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Genetic Biomarker Prevalence is Similar in Fecal Immunochemical Test Positive and Negative Colorectal Cancer Tissue

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Drs. Lidgard and Berger are employees of Exact Sciences, which markets a stool DNA test. Mr. Taylor and Dr. Ahlquist are employees of the Mayo Clinic, an investor in Exact Sciences and who receive royalties from intellectual property. Other coauthors have no financial arrangements to disclose.

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Abstract

Background: Fecal immunochemical test (FIT) screening detects most asymptomatic colorectal cancers. Combining FIT screening with stool-based genetic biomarkers increases sensitivity for cancer, but whether DNA biomarkers (biomarkers) differ for cancers detected versus missed by FIT screening has not been evaluated in a community-based population.

Aims: To evaluate tissue biomarkers among Kaiser Permanente Northern California patients diagnosed with colorectal cancer within two years after FIT screening.

Methods: FIT-negative and FIT-positive colorectal cancer patients 50–77 years of age were matched on age, sex, and cancer stage. Adequate DNA was isolated from paraffin embedded specimens in 210 FIT-negative and 211 FIT-positive patients. Quantitative allele-specific real-time target and signal amplification assays were performed for 7 *K-ras* mutations and 10 aberrantly methylated DNA biomarkers (*NDRG4, BMP3, SFMBT2_895, SFMBT2_896, SFMBT2_897, CHST2_7890, PDGFD, VAV3, DTX1, CHST2_7889*).

Results: One or more biomarkers were found in 414 of 421 CRCs (98.3%). Biomarker expression was not associated with FIT status, with the exception of higher *SFMBT2_897* expression in FIT-negative (194 of 210; 92.4%) than FIT-positive cancers (180 of 211; 85.3%; p=0.02). There were no consistent differences in biomarker expression by FIT status within age, sex, stage, and cancer location subgroups.

Conclusions: The biomarkers of a currently-in-use multi-target stool DNA test (*K-ras*, *NDRG4*, and *BMP3*) and eight newly characterized methylated biomarkers were commonly expressed in tumor tissue specimens, independent of FIT result. Additional study using stool-based testing with these new biomarkers will allow assessment of sensitivity, specificity, and clinical utility.

Keywords

Colorectal Neoplasms/diagnosis; DNA Mutational Analysis; DNA, Neoplasm/analysis; Early Detection of Cancer/methods

INTRODUCTION

Colorectal cancer (CRC) screening is effective for reducing disease-related deaths (1). Testing for occult blood in the stool is the most commonly-used method of CRC screening worldwide (2). With a reported sensitivity of 65–80% for detecting CRC (3–5), the fecal immunochemical test (FIT) outperforms guaiac-based stool tests (3), but is less sensitive than colonoscopy (5).

One new approach, the multi-target stool DNA test, is now included in the screening recommendations of the US Preventive Services Task Force (1). It combines FIT screening with assays for mutated and methylated genes that are commonly observed in colorectal neoplastic lesions (6). For example, *K-ras* gene point mutations are expressed in about 40% of CRCs, occur early in the carcinogenetic process, and are detectable in the stool (7).

Various methylated DNA markers are substantially more broadly informative than *K-ras* in tissue and in stool (8–10). A recent large study found that adding quantitative molecular assays for *K-ras* mutations and aberrantly methylated DNA biomarkers (i.e., *NDRG4* and *BMP3*) to a quantitative fecal hemoglobin immunoassay yielded a sensitivity of 93.3% for CRC stages I-III compared with 73.3% for FIT screening alone (7).

The aim of this study was to evaluate whether CRCs not detected by FIT differed in their DNA biomarker profiles from CRCs detected by FIT, overall and within subgroups by age, sex, stage, and CRC location. We evaluated DNA markers currently in clinical use for CRC screening as components of a multi-target stool DNA test (panel A), and newly-characterized colorectal neoplasia-associated DNA biomarkers (panel B). The identification of new markers highly expressed in CRC tissue may allow their further evaluation in stool-based tests, to decrease the potential for screening tests to miss cancers. Better understanding of the molecular profile differences between FIT-positive and FIT-negative colorectal tumors could allow better patient selection for the use of FIT alone versus FIT in combination with DNA biomarkers in mass screening.

METHODS

Study Oversight

This study was approved by the institutional review board at Kaiser Permanente Northern California (KPNC). The listed authors had sole responsibility for the study's design, data collection, decision to submit the manuscript for publication, and drafting of the manuscript. This study was conducted within the National Cancer Institute-funded *Population-based Research Optimizing Screening through Personalized Regimens* (PROSPR) consortium which conducts multisite, coordinated, transdisciplinary research to evaluate and improve cancer-screening processes.

Study Design and Setting

This was a cross-sectional study of KPNC health plan members who were diagnosed with CRC in the two-year period following FIT screening. KPNC is an integrated health care delivery organization with over 3.8 million members. Each year, KPNC's screening program identifies and mails FIT kits (OC FIT-CHEK, Polymedco, Inc.) to screening-eligible persons (11).

Patient Population and Selection

KPNC health plan members were eligible for inclusion if they were diagnosed with an incident colorectal adenocarcinoma with available staging data between January 1, 2009 and December 31, 2010; were 50–77 years of age on the date of CRC diagnosis; and completed a FIT in the 2 years prior to diagnosis. The 2-year timeframe was based on the biological slow growth assumption that CRCs are potentially detectable by fecal blood tests for at least 2 years prior to clinical presentation/diagnosis (4, 12).

We identified 509 FIT-negative and 710 FIT-positive CRC patients who met the study eligibility criteria (Figure 1). We randomly selected 275 FIT-positive CRC patients and

frequency matched them 1:1 by sex, age in 5-year intervals, and stage (localized, regional, and distant) to 275 FIT-negative CRC patients. CRC tissue blocks were retrieved from KPNC pathology tissue repositories and evaluated for adequacy by the study pathologist. An additional 68 FIT-negative and 56 FIT-positive CRC patients were randomly selected to replace patients whose tissue samples were either unavailable or, after pathology evaluation, were found to be inadequate or lacking sufficient tumor tissue for analysis. After pathology review, 239 FIT-negative and 250 FIT-positive formalin-fixed paraffin-embedded CRC tissue blocks were sent for genetic analyses. A total of 210 FIT-negative and 211 FIT-positive CRC tissue specimens had adequate isolatable DNA for all DNA biomarker assays.

Data Sources

CRC diagnosis date, location, stage, grade, and patient demographics were obtained from the KPNC cancer registry, which reports to the Surveillance Epidemiology and End Results program. Cancers were designated localized, regional (regional, direct extension only; regional, lymph nodes only; regional, direct extension and lymph nodes; and regional, not otherwise specified), or distant (distant metastases or systemic disease (remote)) based on the reported cancer registry summary stage. Right-sided (proximal) cancers were those in the cecum, ascending colon, hepatic flexure, and transverse colon; left-sided (distal) cancers were those in the splenic flexure, descending colon, sigmoid colon, and rectum. Late-stage cancers were defined as stage IIb and higher according to the American Joint Committee on Cancer staging system (13). FIT results were obtained from laboratory databases. Pathology tissue samples were identified using pathology databases, and tumor tissue samples were retrieved from KPNC biospecimen repositories.

Laboratory Procedures

Completed FITs were mailed by patients to a central KPNC laboratory and analyzed using the automated Polymedco, Inc., OC Sensor DIANA fecal occult blood analyzer system. Positive tests had >20 micrograms hemoglobin/gram feces (>100 nanogram hemoglobin/milliliter buffer).

Available CRC tissue specimens were shipped to Exact Sciences (Madison, WI), which was blinded to the FIT result; tissue sample adequacy was assessed prior to performing DNA biomarker assays (14). Sequence-specific DNA biomarker targets were isolated directly from dissected formalin-fixed paraffin-embedded CRC tissue blocks using a magnetic bead-based oligonucleotide hybrid capture method. Testing consisted of molecular assays for 7 mutant *K-ras* genes and 10 aberrantly methylated promoter regions in CRC tissue DNA using quantitative, allele-specific, real-time target and signal amplification assays. One portion of the DNA was used to determine the presence of *K-ras* point mutations, a second portion was subjected to the bisulfite reaction for the identification of aberrantly methylated biomarkers *NDRG4* and *BMP3* (panel A), and a third portion was tested for *SFMBT2_895, SFMBT2_896, SFMBT2_897, CHST2_7890, PDGFD, VAV3, DTX1*, and *CHST2_7889* (panel B).

Clinical Use of DNA Biomarkers

The 7 *K-ras* mutations and 2 methylation biomarkers (*NDRG4* and *BMP3*) in panel A are part of a Food and Drug Administration-approved commercially-available multi-target stool DNA test that includes an assay for hemoglobin (7) (Cologuard, Exact Sciences Corporation, Madison, WI). Panel B biomarkers were identified from a prior whole methylome discovery effort (summarized below) and are not part of any current clinically available biomarker panel.

Whole Methylome Biomarker Discovery

DNA was extracted from 54 archived fresh frozen tissue samples from the Mayo Clinic biorepository including 18 tissue samples each of CRC, adenomas >1.0 cm in greatest dimension, and normal colon epithelium. Extracted DNA was subjected to reduced representation bisulfite sequencing (RRBS), a method for targeting gene regulatory regions with elevated CpG content. Sequencing was performed on the Illumina HiSeq 2000 for 101 cycles. Each flow cell lane generated 100–120 million reads, sufficient for a median coverage of 30–50 fold sequencing depth for aligned sequences. Standard Illumina pipeline software called bases and generated reads in the fastq format. Streamlined analysis and annotation pipeline for reduced representation bisulfite sequencing (SAAP-RRBS) was used for sequence read assessment and clean-up, alignment to reference genome, methylation status extraction, and CpG reporting and annotation. Tertiary analysis consisted of removing non-informative CpGs, using a beta binomial model to set coverage cut-offs, and identifying differentially methylated regions (DMRs) with a BiSeq-like algorithm which defines CpG dense clusters from the sequencing reads. The differential methylation was modeled through beta regression on the smoothed methylation level at each CpG. Statistical significance was determined by logistic regression of the methylation percentage per DMR, based on read counts. This resulted in hundreds of potential candidates. Additional filters utilized included area under the receiver operating characteristic curve (AUC) and percentage methylation ratios between cases and controls in the sample (10).

Panel B Biomarker Validation and Selection

Real time quantitative methylation specific PCR assays (QuARTS) were constructed for the subset of biomarker candidates with high discrimination potential (AUC ≥ 0.85 and case/control percentage methylation ratio ≥20). These QuARTS assays were performed on an independent set of fresh frozen tissues, including 36 tissue samples each of CRC, adenoma >1.0 cm, and normal colon epithelium. Results were expressed as the ratio of methylated candidate biomarker strands to beta-actin strands, a reference gene used to normalize the data percentage methylation). Biomarkers were ranked by AUC and methylation ratios. The 8 top candidates were chosen for the panel B biomarker set in this study (10).

Statistical Analysis

The frequency of DNA biomarker expression in CRC tissue, by FIT status (negative or positive), was compared using chi-square tests for the following outcomes: positive for any biomarker; positive for individual biomarker categories (*K-ras*, panel A, and panel B); and positive for individual methylation biomarkers. Using logistic regression models, we also

evaluated the odds of higher expression of DNA biomarkers in FIT-negative cancers versus FIT-positive cancers (referent), adjusting for age (50–54, 55–59, 60–64, 65–69, 70–74, or 75–77 years), sex, race/ethnicity (non-Hispanic white versus other), and cancer stage (localized, regional, or distant). Logistic regression models were also used to evaluate the odds of higher expression of DNA biomarkers in right-sided cancers vs. left-sided cancers (referent), adjusting for age, sex, and race/ethnicity. Finally, we performed exploratory analyses to evaluate biomarker expression by FIT status within subgroups by patient age (50–64 years and 65–77 years), sex, cancer stage (late and early), and cancer location (right and left colon). The study has 80% power to detect a 5% to 13% absolute difference in the frequency of biomarker expression according to a two-sided test with 5% type I error. The 5% difference applies for 95% incidence while the 13% difference applies for 56% incidence. The study is not powered to assess differences in subgroups and subgroup analyses are exploratory only.

RESULTS

Patient Characteristics

Among 674 patients whose CRC tissue specimens were selected for retrieval, 210 FIT-negative and 211 FIT-positive patients ultimately had adequate DNA for all biomarker analyses (Figure 1). Patients averaged 65.9 years of age at CRC diagnosis. Of the 421 patients, 216 (51.3%) were female and 261 (62.0%) were non-Hispanic white (Table 1). A higher percentage of FIT-negative (109 of 210; 51.9%) than FIT-positive patients (83 of 211; 39.3%) had right colon cancers (p=0.01), but cancers were similar regarding stage and grade.

DNA Biomarker Expression

Overall, 414 of 421 CRCs (98.3%) tested positive for at least one of the biomarkers (*K-ras* or aberrant methylation biomarkers), and this did not differ by FIT status (Table 2). The frequencies of *K-ras*, panel A, and panel B biomarkers were also similar for FIT-negative and FIT-positive cancers; 83 of 210 FIT-negative cancers (39.5%) and 74 of 211 FIT-positive cancers (35.1%) had *K-ras* point mutations; 184 of 210 (87.6%) and 181 of 211 (85.8%) expressed any panel A methylation biomarker, respectively; and 206 of 210 (98.1%) and 204 of 211 (96.7%) expressed any methylated DNA marker in panel B, respectively (Table 2 and Figure 2). In addition, the frequency of the individual methylation biomarkers was similar for FIT-negative and FIT-positive cancers (Table 2 and Figure 2), with the exception of a higher expression of *SFMBT2_897* in FIT-negative compared to FIT-positive cancers (194 of 210; 92.4%) than FIT-positive cancers (180 of 211; 85.3%; p=0.02; adjusted odds ratio: 1.93; 95% confidence interval: 1.01–3.69). Biomarkers were consistently expressed more frequently in cancers in the right colon than left colon (Table 3 and Figure 3); the significance of the differences in frequency of expression persisted for most of the panel A and B biomarkers even after adjustment for age, sex, and race/ethnicity (Table 3).

Exploratory Subgroup Analyses

The frequency of biomarker expression in FIT-negative and FIT-positive cancers did not differ within the two age subgroups (Table 4), but did for males and females (Table 5). Men

were more likely to have *K-ras* point mutations in FIT-negative cancers (44 of 96; 45.8%) than FIT-positive cancers (33 of 109; 30.3%; p=0.02). For women, FIT-negative cancers were more likely to express *NDRG4* (103 of 115; 90.4% vs. 77 of 102; 75.5%; p=0.003) and *PDGFD* (99 of 115; 86.8% vs. 78 of 102; 76.5%; p=0.05). Among late-stage cancers, FIT-negative cancers were more likely than FIT-positive cancers to express *SFMBT2_895* (71 of 75; 94.7% vs. 69 of 81; 85.2%; p=0.05) and *SFMBT2_897* (70 of 75; 93.3% vs. 65 of 81; 80.3%; p=0.02), while no differences were observed for early-stage cancers (Table 6). Finally, similar frequencies of biomarker expression were found for FIT-negative and FIT-positive cancers within the right colon and within the left colon (Table 7).

DISCUSSION

We found similar high frequencies of DNA biomarker expression among patients with FIT-negative and FIT-positive CRC, overall and within subgroups by patient age, sex, and cancer stage, and location. This suggests that the expression of these DNA biomarkers is not directly related to the tendency of cancers to bleed, the mechanism by which FIT screening detects occult cancers.

Our findings have important potential clinical implications. Nearly all CRCs expressed at least one biomarker, particularly aberrant methylation biomarkers (98.1% of FIT-negative and 96.7% of FIT-positive CRCs). This suggests that detection of these DNA biomarkers in stool could help improve non-invasive CRC detection and reduce false-negative rates of stool-based screening. Also, the sensitivity of FIT is reportedly lower for right-sided or proximal CRC than for left-sided or distal cancers (15). Since, in our study, randomly selected FIT-negative cancers were more likely to be right-sided, but the likelihood of biomarker positivity was independent of FIT status, the use of such biomarkers may have the greatest proportional impact on the detection of right-sided cancers.

The molecular biology of right-sided CRC may influence the effectiveness of different screening modalities. A greater proportion of right-sided than left-sided cancers are characterized by micro-satellite instability and exhibit a phenotype showing high rates of aberrant DNA methylation (16). Furthermore, this CRC subset appears to arise from sessile serrated polyp precursors rather than classical adenomas (17).

The 7 *K-ras* mutations and 2 methylation biomarkers in panel A are already in clinical use combined with FIT in a multi-target stool DNA test (Cologuard, Exact Sciences Corporation, Madison, WI). The panel B biomarkers are the products of an extensive colorectal neoplasia biomarker discovery program that have been selected as potential candidates for future versions of the multi-target stool DNA test panel (10). Our study results show that the DNA biomarkers evaluated, with few exceptions, are not linked to the presence or absence of fecal hemoglobin at clinically significant levels (>20 micrograms hemoglobin/gram feces, the cutoff for a positive FIT test). This finding offers empiric evidence at the tissue level to explain the increased sensitivity for CRC that stool DNA biomarker testing provides beyond FIT screening alone for the current clinical biomarkers, and shows the potential of the new panel B biomarkers to provide additional sensitivity. While the translation of the panel A biomarkers from tissue to stool for the CRC screening

population has already been demonstrated (7), the ability to detect the panel B biomarkers in stool must be established in future studies.

There are additional limitations to the study that should also be considered. Our analyses were dependent on adequacy of DNA available for study in archival tumor specimens, and several specimens did not contain adequate DNA. This may have biased our sample toward larger tumors or more advanced disease. Our relatively small sample size may have limited our ability to detect statistically significant differences within subgroups, whereas the performance of multiple comparisons increases the possibility of observing statistically significant differences by chance alone. This study did not evaluate precursor lesions, which are known to harbor many of the same methylated DNA anomalies as CRC (10). Also, documentation of the candidate biomarkers to identify pre-malignant lesions (adenomas) and to separate CRCs from age-related methylation in normal colonic tissue from patients without CRC was performed previously in the biomarker discovery process and was not repeated in this study, which was focused entirely on CRC.

In conclusion, DNA biomarkers were expressed in the majority of CRC tissue specimens, with no substantive differences by FIT positive/negative status. The results suggest that non-invasive testing for DNA biomarkers, in conjunction with FIT screening, could potentially increase the detection of CRCs, particularly for right-sided CRCs which are less frequently detected by FIT alone. The presence of biomarkers in tissue is a requirement, but by itself insufficient to establish the clinical use of a biomarker in a stool-based assay; thus, confirmation of their ability to be detected reliably in stool or body fluid at clinically acceptable specificity is required. Given that the use of multiple biomarkers may increase costs and the number of false positive test results, the cost-effectiveness of varying combinations of FIT and DNA biomarker screening will need to be evaluated.

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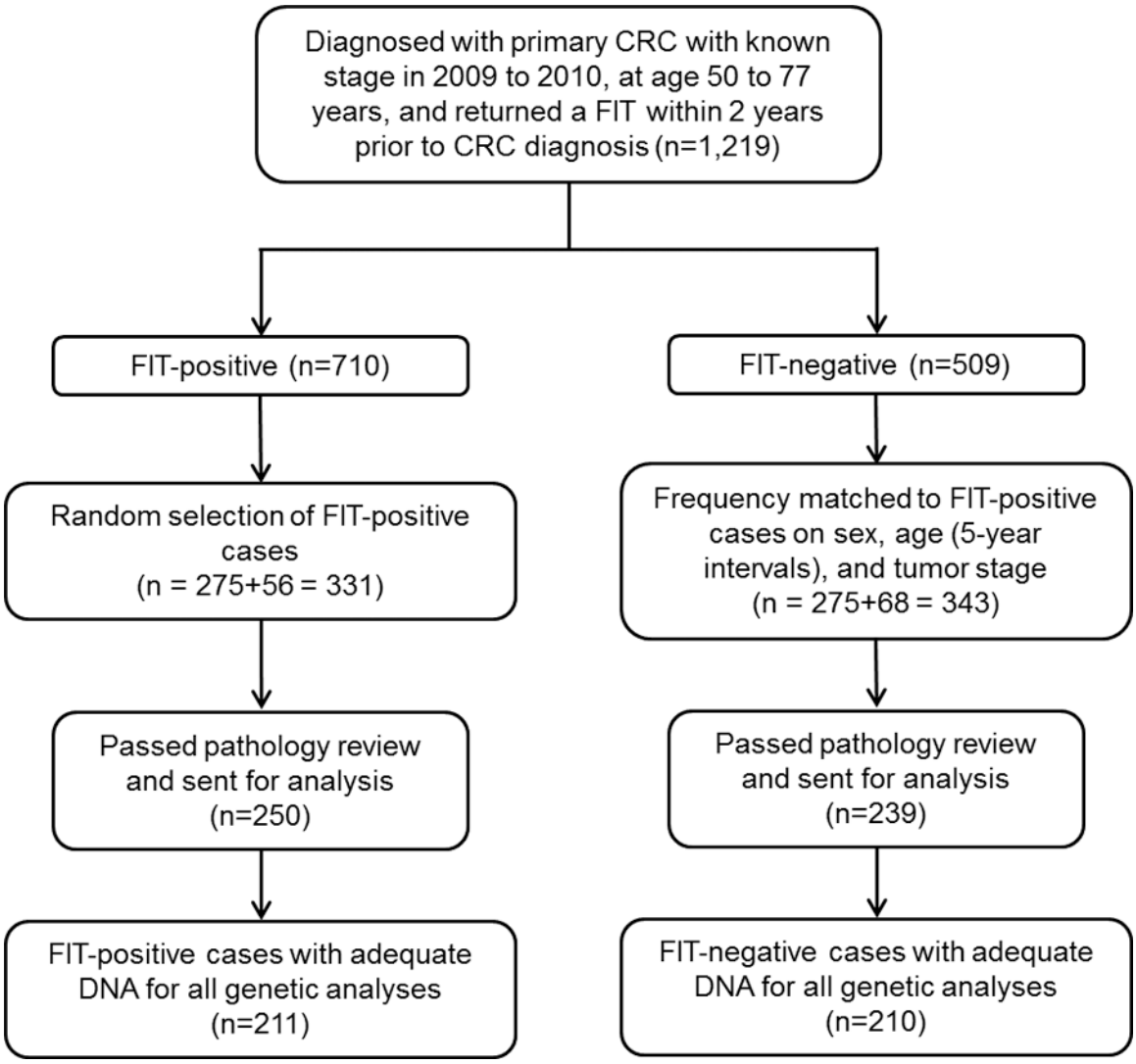

Figure 1.
Flow diagram of study population

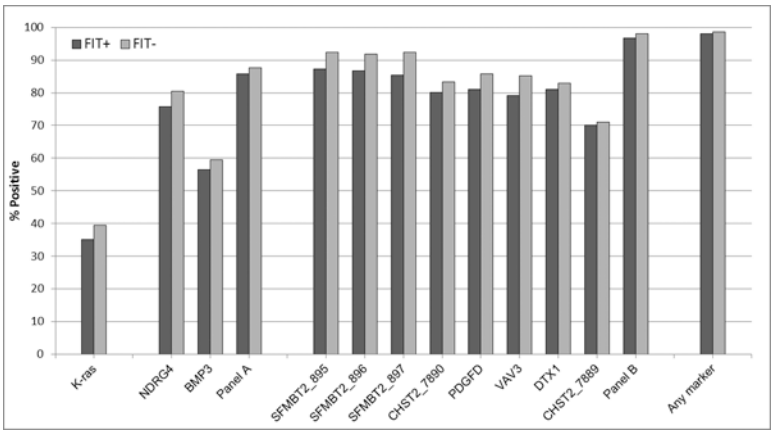

Figure 2.

Expression of DNA biomarkers in CRC tissue by FIT status.

***** $p<0.05$

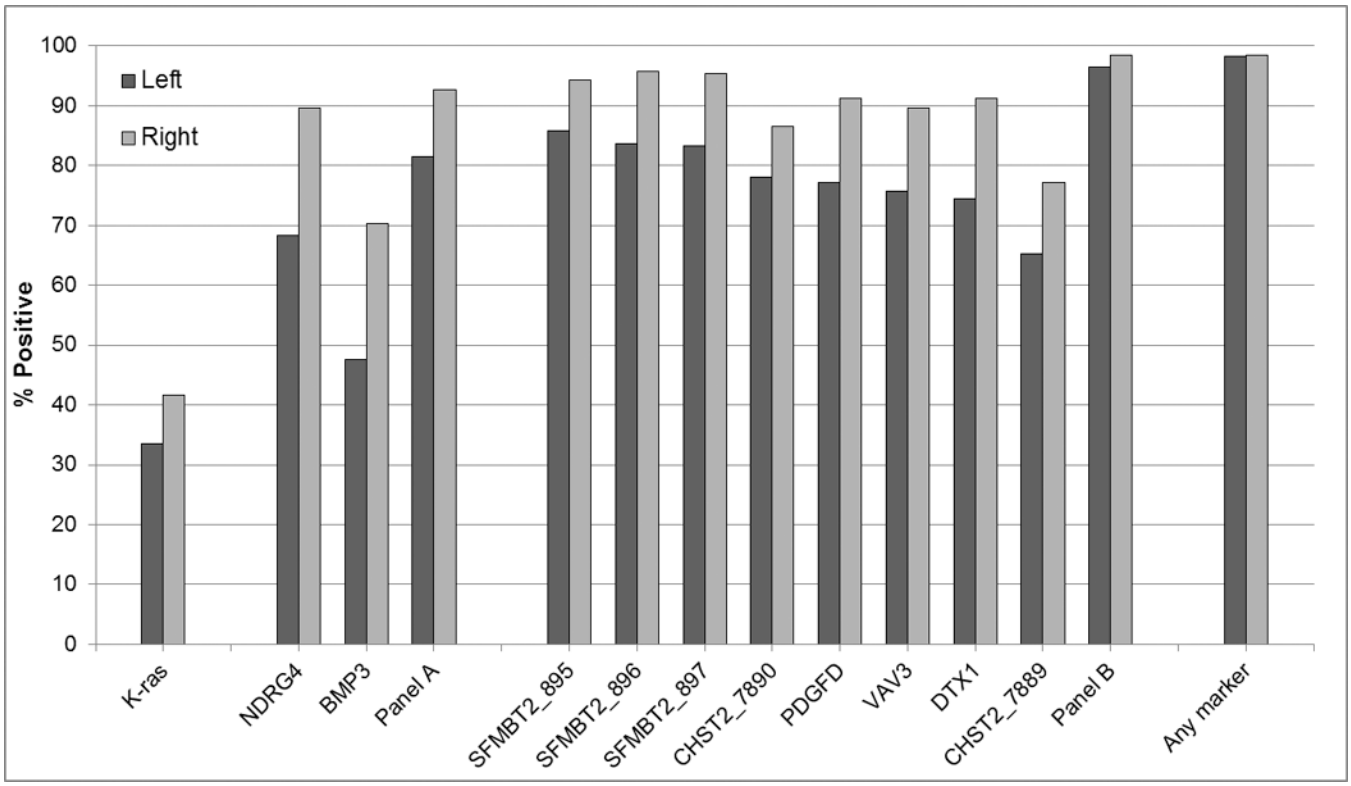

Figure 3:
Expression of DNA biomarkers in CRC tissue by CRC location.
***** $p<0.05$

Table 1:

Patient characteristics overall and by FIT status

Patient Characteristics	All CRCs n=421 n (%)	FIT+ CRCs, n=211 n (%)	FIT- CRCs, n=210 n (%)
Age at CRC Diagnosis, y			
50–54	35(8.3)	21(10.0)	14(6.7)
55–59	52(12.4)	30(14.2)	22(10.5)
60–64	91(21.6)	42(19.9)	49(23.3)
65–69	82(19.5)	38(18.0)	44(21.0)
70–74	103(24.5)	53(25.1)	50(23.8)
75–77	58(13.8)	27(12.8)	31(14.8)
Mean (SD)	65.9(7.4)	65.4(7.6)	66.4(7.1)
Female	216(51.3)	102(48.3)	114(54.3)
Race/Ethnicity			
Non-Hispanic White	261(62.0)	121(57.4)	140(66.7)
Hispanic	43(10.2)	25(11.9)	18(8.6)
African American	35(8.3)	18(8.5)	17(8.1)
Asian/Pacific Islanders	56(13.3)	31(14.7)	25(11.9)
Other	26(6.2)	16(7.6)	10(4.8)
CRC Location in Colon			
Left-sided (distal)	227(53.9)	128(60.7)	99(47.1)
Right-sided (proximal)	192(45.6)	83(39.3)	109(51.9)
Unspecified	2(0.5)	0(0.0)	2(1.0)
CRC Stage (Cancer Registry)			
Localized	265(63.0)	130(61.6)	135(64.3)
Regional	114(27.1)	61(28.9)	53(25.2)
Distant	42(10.0)	20(9.5)	22(10.5)
Late-Stage CRC (≥ Stage IIB)	156(37.1)	81(38.4)	75(35.7)
CRC Grade			
Grade I – Well differentiated	34(8.1)	17(8.1)	17(8.1)
Grade II – Moderately differentiated	311(73.9)	161(76.3)	150(71.4)
Grade III – Poorly differentiated	35(8.3)	14(6.6)	21(10.0)
Grade IV – Undifferentiated	9(2.1)	4(1.9)	5(2.4)
Not determined	32(7.6)	15(7.1)	17(8.1)

CRC=colorectal cancer; FIT=fecal immunochemical test; n=number; SD=standard deviation

Table 2:

Expression of DNA biomarkers in CRC tissue by FIT status

DNA Biomarkers	Total CRCs n=421 n (%)	FIT+ CRCs, n=211 n (%)	FIT- CRCs, n=210 n (%)	p-value	Adjusted OR† (95% CI)
K-ras*	157 (37.3)	74 (35.1)	83 (39.5)	0.35	1.24 (0.83, 1.86)
Panel A*					
NDRG4	329 (78.2)	160 (75.8)	169 (80.5)	0.25	1.25 (0.77, 2.03)
BMP3	244 (58.0)	119 (56.4)	125 (59.5)	0.52	1.08 (0.72, 1.61)
Any from panel A	365 (86.7)	181 (85.8)	184 (87.6)	0.58	1.08 (0.60, 1.92)
Panel B					
SFMBT2_895	378 (89.8)	184 (87.2)	194 (92.4)	0.08	1.66 (0.86, 3.22)
SFMBT2_896	376 (89.3)	183 (86.7)	193 (91.9)	0.09	1.71 (0.90, 3.27)
SFMBT2_897	374 (88.8)	180 (85.3)	194 (92.4)	0.02	1.93 (1.01, 3.69)
CHST2_7890	344 (81.7)	169 (80.1)	175 (83.3)	0.39	1.23 (0.74, 2.04)
PDGFD	351 (83.4)	171 (81.0)	180 (85.7)	0.20	1.36 (0.80, 2.32)
VAV3	346 (82.2)	167 (79.2)	179 (85.2)	0.10	1.48 (0.88, 2.50)
DTX1	345 (82.0)	171 (81.0)	174 (82.9)	0.63	1.10 (0.66, 1.83)
CHST2_7889	297 (70.6)	148 (70.1)	149 (71.0)	0.86	0.99 (0.65, 1.53)
Any from panel B	410 (97.4)	204 (96.7)	206 (98.1)	0.36	1.85 (0.53, 6.53)
Any Biomarker	414 (98.3)	207 (98.1)	207 (98.6)	0.71	1.28 (0.28, 5.94)

CI=confidence interval; CRC=colorectal cancer; FIT=fecal immunochemical test; n=number; FIT+=FIT positive; FIT-=FIT negative; OR=odds ratio

Note: Used 100% assay specificity cutoff.

*_K-ras_ and panel A biomarkers are included in an available multitarget stool DNA test (7).

†Odds of higher DNA biomarker expression in FIT-negative vs. FIT-positive cancers (referent), adjusted for sex, age, race/ethnicity, and cancer stage.

Table 3:

Expression of DNA biomarkers by CRC location

DNA Biomarkers	CRC Location			
	Left Colon n=227 n (%)	Right Colon n=192 n (%)	p-value	Adjusted OR† (95% CI)
K-ras*	76 (33.5)	80 (41.7)	0.08	1.42 (0.93, 2.15)
Panel A*				
NDRG4	155 (68.3)	172 (89.6)	<0.001	3.52 (2.00, 6.18)
BMP3	108 (47.6)	135 (70.3)	<0.001	2.28 (1.49, 3.48)
Any from panel A	185 (81.5)	178 (92.7)	0.001	2.43 (1.25, 4.71)
Panel B				
SFMBT2_895	195 (85.9)	181 (94.3)	0.005	2.54 (1.21, 5.31)
SFMBT2_896	190 (83.7)	184 (95.8)	<0.001	4.54 (2.02, 10.23)
SFMBT2_897	189 (83.3)	183 (95.3)	<0.001	3.72 (1.71, 8.11)
CHST2_7890	177 (78.0)	166 (86.5)	0.03	1.67 (0.97, 2.87)
PDGFD	175 (77.1)	175 (91.2)	<0.001	3.01 (1.64, 5.52)
VAV3	172 (75.8)	172 (89.6)	<0.001	2.52 (1.41, 4.48)
DTX1	169 (74.5)	175 (91.2)	<0.001	3.50 (1.92, 6.39)
CHST2_7889	148 (65.2)	148 (77.1)	0.008	1.74 (1.11, 2.74)
Any from panel B	219 (96.5)	189 (98.4)	0.21	2.89 (0.74, 11.31)
Any Biomarker	223 (98.2)	189 (98.4)	0.87	1.19 (0.25, 5.58)

CI=confidence interval; CRC=colorectal cancer; FIT=fecal immunochemical test; n=number; OR=odds ratio

Note: Used 100% assay specificity cutoff.

**K-ras* and panel A biomarkers are included in an available multitarget stool DNA test (7).

†Odds of higher DNA biomarker expression in right-sided vs. left-sided cancers (referent), adjusted for sex, age, and race/ethnicity.

Table 4:

Expression of DNA biomarkers in CRC tissue by FIT status in age subgroups

DNA Biomarkers	50–65 years			66–75 years		
	FIT+ CRCs n=108 n (%)	FIT- CRCs n=102 n (%)	p-value	FIT+ CRCs n=103 n (%)	FIT- CRCs n=108 n (%)	p-value
K-ras*	34(31.5)	40(39.2)	0.24	40(39.2)	43(39.8)	0.88
Panel A*						
NDRG4	77(71.3)	75(73.5)	0.72	83(80.6)	94(87.0)	0.20
BMP3	57(52.8)	52(51.0)	0.79	62(60.2)	73(67.6)	0.26
Any from panel A	89(82.4)	85(83.3)	0.86	92(89.3)	99(91.7)	0.56
Panel B						
SFMBT2_895	92(85.2)	93(91.2)	0.18	92(89.3)	101(93.5)	0.28
SFMBT2_896	92(85.2)	95(93.1)	0.07	91(88.4)	98(90.7)	0.57
SFMBT2_897	90(83.3)	92(90.2)	0.14	90(87.4)	102(94.4)	0.07
CHST2_7890	83(76.9)	82(80.4)	0.53	86(83.5)	93(86.1)	0.60
PDGFD	86(79.6)	86(84.3)	0.38	85(82.5)	94(87.0)	0.36
VAV3	83(76.9)	84(82.4)	0.32	84(81.6)	95(88.0)	0.19
DTX1	86(79.6)	85(83.3)	0.49	85(82.5)	89(82.4)	0.98
CHST2_7889	71(65.7)	74(72.6)	0.29	77(74.8)	75(69.4)	0.39
Any from panel B	107(99.1)	100(98.0)	0.53	97(94.2)	106(98.2)	0.13
Any Biomarker	107(99.1)	100(98.0)	0.53	100(97.1)	107(99.1)	0.29

CRC=colorectal cancer; FIT=fecal immunochemical test; n=number; FIT+=FIT positive; FIT-=FIT negative

**K-ras* and panel A biomarkers are included in an available multitarget stool DNA test (7).

Note: Used 100% assay specificity cutoff.

Table 5:

Expression of DNA biomarkers in CRC tissue by FIT status in males and females

DNA Biomarkers	Male			Female		
	FIT+ CRCs n=109 n (%)	FIT- CRCs n=96 n (%)	p-value	FIT+ CRCs n=102 n (%)	FIT- CRCs n=115 n (%)	p-value
K-ras*	33(30.3)	44(45.8)	0.02	41(40.2)	39(34.2)	0.36
Panel A*						
NDRG4	83(76.2)	66(68.8)	0.24	77(75.5)	103(90.4)	0.003
BMP3	57(52.3)	53(55.2)	0.68	62(60.8)	72(63.2)	0.72
Any from panel A	94(86.2)	78(81.3)	0.33	87(85.3)	106(93.0)	0.07
Panel B						
SFMBT2_895	95(87.2)	87(90.6)	0.43	89(87.3)	107(93.9)	0.10
SFMBT2_896	94(86.2)	87(90.6)	0.33	89(87.3)	106(93.0)	0.16
SFMBT2_897	92(84.4)	87(90.6)	0.18	88(86.3)	107(93.9)	0.06
CHST2_7890	86(78.9)	79(82.3)	0.54	83(81.4)	96(84.2)	0.58
PDGFD	93(85.3)	81(84.4)	0.85	78(76.5)	99(86.8)	0.05
VAV3	82(75.2)	76(79.2)	0.50	85(83.3)	103(90.4)	0.13
DTX1	84(77.1)	74(77.1)	1.00	87(85.3)	100(87.7)	0.60
CHST2_7889	74(67.9)	66(68.8)	0.90	74(72.6)	83(72.8)	0.97
Any from panel B	105(96.3)	93(96.9)	0.83	99(97.1)	113(99.1)	0.26
Any Biomarker	106(97.3)	94(97.9)	0.76	101(99.0)	113(99.1)	0.94

CRC=colorectal cancer; FIT=fecal immunochemical test; n=number; FIT+=FIT positive; FIT-=FIT negative

**K-ras* and panel A biomarkers are included in an available multitarget stool DNA test (7).

Note: Used 100% assay specificity cutoff.

Table 6:

Expression of DNA biomarkers in CRC tissue by FIT status in CRC stage subgroups

DNA Biomarkers	Late-Stage CRC			Early-Stage CRC		
	FIT+ CRCs n=81 n (%)	FIT- CRCs n=75 n (%)	p-value	FIT+ CRCs n=130 n (%)	FIT- CRCs n=135 n (%)	p-value
K-ras*	27(3.3)	28(37.3)	0.60	47(36.2)	55(40.7)	0.44
Panel A*						
NDRG4	56(69.1)	60(80.0)	0.12	104(80.0)	109(80.7)	0.88
BMP3	45(55.6)	45(60.0)	0.58	74(56.9)	80(59.3)	0.70
Any from panel A	64(79.0)	66(88.0)	0.13	117(90.0)	118(87.4)	0.51
Panel B						
SFMBT2_895	69(85.2)	71(94.7)	0.05	115(88.5)	123(91.1)	0.48
SFMBT2_896	69(85.2)	66(88.0)	0.61	114(87.7)	127(94.1)	0.07
SFMBT2_897	65(80.3)	70(93.3)	0.02	115(88.5)	124(91.9)	0.35
CHST2_7890	59(72.8)	64(85.3)	0.06	110(84.6)	111(82.2)	0.60
PDGFD	61(75.3)	59(78.7)	0.62	110(84.6)	121(89.6)	0.22
VAV3	61(75.3)	63(84.0)	0.18	106(81.5)	116(85.9)	0.33
DTX1	62(76.5)	64(85.3)	0.16	109(83.9)	110(81.5)	0.61
CHST2_7889	54(66.7)	52(69.3)	0.72	94(72.3)	97(71.9)	0.93
Any from panel B	77(95.1)	73(97.3)	0.46	127(97.7)	133(98.5)	0.62
Any Biomarker	79(97.5)	73(97.3)	0.94	128(98.5)	134(99.3)	0.54

CRC=colorectal cancer; FIT=fecal immunochemical test; n=number; FIT+=FIT positive; FIT-=FIT negative

**K-ras* and panel A biomarkers are included in an available multitarget stool DNA test (7).

Note: Used 100% assay specificity cutoff.

Table 7:

Expression of DNA biomarkers in CRC tissue by FIT status within the left and right colon

DNA Biomarkers	Left Colon			Right Colon		
	FIT+ CRCs n=128 n (%)	FIT- CRCs n=99 n (%)	p-value	FIT+ CRCs n=83 n (%)	FIT- CRCs n=109 n (%)	p-value
K-ras*	38(29.7)	38(38.4)	0.17	36(43.4)	44(40.4)	0.68
Panel A*						
NDRG4	87(68.0)	68(68.7)	0.91	73(88.0)	99(90.8)	0.52
BMP3	59(46.1)	49(49.5)	0.61	60(72.3)	75(68.8)	0.60
Any from panel A	104(81.3)	81(81.8)	0.91	77(92.8)	101(92.7)	0.98
Panel B						
SFMBT2_895	106(82.8)	89(89.9)	0.13	78(94.0)	103(94.5)	0.88
SFMBT2_896	105(82.0)	85(85.9)	0.44	78(94.0)	106(97.3)	0.26
SFMBT2_897	103(80.5)	86(86.9)	0.20	77(92.8)	106(97.3)	0.15
CHST2_7890	97(75.8)	80(80.8)	0.37	72(86.8)	94(86.2)	0.92
PDGFD	94(73.4)	81(81.8)	0.14	77(92.8)	98(89.9)	0.49
VAV3	94(73.4)	78(78.8)	0.35	73(88.0)	99(90.8)	0.52
DTX1	93(72.7)	76(76.8)	0.48	78(94.0)	97(89.0)	0.23
CHST2_7889	83(64.8)	65(65.7)	0.90	65(78.3)	83(76.2)	0.72
Any from panel B	123(96.1)	96(97.0)	0.72	81(97.6)	108	0.41
Any Biomarker	126(98.4)	97(98.0)	0.80	81(97.6)	108(99.1)	0.41

CRC=colorectal cancer; FIT=fecal immunochemical test; n=number; FIT+=FIT positive; FIT-=FIT negative

**K-ras* and panel A biomarkers are included in an available multitarget stool DNA test (7).

Note: Used 100% assay specificity cutoff.