 (25.0)	1 (5.3)	0 (0)	
Location, *n* (%)						0.680
Proximal	6 (20.7)	5 (20.0)	1 (25.0)	2 (10.5)	4 (40.0)	
Distal	23 (79.3)	20 (80.0)	3 (75.0)	17 (89.5)	6 (60.0)	
Tumor size (mm), *n* (%)						0.761
<10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
10–30	16 (55.2)	14 (56.0)	2 (50.0)	9 (47.4)	7 (70.0)	
≥30	13 (44.8)	11 (44.0)	2 (50.0)	10 (52.6)	3 (30.0)	

CRC = colorectal cancer, FIT = fecal immunochemical test, mSDC2 = methylated *SDC2*.
Chi-squared or Fisher's exact tests, as appropriate, were used to compare the differences in categorical variables, while a two-sample *t*-test was used to compare the differences in continuous variables. A two-tailed *P*-value of <0.05 indicates a significant difference between the mSDC2-positive and FIT-positive groups.

Table 3. mSDC2 vs FIT stratified analysis of AAs according to clinical and pathological characteristics

Characteristic	AA (*n* = 298)	mSDC2		FIT		P-value
		+ (*n* = 189)	– (*n* = 109)	+ (*n* = 151)	– (*n* = 147)	
Age (years)						0.866
Median (range)	59 (40–74)	59 (40–74)	58 (40–74)	59 (40–74)	59 (40–74)	
Gender, *n* (%)						0.542
Male	187 (62.3)	120 (63.5)	67 (61.5)	91 (60.3)	96 (65.3)	
Female	111 (37.7)	69 (36.5)	42 (38.5)	60 (39.7)	51 (34.7)	
Location, *n* (%)						0.609
Proximal	92 (30.9)	54 (28.6)	38 (34.9)	47 (31.1)	45 (30.6)	
Distal	206 (79.1)	135 (71.4)	71 (65.1)	104 (68.9)	102 (69.4)	
Tumor size (mm), *n* (%)						0.447
<10	44 (14.8)	30 (15.9)	14 (12.8)	17 (11.3)	27 (18.4)	
10–30	243 (81.5)	148 (78.3)	95 (87.2)	126 (83.4)	117 (79.6)	
≥30	11 (3.7)	11 (5.8)	0 (0)	8 (5.3)	3 (2.0)	
Pathological type, *n* (%)						0.825
HGD	23 (7.7)	16 (8.5)	7 (6.4)	13 (8.6)	10 (6.8)	
Villous	71 (23.8)	47 (24.9)	24 (22.0)	45 (29.8)	26 (17.7)	
Tubulovillous	114 (38.3)	72 (38.1)	42 (38.5)	51 (33.8)	63 (42.8)	
Tubular	75 (25.2)	46 (24.3)	29 (26.6)	34 (22.5)	41 (27.9)	
SSA	15 (5.0)	8 (4.2)	7 (6.4)	8 (5.3)	7 (4.8)	

AA = advanced adenoma, FIT = fecal immunochemical test, HGD = high-grade dysplasia, mSDC2 = methylated *SDC2*, SSA = sessile serrated adenoma.
Chi-squared or Fisher's exact tests, as appropriate, were used to compare the differences in categorical variables, while a two-sample *t*-test was used to compare the differences in continuous variables. A two-tailed *P*-value of <0.05 indicates a significant difference between the mSDC2-positive and FIT-positive groups.

the subjects were stratified based on lesion location ($P = 0.680$, Table 2; $P = 0.609$, Table 3) and size ($P = 0.761$, Table 2; $P = 0.447$, Table 3). Both tests showed that CRCs and AAs tend to be located in the distal region. Furthermore, when CRCs and AAs were stratified based on their sizes, both the mSDC2 and FIT tests detected them in similar proportions ($P = 0.761$, Table 2; $P = 0.447$, Table 3). Finally, both the mSDC2 and FIT tests could detect various types of AAs, most evidently those of tubulovillous nature, and exhibited a similar capacity to identify precancerous lesions with HGD and sessile serrated adenomas (SSAs) ($P = 0.825$, Table 3).

Enhanced yield of diagnosed colorectal neoplasms when combining mSDC2 and FIT tests

When the mSDC2-positive group ($n = 507$) and FIT-positive group ($n = 361$) were combined, a total of 792 individuals were subjected to further analysis (Figure 2). Compared with the mSDC2-positive individuals, the DRs for CRC, AAs, and NAPs increased by 16% (4/25), 57.7% (109/189), and 41.8% (69/165), respectively. Compared with the FIT, the DR for combined results increased by 52.6% (10/19), 97.4% (147/151), and 188.9% (153/81) (Table 1 and Figure 2). A total of 76 participants tested positive in both mSDC2 and FIT, representing only a small fraction (76/792, 9.6%) of the combined cases compared with mSDC2 single-positive cases (76/431, 17.6%) or FIT single-positive cases (76/285, 26.7%) (Figure 2). However, the DR for ACNs among the double-positive cases drastically increased to 75.0% (57/76), which was significantly higher than that achieved with either single-target test (mSDC2, 42.2% [214/507]; FIT, 47.1% [170/361]). If the NAPs were added, the DR could be elevated to 90.8% (69/76).

To further elucidate the effects of risk factors such as gender and age on the diagnostic yields of the mSDC2 and FIT tests, we conducted a logistic regression analysis of the detection rates among the various groups of neoplastic lesions, polyps, and controls. If CRCs and AAs are considered positive findings compared

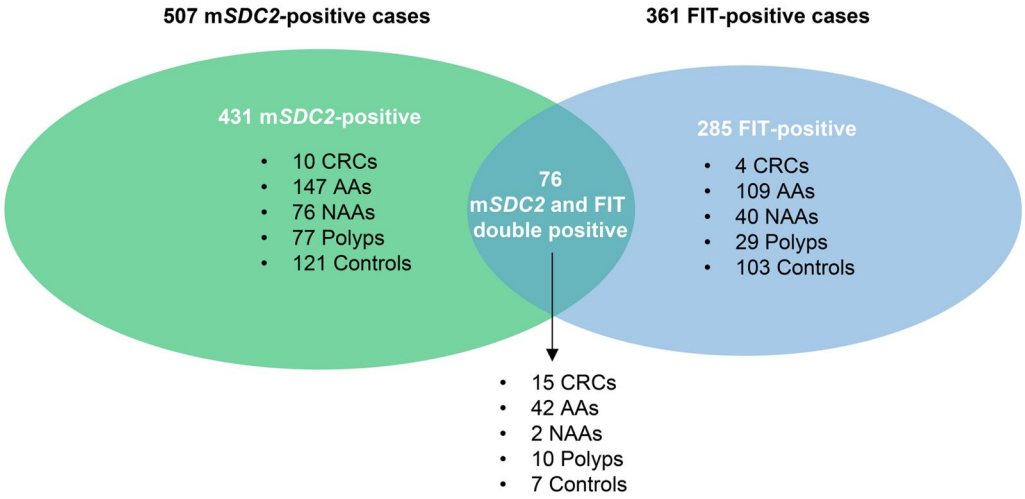

Figure 2. Overlap of mSDC2- and FIT-positive cases and subcategory distribution.

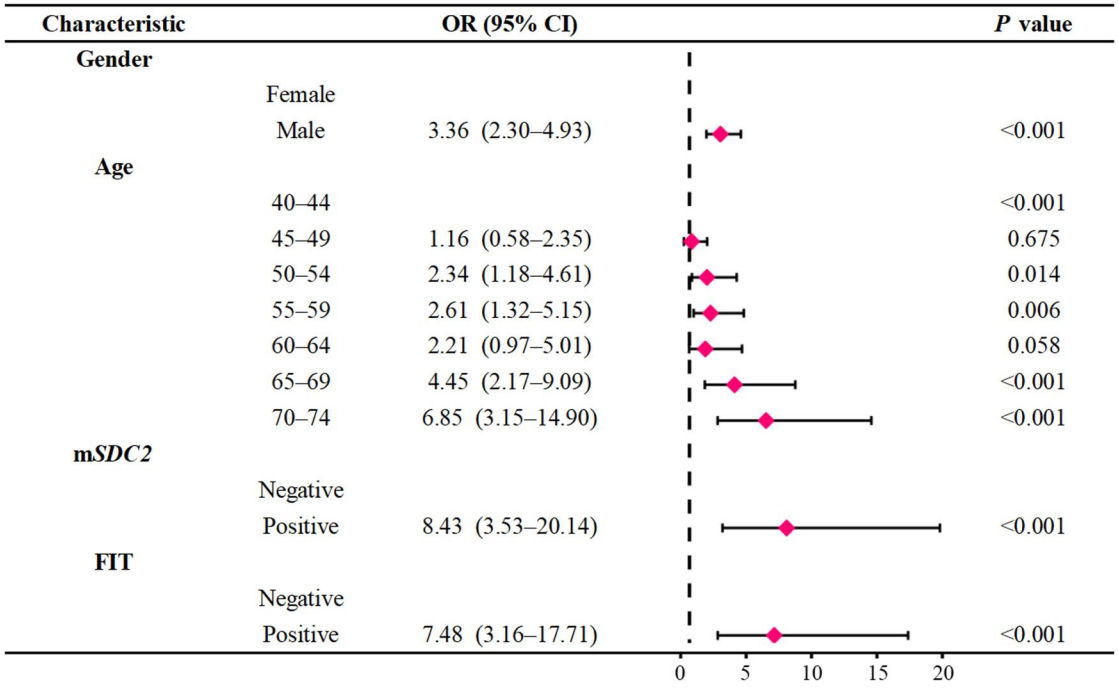

Figure 3. Logistic regression analysis of the effect of gender and age on detecting ACNs vs controls. Logistic regression models were constructed with four covariates (age, gender, mSDC2, and FIT). P-values were adjusted by using the FDR method. The OR values were used to represent the risk of exposure for each variable (e.g. male vs female and positive vs negative). In this plot, the square dot represents the OR value and the horizontal line represents the 95% CI.

with controls, then ACNs were more likely to be detected in males than in females (OR, 3.364, 95% CI, 2.295–4.932, $P < 0.001$, Figure 3). As the age increased, the likelihood of detecting ACNs also increased significantly, peaking in the age group of 70–74 years (OR, 6.853, 95% CI, 3.152–14.902, $P < 0.001$, Figure 3). Consistently, higher methylation levels of SDC2 and the amount of Hb in stool were more likely to be associated with ACNs than with controls, showing higher odds for mSDC2 than for FIT (OR for mSDC2: 8.426, 95% CI: 3.525–20.14, $P < 0.001$; OR for FIT: 7.481, 95% CI: 3.16–17.711, $P < 0.001$, Figure 3). If NAPs were considered positive findings that required medical treatment, then similar findings to ACNs and controls could be observed between NAPs and controls as well as between colorectal neoplasms (CNs) and controls (Supplementary Tables S3 and S4). Hence, it could be suggested that the mSDC2 test performs significantly better than FIT in effectively detecting both neoplastic and non-neoplastic growths in a large population with a low prevalence of CRC.

In the current population screening, the Ct and Hb concentration thresholds for determining mSDC2 and FIT results as positive were 38 and 100 ng/μL, respectively. If a higher Ct cut-off value of 39 is employed for the mSDC2 test, then at least 21 and 3 additional AAs and NAPs, respectively, could be detected. Similarly, 14 and 18 more cases of AAs and NAPs could be identified when the cut-off values for FIT were made as low as

50 ng/mL (Supplementary Table S5). However, no extra cases of CRC could be detected within the group of 792 subjects when the threshold values for both the mSDC2 and FIT tests were reduced.

Discussion

The exploration of new methods for CRC screening is becoming increasingly scrutinized. The potential of the mSDC2 test (emerging non-invasive CRC detection technology) to demonstrate value in large-scale population screening is pivotal in determining its future application prospects. This study utilized FIT as a control to assess the performance of the mSDC2 test. The results indicated that both tests were effective in identifying early-stage CRC and AAs, with mSDC2 showing superior sensitivity in detecting advanced neoplasms compared with FIT. Furthermore, the complementary nature of mSDC2 and FIT in colorectal lesions detection offers a novel approach for screening and diagnosis.

Early detection of CRC and precancerous lesions has been shown to significantly reduce cancer-related mortality rates in screening-aged populations [42]. Notably, 72.0% and 57.9% of CRCs that were detected in our study by using the mSDC2 test and FIT, respectively, were early-stage (stage 0/I) diseases. The remaining CRCs were mostly stage II cancers, except for one case of stage III CRC that was identified by FIT. Notably, these results are consistent with those achieved by screening an asymptomatic population. Additionally, both tests identified a greater number of AAs, predicting a further improved survival outcome, absolute reduction in the number of deaths, and an incremental gain in life-years because of timely surgical interventions. Interestingly, a large proportion of these advanced precancerous lesions had substantial villous components (63.0% for mSDC2 and 63.5% for FIT), which had not been reported previously. Additionally, mSDC2 seemed to perform significantly better than FIT in detecting advanced neoplasms ($P < 0.001$). As the two tests afford similar diagnostic yields for CRC, the significant difference between ACNs and controls can be attributed to the better detection capability of the mSDC2 test. AAs were also indicated in previous clinical trials [25, 26]. The mSDC2 test was more sensitive in detecting NAPs than FIT ($P < 0.001$), which conforms with the significantly higher PR of this test (7.6%). However, as NAPs can be considered positive findings that require treatment, the FDR of the mSDC2 test (25.2%, 128/507) was not significantly different from that of FIT (30.5%, 110/361) ($P = 0.113$). Therefore, mSDC2 can be expected to be a more sensitive biomarker than Hb in mass screening programs for CRC. However, because of the lack of colonoscopy data for subjects who were negative for both mSDC2 and FIT, and owing to incomplete colonoscopy uptake in subjects with positive results, it is impossible to compute the exact sensitivity and specificity for both tests and compare their performance head to head in terms of these two study end points.

The adherence rate to colonoscopy can greatly impact the outcomes of non-invasive screening modalities such as the mSDC2 test and FIT [43]. The adherence rate to colonoscopy as the sole screening modality for CRC is notoriously low in China (9.7%–25.2%), even among high-risk populations in urban areas [13, 44]. In addition, the adherence rate to colonoscopy referral after a single FIT-positive result only marginally improved to 30.7%, as reported by large-scale screening that involved over half a million people in China [45]. In the current study, we conducted all colonoscopy examinations under general anesthesia. These examinations were fully subsidized by the mSDC2 screening program (Shipai Town Colorectal Cancer Free Screening Project, 20210410 Shipai Town Party and Government Comprehensive Office [2021] No. 2). Therefore, an adherence rate of 63.8% to colonoscopy recommendation in the present study is more than double that reported in some of the previous studies [13, 44, 45]. Concomitantly, FIT-positive results demonstrated a similar adherence rate to colonoscopy (63.5%). Hence, the significantly higher colonoscopy uptake played a determining role in ensuring the high effectiveness of mSDC2 and FIT testing in real-world screening. In addition to increased uptake of colonoscopy examinations and enhanced performance of the mSDC2 test and FIT combination method, our study has several other advantages as well. The mSDC2 test is non-invasive, user-friendly, and the most affordable so far [26]. Moreover, stool-based sampling is a private, easy, and convenient method, and fecal DNA remains intact in a preservation solution at ambient temperature for as long as a week [23, 26]. The mSDC2 biomarker that was employed in the current population screening for CRC has been extensively tested in numerous preclinical studies and clinical trials, which have proved its performance in hospital-based cohorts [22–26, 34, 37, 38, 41]. The background methylation profile of the SDC2 gene is immaculate in normal and adjacent normal epithelial tissues, making it an optimal target for accurate quantification of mSDC2 in CRC and precancerous lesions by quantitative methylation-specific polymerase chain reaction [23].

FIT products from the OC-Sensor® family are the most assessed and utilized tools globally for CRC-screening programs [46]. A previous study on carefully selected asymptomatic participants (>32,000) reported sensitivity and specificity as high as 67.3%–73.8% and 96.0%–96.4%, respectively, using OC-Sensor® FIT [19, 47]. However, FIT has several limitations as well, such as its unavoidable degradation of Hb and intermittent bleeding patterns. Consequently, one in four CRC cases is still diagnosed at a more advanced stage, leading to a poor prognosis [48]. In addition, its sensitivity to AAs is notoriously low because of infrequent bleeding in precancerous lesions [46]. In the current study, the absolute number of AAs (189) detected by the mSDC2 test is significantly higher than that detected by FIT (151). Moreover, 147 AAs detected by mSDC2 were not detected by FIT compared with 109 AAs detected by FIT but not the mSDC2 methylation test. The effectiveness of such a combined approach has been illustrated in a multi-target sDNA test (Cologuard®) in which adding FIT to methylation markers increased the sensitivity of the FDA-approved test to >90% [17, 19]. In our current investigation, adding mSDC2 testing to FIT afforded an additional gain of 157 ACNs—a whopping increase of 92.4% (157/170) in the detection yield. Hence, the two biomarkers of entirely different natures—a methylated DNA fragment and a hem-containing protein—complemented each other exceptionally well in detecting ACNs and NAPs. Though the mSDC2 test is currently more expensive than FIT, the nearly doubled gain in positive findings cannot be ignored, particularly for individuals with early-stage CRC for whom any delay in diagnosis could allow the tumor to further progress to an advanced stage. In addition, our study has certain limitations as well, which require specific improvements. First, the participants were not asked to fill out any questionnaires that were designed for the CRC-screening program [49, 50]. Information regarding factors such as an individual's family history, smoking habit, and body mass index were unavailable for any subsequent in-depth analysis. The study population may have included both average-risk and high-risk populations, making it heterogeneous and the results difficult to extrapolate to a true average-risk population. Another major weakness in the current study is the lack of colonoscopy data on the subjects

with negative results from both m*SDC2* and FIT, precluding evaluation of the true sensitivity and specificity of these tests for the community-based screening population. Therefore, the relevance of the study results in the current screening setting is limited for interpretation. Third, single cut-off values in two different analytical systems—Ct = 38 for Colosafe® and 100 ng/mL of Hb for OC-Sensor® FIT—were used as the criteria for recommending colonoscopy examinations and statistical analyses. These empirical values have been recommended by the manufacturer and adopted by most other large screening programs [9, 51–53]. Hence, we could not accurately evaluate, particularly for OC-Sensor® FIT, the impact of other lower threshold values, including 50 and 75 ng/mL of Hb on PR, PPV, and DR. Fourth, the colonoscopy uptake was still lower than those in other screening programs, which ranged from 82.6% to 93.1% [9, 48–50]. Additionally, adherence to colonoscopy in our study was also lower than in some of the other domestic programs with colonoscopy subsidies. For example, the adherence rates were 76.2% in Haining (Zhejiang, China) [54] and 78.7% in Jingzhou (Hubei, China) [55]. Public awareness of the effectiveness of CRC screening, strong monetary support to fully reimburse the participants for the cost of colonoscopy, and keen promotion by well-respected physicians and experienced endoscopists should be conducted to further enhance colonoscopy uptake. Fifth, even though numerous additional ACNs could be detected when the two fecal tests were combined, whether the solid additive effect that we have observed here can be replicated in future organized mass screenings has yet to be determined.

In conclusion, this study employed FIT—a non-invasive method for detecting occult blood in stool, as recommended by CRC-screening guidelines—as a control to examine the performance of the m*SDC2* test. The lessons learned and experiences gained in the current trial can help in developing future screening strategies such as a streamlined enrolment process, convenient sampling procedure, fully subsidized colonoscopy, timely and persistent follow-up of positive results for colonoscopy, and combination of proven stool tests to maximize the yield and minimize false positives in a screening-naïve population with a low prevalence of CRC. As a result, the study outcomes provide vital information to help formulate future screening strategies for CRC in regional populations, which may further improve the compliance rate for testing and colonoscopy, reduce the risk of adverse outcomes, and enhance the effectiveness and diagnostic accuracy of the m*SDC2* test.

Ethics approval

This study was performed as per the principles of the Helsinki Declaration and approved by the Institutional Review Board of the Shipai Hospital of Dongguan. All participants have acknowledged and signed informed consent.

Supplementary data

Supplementary data is available at *Gastroenterology Report* online.

Authors' contributions

X.K. and H.Z. conceptualized the study design, obtained funding, and supervised the study. Z.Z., Z.Y., Q.W., F.N., and X.W. acquired data and performed analyses. X.W. and H.Z. drafted and revised the manuscript. X.K., F.N., and H.Z. performed critical reviews of the manuscript. X.K., Z.Z., Z.Y., and F.N. provided administrative, technical, and material support. All authors have read and approved the final manuscript.

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Conflicts of interest

X.W. and F.N. are employees of Creative Biosciences (Guangzhou) Co., Ltd. H.Z. is the shareholder of Creative Biosciences (Guangzhou) Co., Ltd. The other authors declared no competing interests.

Data availability

This article and related supplementary information files include all data generated or analysed during this study.

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