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Timothy Shu Date

Associations of Dietary, Lifestyle, and Other Participant Characteristics with APC, β-catenin, E-cadherin, and MSH2 Expression in the Normal Mucosa of Sporadic Colorectal Adenoma Patients

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Abstract

Associations of Dietary, Lifestyle, and Other Participant Characteristics with APC, β-catenin, E-cadherin, and MSH2 Expression in the Normal Mucosa of Sporadic Colorectal Adenoma Patients

By Timothy Shu

Abnormal expression of the Wnt pathway proteins APC, β-catenin, and E-cadherin, as well as of the DNA mismatch repair protein MSH2 is common during colorectal carcinogenesis. To investigate associations of selected demographic, lifestyle, dietary, and medical characteristics with the expression of these proteins in the normal-appearing colorectal mucosa of sporadic colorectal adenoma patients, we measured APC, β-catenin, E-cadherin, and MSH2 colorectal crypt expression in biopsies of the normal-appearing colorectal mucosa from 104 participants using automated immunohistochemistry and quantitative image analysis. We used multivariable general linear models to compare mean biomarker expression across categories of participant characteristics. For those with the highest total meat consumption versus the lowest, the adjusted mean ratio of APC expression to β-catenin expression (APC/β-catenin) was 33% lower (p=0.03) in the whole crypt. For those with the highest vegetable and fruit consumption versus the lowest, mean E-cadherin expression was 29% higher (p=0.02) in the whole crypt. For those with the highest serum 25-OH vitamin D concentrations versus the lowest, the ratio of MSH2 expression to mib-1 expression (MSH2/mib-1 score) was 29% higher (p=0.03) in the whole crypt. These findings support that (i) lower total meat consumption, higher vegetable and fruit consumption, and higher vitamin D exposure may be favorably associated with the expression of biomarkers of risk for colorectal neoplasms in the normal-appearing colorectal mucosa, and (ii) further investigation into associations of demographic, lifestyle, dietary, and medical characteristics with biomarkers of risk for colorectal neoplasms is warranted.

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Introduction

While colorectal cancer (CRC) mortality and incidence in the US has steadily decreased over the last 30 years, CRC is still the 2nd leading cause of cancer-related death in the US, with 14.8 deaths per 100,000 in 2014 (1-2). Targeting this cancer as a public health concern requires an understanding of the causal components at play. A large proportion of CRC incidence can be attributed to a combination of environmental exposures, such as smoking, alcohol consumption, and a sedentary lifestyle (3). At the molecular level, these risk factors often lead to alterations in specific pathways tied to cellular functions, such as transcription or DNA repair. For instance, the adenomatous polyposis coli (APC) protein drives ubiquitination to regulate transcriptional pathways and is defective in many cases of CRC (4). Another well-documented molecular cause of disease stems from mutations or epigenetic silencing of DNA mismatch repair genes, such as MSH2 (5). Because of the associations between the aforementioned pathway defects and CRC, these molecules have potential as easily measurable endpoints for colorectal cancer-related outcomes.

There is strong biological plausibility and observational evidence for the association of environmental risk factors with the aforementioned biomarkers. Calcium and vitamin D intake was found to modulate the APC pathway and the expression of pathway-specific proteins: APC, β-catenin, and E-cadherin (6-7). Calcium and vitamin D was also associated with differential expression of the mismatch repair protein MSH2 (8). While the associations of these specific dietary factors with biomarkers of risk were previously studied, it is unknown whether other modifiable environmental factors are also associated with these biomarkers.

A goal of our research group is to identify and utilize modifiable, pre-neoplastic biomarkers of risk for colorectal neoplasms. The adenomatous polyp, a benign neoplasm that is a precursor of CRC, is currently the only accepted biomarker of risk for CRC, so understanding other biomarkers could improve CRC risk assessment and management (9). Our goal is to investigate APC, β-catenin, E-cadherin, and MSH2 as pre-neoplastic biomarkers to: (i) identify demographic, lifestyle, dietary, and medical exposures that are associated with differences in biomarker expression; (ii) corroborate the validity of these biomarkers as screening endpoints for the potential efficacy and optimal doses of preventive interventions against colorectal carcinogenesis; and (iii) further understand relevant mechanisms of CRC etiology in humans.

Previous studies tested the effects of vitamin D and calcium on the expression of APC, β-catenin, E-cadherin, and MSH2 in the colorectal mucosa, but our aim was to observationally assess associations of a wide selection of participant characteristics with these biomarkers (10-11). Based on the aforementioned biological plausibility and the previous biomarker trials, we hypothesized that pro-colorectal carcinogenic risk factors would be associated with lower APC expression, higher β-catenin expression, lower E-cadherin expression, and lower MSH2 expression, while anti-carcinogenic risk factors would be associated with higher APC expression, lower β-catenin expression, higher E-cadherin expression, and higher MSH2 expression in the normal-appearing colorectal mucosa of sporadic colorectal adenoma patients.

Materials and Methods

Participant Population

Study participants were recruited from two clinical centers as part of a larger randomized, placebo-controlled, chemoprevention clinical trial testing the efficacy of supplemental calcium and vitamin D for preventing colorectal adenoma recurrence. Eligible participants were 45-75 years old, in general good health, and within 4 months of study entry had a colonoscopy resulting in a histologically identified neoplastic polyp ≥2mm in diameter. Exclusion criteria included an invasive carcinoma in any polyp removed, familial colonic polyposis syndromes, inflammatory bowel diseases, malabsorption syndromes, history of large bowel resection, alcohol or narcotic dependence, serum calcium outside the normal range, creatinine greater than 20% over the upper limit of normal, serum 25-hydroxy vitamin D <12ng/mL or >90ng/mL, history of kidney stones or hyperparathyroidism, and history of osteoporosis or other medical condition that could require supplemental vitamin D or calcium. Additional exclusion criteria for the adjunct study included being unable to be off aspirin for 7 days, history of a bleeding disorder, or current use of an anticoagulant medication.

Clinical Trial Protocol

For the parent study, between May 2004 and July 2008, 19,083 apparently eligible patients were identified through initial screening of colonoscopy and pathology reports. Of these, 2,259 met the final eligibility criteria, consented to participate, and were randomized. After the initial parent study was underway, funding was received for the adjunct biomarker study. For the adjunct study, near the end of the placebo run-in period, without knowledge of treatment assignment, 231 apparently eligible parent study participants at two clinical centers (South Carolina and Georgia) were offered participation in the biomarker study. Of these, 109 met the final eligibility criteria, signed consent, and had baseline rectal biopsies taken; of these, sufficient biopsy tissue for biomarker measurements was obtained at baseline and one year follow up on 104. All participants signed a consent form upon enrollment and the Institutional Review Boards at each center approved the research.

At enrollment, the coordinator collected information from each study participant on medical history, medication and nutritional supplement use, diet, and lifestyle. Diet was assessed using a semi-quantitative Block Brief 2000 food frequency questionnaire (NutritionQuest, Berkeley, CA). Blood to measure calcium, creatinine, 25(OH)D, and 1,25(OH)$_2$D concentrations was also obtained at baseline.

Biopsy Collection

Participants underwent biopsies of normal-appearing rectal mucosa without any preceding bowel cleansing procedure. Six 1mm thick biopsies of were collected from the rectal mucosa 10cm above the external anal aperture through a proctoscope using jumbo cup biopsy forceps. Biopsies were immediately placed into saline, oriented, transferred to 10% normal-buffered formalin for 24 hours, then transferred to 70% ethanol for up to a week, and then embedded in paraffin blocks. (two blocks of three biopsies per participant, per visit). APC, β-catenin, E-cadherin, and MSH2 (plus mib-1 [Ki-67 epitope]) were measured in the biopsies using automated immunohistochemistry with image analysis. (Mib-1 was included for the present analyses as a marker of proliferation, strictly to assess MSH2 expression relative to proliferation.)

Immunohistochemistry Protocol

Five slides with three levels of 3μm-thick biopsy sections taken 40μm apart were prepared for each biomarker. Heat-mediated antigen retrieval was then used to uncover the epitope. Slides were placed into a preheated Pretreatment Module (Lab Vision Corp., Fremont, CA) with 100x citrate buffer pH 6.0 (DAKO S1699, DAKO Corp., Carpinteria, CA) and steamed for 40 minutes. The slides were then put into a DakoCytomation Autostainer Plus system that immunohistochemically processed the slides using a streptavidin-biotin method (LSAB2 Detection System[DAKO K0675]) and a monoclonal antibody to each biomarker (for APC, Oncogene OP80 at a concentration of 1:50; for β-catenin, BD Pharmingen [formerly Transduction Laboratories 610154] at a concentration of 1:300; for E-cadherin, Zymed 33-4000 at a concentration of 1:50; for MSH2, Calbiochem NA27 at a concentration of 1:500; and for mib-1, DAKO M7240 at a concentration of 1:350). The slides were not counterstained and were coverslipped with a Leica CV5000 Coverslipper (Leica Microsystems, Inc., Buffalo Grove, IL). Positive and negative control slides were included in each staining batch.

Quantifying Labeling Densities of Biomarkers in Normal Colon Crypts

Immunohistochemically-detected levels of the biomarkers in colon crypts were measured using quantitative image analysis. First, slides were scanned using the Aperio Scanscope CS digital scanner (Aperio Technologies, Inc., Vista, CA). Next, these electronic images were reviewed with the CellularEyes program (DivEyes LLC, Atlanta, GA) to identify colon crypts that would be acceptable for analysis. A "scorable" crypt was defined as an intact crypt that extended from the muscularis mucosa to the colon lumen. Before analysis, images of the negative and positive control slides were checked to ensure staining adequacy. Standardized settings were used for all equipment throughout the scoring process. The technician, who was blinded to treatment assignment, selected two of three biopsies with 16-20 "scorable" hemicrypts (one half of the crypt) per biopsy and used a digital drawing board to trace the border of each hemicrypt. The program then divided the hemicrypt outline into equally spaced segments (with the average widths of normal colonocytes) and measured the background-corrected optical density of the labeled biomarker across the entire hemicrypt and for each individual segment. Resulting data were automatically transferred into the MySQL database (Sun Microsystems Inc., Redwood Shores, CA). These analysis steps were then repeated for the next identified hemicrypt. A reliability control sample that had been previously analyzed by the reader was re-analyzed over the course of the trial to determine intra-reader "scoring" reliability. The intra-class correlation coefficient for each biomarker was >0.90.

Statistical Analysis

All analyses were cross-sectional, using baseline data only. We summarized participant characteristics using simple descriptive statistics, such as means, ranges, and standard deviations for continuous variables, and proportions as percentages for categorical variables.

To assess associations of the biomarkers with selected participant characteristics, we used multivariable general linear models to compare adjusted mean biomarker expression across categories (e.g., tertiles of dietary intakes) of the participant characteristics. These models contained the intercept, the characteristic of interest, staining batch, and potential confounders. The criteria for including a baseline characteristic as a potential confounder was whether its inclusion/exclusion led to a change of ≥10% in the estimated difference in mean biomarker expression between the lowest and highest categories of the characteristic of interest. The covariates included in the final model for each characteristic are reported in Supplementary Table S1. We analyzed biomarker expression in the whole crypt and in crypt functional zones, including the upper 40% of crypts (the differentiation zone), the lower 60% of crypts (the proliferation zone), and the ratio of upper 40% to the whole crypt (the ϕh of crypts, the distribution index).. Also, to assess anti-proliferative APC relative to pro-proliferative β-catenin expression, we calculated an APC/β-catenin ratio. To assess MSH2 expression relative to proliferation, we calculated an MSH2/mib-1 ratio.

All statistical analyses were conducted using SAS software Version 9.4 (SAS Institute, Cary, NC). Two-sided p-values ≤0.05 were considered statistically significant. For this pilot study, we also noted participant characteristics for which estimated adjusted mean biomarker proportional differences between the lowest and highest participant characteristic category was ≥20% and/or had a p-value ≤0.20, plus there was a roughly dose-response pattern to the means across the categories (if > 2 categories).

Results

Participant Characteristics

Selected characteristics of the study participants are summarized in Table 1. The mean age of participants was 59 years (range from 47–75 years), 46% of participants were male, and 79% were white. 97% of the participants had at least a high school education. Also, 8% were current smokers and 38% characterized their physical activity level as "high." Participant's BMI ranged from 21.0–54.1 kg/m^2 (mean 29.6 kg/m^2) and their serum 25-OH-vitamin D concentrations ranged from 12.9–68.8 ng/mL (mean 24.1 ng/mL).

Biomarker Expression by Characteristic

Adjusted mean expression of APC, β-Catenin, E-cadherin, and MSH2 in the whole crypt, the upper 40% of the crypt, and the lower 60% of the crypt, by level of selected participant characteristics is presented in Tables 2–7. In Table 2, we summarize the strongest findings for all of the biomarkers. The criteria for inclusion in this table were: estimated proportional mean differences in biomarker expression between the highest and lowest categories of the exposure variable ≥20% and/or a p-value of <0.20, plus at least an approximate dose-response pattern. More comprehensive findings and exact values are provided in Tables 3–6. The findings for minimally-adjusted mean biomarker expression and the ϕh of crypts are presented in Supplementary Tables S2–S7.

APC

The mean adjusted APC expression among participants in the highest relative to those in the lowest tertile of total meat consumption was estimated to be 18.8% lower (p=0.12) in whole crypts and 22.7% lower (p=0.14) in the lower 60% of crypts. Similarly the mean adjusted APC expression among participants in the highest relative to those in the lowest tertile of saturated fat consumption was estimated to be 14.2% lower (p=0.18) in whole crypts and 18.8% lower (p=0.19) in the lower 60% of crypts. The mean adjusted APC expression among participants in the highest relative to those in the lowest tertile of total fat consumption was estimated to be 17.8% lower (p=0.19) in the lower 60% of crypts. The mean adjusted APC expression among participants in the highest relative to those in the lowest tertile of vegetable and fruit consumption was also estimated to be 22.0% lower (p=0.20) in the lower 60% of crypts, although the direction of this association was opposite to that hypothesized. The estimated associations of the following participant characteristics with APC did not meet our criteria outlined in the statistical analysis section for inclusion in Table 2: age, aspirin use, other NSAID use, smoking, physical activity, BMI, serum 25-OH vitamin D concentrations, total vitamin E intake, total calcium intake, and dietary fiber intake.

β-Catenin

The mean adjusted β-catenin expression among participants in the highest relative to those in the lowest tertile of total meat consumption was estimated to be 27.2% higher (p=0.06) in the whole crypts. The mean adjusted β-catenin expression among participants who were current smokers relative to those who were not was estimated to be 20.3% higher (p=0.39) in the upper 40% of crypts, and the mean adjusted β-catenin expression among participants who regularly took aspirin relative to those who did not was estimated to be 11.3% lower (p=0.06) in the lower 60% of crypts. The mean adjusted β-catenin expression among participants in the highest relative to those in the lowest tertile of saturated fat consumption was estimated to be 20.4% lower (p=0.04) in the whole crypts. Similarly, the mean adjusted β-catenin expression among participants in the highest relative to those in the lowest tertile of total fat consumption was estimated to be 14.2% lower (p=0.08) in the whole crypts. The mean adjusted β-catenin expression among participants in the highest relative to the lowest tertile of total calcium intake was estimated to be 12.0% higher (p=0.17) in the whole crypts. Also, the mean adjusted β-catenin expression among participants in the highest relative to the lowest tertile of serum 25-OH vitamin D was estimated to be 23.6% higher (p=0.09) in the lower 60% of crypts. The estimated associations of β-catenin expression with saturated fat consumption, total fat consumption, total calcium intake, and serum 25-OH vitamin D were in directions opposite to those hypothesized. The estimated associations of the following participant characteristics with β-catenin expression did not meet our criteria outlined in the statistical analysis section for inclusion in Table 2: age, other NSAID use, physical activity, BMI, total vitamin E intake, dietary fiber intake, and vegetable and fruit consumption.

Ratio of APC to β-Catenin

The ratio of mean adjusted APC expression to β-catenin expression among participants in the highest relative to the lowest tertile of total meat consumption was estimated to be 32.8% lower (p=0.03) in whole crypts, 35.2% lower (p=0.03) in the upper 40% of crypts, and 30.7% lower (p=0.08) in the lower 60% of crypts. The ratio of mean adjusted APC expression to β-catenin expression among participants in the highest relative to the lowest tertile of serum 25-OH vitamin D was estimated to be 25.0% higher (p=0.24) in the whole crypts and 30.8% higher (p=0.19) in the upper 40% of crypts. The ratio of mean adjusted APC expression to β-catenin expression among participants who were current smokers relative to those who were not was estimated to be 33.7% lower (p=0.18) in the whole crypts, 36.7% lower (p=0.20) in the upper 40% of crypts, and 29.2% lower (p=0.34) in the lower 60% of crypts. The ratio of mean adjusted APC expression to β-catenin expression among participants in the highest relative to the lowest tertile of dietary fiber intake was estimated to be 34.3% higher (p=0.31) in the whole crypts and 54.6% higher (p=0.07) in the upper 40% of crypts. Also, the ratio of mean adjusted APC expression to β-catenin expression among participants in the highest relative to the lowest tertile of total calcium intake was estimated to be 21.1% lower (p=0.25) in the upper 40% of crypts, although the direction of this association was opposite to those hypothesized. The estimated associations of the following participant characteristics with the ratio of APC expression to β-catenin expression did not meet our criteria outlined in the statistical analysis section for inclusion in Table 2:

age, aspirin use, other NSAID use, physical activity, BMI, saturated fat consumption, total fat consumption, total vitamin E intake, and vegetable and fruit consumption.

E-Cadherin

The mean adjusted E-cadherin expression among participants in the highest relative to those in the lowest tertile of vegetable and fruit consumption was estimated to be 28.5% higher (p=0.02) in the whole crypts and 31.4% higher (p=0.16) in the lower 60% of crypts. Similarly, the mean adjusted E-cadherin expression among participants in the highest relative those in the lowest tertile of total calcium intake was estimated to be 19.0% higher (p=0.10) in the whole crypts and 20.2% higher (p=0.11) in the lower 60% of crypts. The mean adjusted E-cadherin expression among participants in the highest relative those in the lowest tertile of dietary fiber intake was estimated to be 35.4% higher (p=0.19) in the upper 40% of crypts. However, the mean adjusted E-cadherin expression among the oldest relative to the youngest tertile of participants was estimated to be 38.7% lower (p=0.25) in the upper 40% of crypts. The mean adjusted E-cadherin expression among participants in the highest relative to those in the lowest tertile of serum 25-OH vitamin D was estimated to be 23.7% lower (p=0.01) in the whole crypts, 35.4% higher (p=0.48) in the upper 40% of crypts, and 24.2% lower (p=0.01) in the lower 60% of crypts. The mean adjusted E-cadherin expression among the participants in the three highest categories relative to the lowest category of BMI was estimated to be 67.8% higher (p=0.16) in the upper 40% of crypts. However, the mean adjusted E-cadherin expression among the participants with high relative to low physical activity was estimated to be 6.2% lower (p=0.20) in the whole crypts and 7.3% lower (p=0.18) in the lower 60% of crypts. The estimated associations of E-cadherin expression with serum 25-OH vitamin D (in the whole crypts and lower 60% of crypts), BMI, and physical activity were in directions opposite to those hypothesized. The estimated associations of the following participant characteristics with E-cadherin expression did not meet our criteria outlined in the statistical analysis section for inclusion in Table 2: aspirin use, other NSAID use, smoking, saturated fat consumption, total fat consumption, total vitamin E intake, and total meat consumption.

MSH2

The mean adjusted MSH2 expression among participants in the highest relative to those in the lowest tertile of serum 25-OH vitamin D was estimated to be 25.2% higher (p=0.08) in the whole crypts and 23.6% higher (p=0.09) in the lower 60% of crypts. The mean adjusted MSH2 expression among participants who took aspirin relative those who did not was estimated to be 12.7% lower (p=0.03) in the whole crypt, 30.5% higher (p=0.70) in the upper 40% of crypts, and 11.3% lower (p=0.06) in the lower 60% of crypts. The mean adjusted MSH2 expression among participants who were current smokers relative those who were not was estimated to be 259.4% lower (p=0.20) in the upper 40% of crypts. Similarly, the mean adjusted MSH2 expression among participants in the three highest categories relative to those in the lowest category of BMI was estimated to be 93.2% lower (p=0.06) in the upper 40% of crypts. The mean adjusted MSH2 expression among participants in the highest relative to those in the lowest tertile of total calcium intake was estimated to be 20.5% lower (p=0.05) in the whole crypts, 34.0% lower (p=0.91) in the upper 40% of crypts, and 16.4% lower (p=0.11) in the lower 60% of crypts. The estimated associations of MSH2 expression with aspirin use (in the whole crypts and lower 60% of crypts) and total calcium intake (in the whole crypts and lower 60% of crypts) were in directions opposite to those hypothesized.

Ratio of MSH2 to Mib-1

The ratio of mean adjusted MSH2 to mib-1 expression among participants in the highest relative to those in the lowest tertile of serum 25-OH vitamin D was estimated to be 28.9% higher (p=0.03) in the whole crypts. The ratio of mean adjusted MSH2 to mib-1 expression among participants who were current smokers relative to those who were not was estimated to be 22.5% lower (p=0.38) in the whole crypts. Similarly, the ratio of mean adjusted MSH2 to mib-1 expression among participants who used aspirin relative to those who did not was estimated to be 15.4% lower (p=0.04). The ratio of mean adjusted MSH2 to mib-1 expression among participants in the highest relative to those in the lowest tertile of total calcium intake was also estimated to be 13.6% lower (p=0.17). However, the estimated associations of the ratio of MSH2 to mib-1 expression with aspirin use and total calcium intake were in directions opposite our hypothesis. The estimated associations of the following participant characteristics with MSH2 expression did not meet our criteria outlined in the statistical analysis section for inclusion in Table 2: age, other NSAID use, physical activity, saturated fat consumption, total fat consumption, total vitamin E intake, dietary fiber intake, meat consumption, and vegetable and fruit consumption.

Discussion

Our findings suggest that age, sex, NSAID use, smoking, physical activity, body fatness, vitamin D exposure, and dietary intakes of total and saturated fats, vitamin E, calcium, fiber, meat, and fruit and vegetables may be associated with biomarkers of colorectal carcinogenesis pathways in the normal-appearing colorectal mucosa of sporadic colorectal adenoma patients. These associations, although cross-sectional, suggest that the exposures may affect colorectal carcinogenesis pathways in the colorectal epithelium and thus, risk for colorectal neoplasms, which supports further investigation in larger studies.

More specifically, our results suggest that based on their estimated associations with APC, β-catenin, and E-cadherin expression, the following may be associated with a colorectal mucosa at higher risk for colorectal carcinogenesis through the APC pathway: being older or male, smoking, and consuming more meat and less dietary fiber. The findings for saturated fat, calcium and vegetable and fruit intakes, and 25-OH-vitamin D concentrations were mixed: saturated fat was inversely associated with APC expression (as hypothesized) in the whole and lower 60% of crypts, but also inversely associated with β-catenin expression (opposite to hypothesis) in the whole crypt; however, it was not associated with the APC/β-catenin ratio. Similarly, total fat was inversely associated with APC in the lower 60% of crypts and with β-catenin in the whole crypt, and not associated with the APC/β-catenin ratio. Calcium intake was directly associated with E-cadherin expression as hypothesized, but directly associated with β-catenin and inversely associated with the APC/β-catenin ratio (neither as hypothesized). Vegetable and fruit intakes were directly associated with E-cadherin in whole crypts and the lower 60% of crypts as hypothesized, but inversely associated with APC in the lower 60% of crypts. The associations of 25-OH-vitamin D with the APC/β-catenin ratio and E-cadherin in the upper 40% of crypts were direct, as hypothesized; however, its associations with β-catenin in the lower 60% of crypts was direct, with the APC/β-catenin ratio was indirect, and with E-cadherin in whole crypts and the lower 60% of crypts was inverse. Finally, physical activity was inversely associated with E-cadherin in the whole and lower 60% of crypts, and BMI was directly associated with E-cadherin in the upper 40% of crypts; these findings were not consistent with our hypotheses.

Our findings also suggest that based on their associations with MSH2 expression, the following may be associated with a colorectal mucosa at higher risk for colorectal carcinogenesis through the DNA mismatch repair pathway: sex, smoking, obesity, and vitamin D exposure. The findings for NSAID use and calcium intakes were mixed: NSAID use was directly associated with MSH2 (as hypothesized) in the upper 40% of crypts, but inversely associated with it in the whole and lower 60% of crypts; it was also inversely associated with the MSH2/mib-1 ratio in the whole crypt.

The cellular mechanisms for the associations of various dietary and medical characteristics with APC, β-catenin, E-cadherin, and MSH2 were previously investigated. Meat, specifically red meat, contains high levels of heme, an iron-porphyrin metalloprotein. Heme consumption by rats was found to induce lipid peroxidation in the colon leading to cell surface damage and APC mutation (12).

Calcium was found to stimulate E-cadherin production through the CaSR promoter system in colon cells *in vitro* (13). Finally, cigarette smoking was found to induce hypermethylation in APC promoter regions, leading to lower expression of APC (14). While inverse associations of vitamin D and vegetable and fruit consumption, and direct associations of saturated fats with colon cancer have been reported, the exact underlying cellular mechanisms are not well understood. Saturated fat consumption increases the production of secondary bile acids, which damages cell structures via an oxidative mechanism that can activate compensatory hyperproliferation via an upregulated *wnt* (and thus APC) pathway (15). On the other hand, secondary bile acids can damage DNA, leading to mutations, such as to APC, which would lead to diminished APC expression. It is possible that these mixed effects of saturated fats may somewhat explain our mixed results for associations of saturated fats with APC pathway biomarkers. Vegetables and fruit contain antioxidant micronutrients that may help mitigate the oxidative effects of the bile acids produced from saturated fat intakes (16).

Previous trials assessed the effects of vitamin D and calcium on the aforementioned biomarkers. Liu et al reported the effects of supplemental vitamin D and calcium over one year on APC, β-catenin, and E-cadherin expression in colon crypts in the same 104-person cohort used for our cross-sectional analyses (10). Our observational findings are consistent with several of the clinical trial findings for vitamin D and calcium. In the trial, vitamin D supplementation increased the APC/β-catenin ratio by 28%, and calcium supplementation increased E-cadherin expression, especially in the upper 40% of the crypt. However, in the trial, it was estimated that the supplemental vitamin D slightly increased E-cadherin expression, whereas in the current observational analysis, the associations of 25-OH-vitamin D with different parameters of the crypt were mixed (consistent with the findings in the trial in the upper 40% of crypts but inverse in the whole and lower 60% of crypts). The reasons for the discrepancies are unclear. The finding in the clinical trial may be more valid because of the randomized, controlled design and the larger vitamin D exposure than in the observational study. A second study used the same 104-person cohort to assess the effects of supplemental vitamin D and calcium on the expression of MSH2, TGFα, and $TGF\beta_1$ (11). Consistent with our observational findings, supplemental vitamin D increased the MSH2/mib-1 ratio, but supplemental calcium decreased it in the upper 40% of the crypt.

Our study had several strengths and limitations. One of the primary limitations was the small sample size, which limited our ability to detect associations, and also increased the potential for chance observations. Despite this limitation, we still detected statistically significant associations of meat consumption with a lower APC/β-catenin expression ratio, vegetable and fruit consumption with greater E-cadherin expression, and serum 25-OH-vitamin D concentrations with a greater MSH2/mib-1 expression ratio. Our study was also restricted to sporadic colorectal adenoma patients, so our findings may not be generalizable to other populations. Study strengths include (i) the comprehensive assessment of multiple dietary, lifestyle, demographic, and medical factors using multivariable general linear modeling, and (ii) the automated immunostaining and novel image analysis software, which enabled quantification of crypt biomarker distributions and high biomarker scoring reliability.

In conclusion, the results of this preliminary, cross-sectional study, taken together with previous literature, suggest that age, sex, NSAID use, smoking, physical activity, body fatness, vitamin D exposure, and dietary intakes of total and saturated fats, vitamin E, calcium, fiber, meat, and fruit and vegetables intakes may be associated with biomarkers of colorectal carcinogenesis pathways in the normal-appearing colorectal mucosa of sporadic colorectal adenoma patients. These associations, although preliminary and cross-sectional, suggest that the exposures may affect colorectal carcinogenesis pathways in the colorectal epithelium, and thus risk for colorectal neoplasms, and so support further investigation in larger studies. These findings also support further study of the use of APC, β-catenin, E-cadherin, and APC/β-catenin and MSH2/mib-1 ratios in the normal-appearing rectal mucosa, as potentially modifiable, pre-neoplastic markers of risk for colorectal neoplasms.

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Table 1. Selected baseline characteristics of the study participants[a] (n = 104)

Characteristics	Mean or proportion	SD	Range
Demographics			
Sex (%)			
Male	46.2		
Female	53.8		
Race (%)			
White	78.9		
Black	19.2		
Other	1.9		
Age (yrs)	58.9	6.7	47 - 75
Lifestyle			
Currently smoke (%)	7.7		
Regularly[b] take aspirin (%)	38.5		
Regularly[b] take non-aspirin NSAID (%)	33.7		
Highest education level (%)			
High school	34.6		
College	36.5		
Graduate	26.0		
Physical activity level (%)			
Low	28.8		
Moderate	31.7		
High	38.5		
Body mass index (kg/m^2)	29.6	5.6	21.0 - 54.1
18.5 - 24.9	21.2		
25.0 - 29.9	41.3		
30.0 - 34.9	22.1		
35.0 - 39.9	11.5		
≥40.0	3.8		
Dietary intake			
Total energy (kcal/day)	1349	763	630.1 - 2936.4
Saturated fat (as % of total energy)	11.5	2.7	4.2 - 17.4
Total fat (as % of total energy)	36.3	7.4	17.8 - 58.5
Total[c] calcium (mg/1,000 kcal)	425	161	170.1 - 1085.2
Vitamin E (mg/1,000 kcal)	5.6	1.9	2.6 - 15.6
Dietary fiber (g/1,000 kcal)	10.7	4.0	4.6 - 22.4
Total vegetables and fruit (servings/day)	4.6	2.2	0.7 - 12.5
Total meat (servings/day)	1.7	0.9	0.2 - 5.0
Serum concentrations			
25-OH vitamin D (ng/mL)	24.1	9.3	12.9 - 68.8

Abbreviations: SD, standard deviation; NSAID, non-steroidal anti-inflammatory drug;
[a]A subset of colorectal adenoma patients participating in the Calcium/Vitamin D, Biomarkers and Colon Polyp Prevention Trial from the South Carolina and Georgia clinical centers.

[b]Take at least one a week.

[c]Dietary plus supplemental intake.

Table 2. Summary of adjusted mean differences[a] in the expression of colon carcinogenesis pathway proteins in the normal-appearing colorectal mucosa of colorectal adenoma patients (n = 104) across categories of participant characteristics

Characteristics	Biomarkers															
	APC			β-catenin			APC/β-catenin[d]			E-cadherin			MSH2			MSH2/mib-1[e]
	Whole crypt	Upper 40%[b]	Lower 60%[c]	Whole crypt	Upper 40%	Lower 60%	Whole crypt	Upper 40%	Lower 60%	Whole crypt	Upper 40%	Lower 60%	Whole crypt	Upper 40%	Lower 60%	Whole crypt
Lifestyle, demographics																
Age											↓					
Take aspirin						↓							(↓*)	↑	(↓)	(↓*)
Take non-aspirin NSAID																
Smoke					↑		↓	↓	↓					↓		↓
Physical activity										(↓)		(↓)				
Body mass index											(↑)			↓		
Dietary intakes																
Saturated fat	↓		↓	(↓*)												
Total fat			↓	(↓)												
Total vitamin E																
Total calcium				(↑)				(↓)		↑		↑	(↓*)	↑	(↓)	(↓)
Dietary fiber							↑	↑			↑					
Meat	↓		↓	↑			↓*	↓*	↓							
Vegetables and fruit			(↓)							↑*		↑				
Serum concentrations																
25-OH vitamin D						(↑)	↑	↑		(↓*)	↑	(↓*)	↑		↑	↑*

Abbreviations: NSAID, non-steroidal anti-inflammatory drug.

[a]Associations assessed using multivariable general linear models containing the characteristic of interest, staining batch, and measured confounding variables. Criteria for inclusion in this table are: estimated proportional mean difference ≥20% and/or a p-value <0.20 for the estimated difference. Up/down arrows indicate the direction (higher or lower, respectively) of the mean biomarker difference between a higher exposure category relative to the reference category. Arrows in brackets [e.g., (↑)] indicate that the direction of the difference was opposite that hypothesized. * Indicates a statistically significant ($p < 0.05$) finding.

[b]Biomarker expression in the upper 40% of the crypt (the canonical differentiation zone).

[c]Biomarker expression in the lower 60% of the crypt (the canonical proliferation zone).

[d]APC expression divided by β-catenin expression in the whole crypt, upper 40% of crypts, and lower 60% of crypts.

[e]MSH2 expression divided by MIB1 expression in the whole crypt.

Table 3. Comparisons[a] of adjusted mean APC expression[b] in the normal-appearing colorectal mucosa of sporadic colorectal adenoma patients (n = 104), by selected participant characteristics

Characteristics	n	Whole crypt, mean (OD)	95% CI	Proportional difference[c] (%)	P-value	Upper 40% of crypts, mean (OD)	95% CI	Proportional difference[c] (%)	P-value	Lower 60% of crypts, mean (OD)	95% CI	Proportional difference[c] (%)	P-value
Age (years)													
47 - 54	34	2,432	(2,069, 2,794)	-		884	(643, 1,126)	-		1,286	(946, 1,627)	-	
55 - 62	35	2,550	(2,195, 2,905)	4.9		978	(731, 1,224)	10.5		1,303	(956, 1,650)	1.3	
63 - 75	35	2,547	(2,188, 2,905)	4.7	0.66	994	(776, 1,213)	12.4	0.35	1,271	(964, 1,579)	-1.2	0.86
Sex													
Male	48	2,839	(2,456, 3,221)	-		1,114	(940, 1,289)	-		1,561	(1,335, 1,788)	-	
Female	56	2,231	(1,879, 2,583)	-21.4	0.03	929	(768, 1,091)	-16.6	0.14	1,161	(951, 1,370)	-25.7	0.01
Regular[d] use of aspirin													
No	64	2,561	(2,239, 2,884)	-		995	(759, 1,231)	-		1,282	(951, 1,612)	-	
Yes	40	2,480	(2,236, 2,724)	-3.2	0.71	922	(747, 1,098)	-7.3	0.49	1,285	(1,039, 1,531)	0.3	0.98
Regular[d] use of other NSAID													
No	69	2,458	(2,103, 2,814)	-		950	(776, 1,123)	-		1,322	(1,076, 1,567)	-	
Yes	35	2,520	(2,285, 2,755)	2.5	0.73	1,054	(946, 1,163)	11.0	0.34	1,338	(1,184, 1,492)	1.2	0.85
Currently smoke													
No	96	2,519	(2,333, 2,706)	-		1,043	(951, 1,134)	-		1,353	(1,225, 1,481)	-	
Yes	8	2,228	(1,445, 3,012)	-11.5	0.48	886	(507, 1,266)	-15.0	0.48	1,192	(659, 1,724)	-11.9	0.64
Physical activity													
Low	30	2,423	(2,043, 2,803)	-		1,031	(847, 1,215)	-		1,250	(995, 1,505)	-	
Moderate	38	2,567	(2,224, 2,910)	5.9		1,061	(896, 1,226)	2.9		1,362	(1,133, 1,591)	8.9	
High	27	2,502	(2,182, 2,821)	3.3	0.80	975	(818, 1,131)	-5.5	0.93	1,386	(1,169, 1,603)	10.9	0.59
Body mass index (kg/m^2)													
<25.0	22	2,323	(1,880, 2,765)	-		956	(747, 1,165)	-		1,258	(969, 1,547)	-	
25.0 - 29.9	43	2,549	(2,251, 2,847)	9.7		1,048	(906, 1,190)	9.6		1,386	(1,190, 1,583)	10.2	
≥30	39	2,577	(2,249, 2,904)	10.9	0.43	1,035	(878, 1,193)	8.3	0.58	1,385	(1,167, 1,604)	10.1	0.50
Total energy, tertiles													
1	34	2,593	(2,162, 3,024)	-		1,104	(926, 1,283)	-		1,516	(1,272, 1,761)	-	
2	35	2,321	(1,983, 2,659)	-10.5		939	(776, 1,102)	-15.0		1,248	(1,024, 1,471)	-17.7	
3	35	2,630	(2,195, 3,066)	1.4	0.25	1,014	(836, 1,193)	-8.1	0.55	1,287	(1,042, 1,532)	-15.1	0.16
Saturated fat, tertiles													
1	34	2,685	(2,344, 3,026)	-		1,100	(941, 1,259)	-		1,450	(1,229, 1,671)	-	
2	35	2,544	(2,206, 2,881)	-5.3		981	(821, 1,140)	-10.9		1,450	(1,227, 1,672)	-0.04	
3	35	2,303	(1,918, 2,689)	-14.2	0.18	990	(806, 1,174)	-10.0	0.36	1,178	(921, 1,434)	-18.8	0.19
Total fat, tertiles													
1	34	2,615	(2,245, 2,986)	-		1,037	(860, 1,214)	-		1,421	(1,182, 1,659)	-	
2	35	2,598	(2,259, 2,936)	-0.7		1,014	(847, 1,180)	-2.2		1,443	(1,215, 1,671)	1.6	
3	35	2,316	(1,935, 2,697)	-11.4	0.32	989	(791, 1,187)	-4.6	0.76	1,167	(899, 1,435)	-17.8	0.20
Total[e] vitamin E, tertiles													
1	34	2,447	(2,055, 2,838)			974	(704, 1,244)	-		1,283	(912, 1,654)	-	
2	35	2,437	(2,087, 2,787)	-0.4		996	(748, 1,245)	2.3		1,236	(895, 1,577)	-3.7	
3	35	2,648	(2,246, 3,050)	8.2	0.55	964	(727, 1,202)	-1.0	0.85	1,407	(1081, 1,733)	9.7	0.44
Total[e] calcium, tertiles													
1	34	2,621	(2,229, 3,013)	-		1,039	(854, 1,225)	-		1,457	(1,201, 1,713)	-	
2	35	2,553	(2,184, 2,923)	-2.6		1,021	(845, 1,198)	-1.7		1,420	(1,177, 1,662)	-2.5	
3	35	2,361	(1,969, 2,753)	-9.9	0.41	998	(812, 1,184)	-4.0	0.86	1,178	(922, 1,435)	-19.1	0.22
Dietary fiber, tertiles													
1	34	2,480	(2,070, 2,890)	-		1,042	(842, 1,243)	-		1,265	(987, 1,542)	-	
2	35	2,509	(2,172, 2,847)	1.2		1,025	(863, 1,186)	-1.7		1,342	(1,119, 1,565)	6.1	
3	35	2,546	(2,146, 2,941)	2.7	0.85	1,005	(807, 1,202)	-3.6	0.50	1,466	(1,193, 1,740)	15.9	0.34
Total meat intake, tertiles													
1	34	2,801	(2,368, 3,234)	-		1,088	(886, 1,289)	-		1,555	(1,275, 1,836)	-	
2	35	2,512	(2,185, 2,840)	-10.3		1,067	(911, 1,223)	-1.9		1,320	(1,103, 1,537)	-15.1	
3	35	2,274	(1,895, 2,653)	-18.8	0.12	917	(736, 1,098)	-15.7	0.29	1,202	(950, 1,454)	-22.7	0.14
Total vegetable and fruit intake, tertiles													
1	34	2,766	(2,373, 3,158)	-		1,124	(931, 1,318)			1,547	(1,282, 1,812)	-	
2	35	2,360	(2,021, 2,698)	-14.7		935	(771, 1,100)	-16.8		1,304	(1,079, 1,530)	-15.8	
3	35	2,418	(2,033, 2,802)	-12.6	0.28	1,017	(825, 1,208)	-9.6	0.58	1,206	(944, 1,469)	-22.0	0.20
Serum 25-OH-vitamin D (ng/mL)													
<17.9	34	2,502	(2,127, 2,876)	-		1,023	(843, 1,202)	-		1,343	(1,096, 1,591)	-	
17.9 - 26.9	35	2,249	(1,891, 2,608)	-10.1		925	(746, 1,104)	-9.5		1,202	(954, 1,450)	-10.5	
>26.9	35	2,791	(2,363, 3,219)	11.6	0.57	1,130	(923, 1,336)	10.5	0.65	1,555	(1,270, 1,841)	15.8	0.54

Abbreviations: CI, confidence interval; NSAID, non-steroidal anti-inflammatory drug; OD, optical density.

[a]Using general linear models, adjusted for staining batch and measured confounding variables listed in Supplementary Table S1.

[b]Measured using automated immunohistochemistry with image analysis.

[c]Calculated as (comparison group mean - reference group mean) / (reference group mean) x 100%.

[d]Take at least once a week.

[e]Dietary plus supplemental intake.

Table 4. Comparisons[a] of adjusted mean β-catenin expression[b] in the normal-appearing colorectal mucosa of sporadic colorectal adenoma patients (n = 104), by selected participant characteristics

Characteristics	n	Whole crypt, mean (OD)	95% CI	Proportional difference[c] (%)	P-value	Upper 40% of crypts, mean (OD)	95% CI	Proportional difference[c] (%)	P-value	Lower 60% of crypts, mean (OD)	95% CI	Proportional difference[c] (%)	P-value
Age (years)													
47 - 54	34	10,673	(9,113, 12,2333)	-		4,025	(2,864, 5,186)	-		1,524	(1,311, 1,737)	-	
55 - 62	35	10,469	(8,943, 11,994)	-1.9		3,901	(2,766, 5,036)	-3.1		1,555	(1,359, 1,751)	2.1	
63 - 75	35	11,115	(9,852, 12,379)	4.1	0.89	4,513	(3,573, 5,453)	12.1	0.63	1,438	(1,246, 1,630)	-5.6	0.69
Sex													
Male	48	10,413	(9,574, 11,252)	-		4,580	(3,945, 5,215)	-		1,525	(1,348, 1,701)	-	
Female	56	10,381	(9,646, 11,115)	-0.3	0.72	3,786	(3,231, 4,342)	-17.3	0.11	1,400	(1,245, 1,554)	-8.2	0.17
Regular[d] use of aspirin													
No	64	10,502	(9,563, 11,441)	-		3,994	(3,294, 4,695)	-		1,561	(1,402, 1,719)	-	
Yes	40	10,519	(9,773, 11,264)	0.2	0.85	4,046	(3,490, 4,602)	1.3	0.99	1,384	(1,263, 1,505)	-11.3	0.06
Regular[d] use of other NSAID													
No	69	10,701	(9,569, 11,834)	-		4,146	(3,343, 4,949)	-		1,570	(1,385, 1,755)	-	
Yes	35	10,325	(9,561, 11,088)	-3.5	0.62	4,100	(3,559, 4,641)	-1.1	0.32	1,377	(1,241, 1,512)	-12.3	0.30
Currently smoke													
No	96	10,421	(9,856, 10,985)	-		3,975	(3,555, 4,396)	-		1,467	(1,363, 1,570)	-	
Yes	8	12,341	(10,032, 14,651)	18.4	0.22	4,783	(3,063, 6,503)	20.3	0.39	1,499	(1,063, 1,936)	2.2	0.96
Physical activity													
Low	30	10,995	(9,484, 12,507)	-		4,351	(3,176, 5,527)	-		1,450	(1,220, 1,680)	-	
Moderate	38	11,536	(10,075, 12,998)	4.9		4,272	(3,136, 5,409)	-1.8		1,487	(1,292, 1,681)	2.5	
High	27	10,997	(9,554, 12,439)	0.0	0.65	4,693	(3,571, 5,815)	7.9	0.33	1,492	(1,315, 1,670)	2.9	0.88
Body mass index (kg/m^2)													
<25.0	22	9,807	(8,461, 11,154)	-		4,470	(3,461, 5,478)	-		1,448	(1,226, 1,671)	-	
25.0 - 29.9	43	10,745	(9,871, 11,619)	9.6		3,768	(3,114, 4,423)	-15.7		1,552	(1,378, 1,726)	7.2	
≥30	39	10,784	(9,817, 11,751)	10.0	0.47	4,066	(3,342, 4,790)	-9.0	0.75	1,468	(1,300, 1,635)	1.3	0.99
Total energy, tertiles													
1	34	9,867	(8,654, 11,080)	-		4,376	(3,447, 5,306)	-		1,234	(1,009, 1,459)	-	
2	35	11,250	(10,267, 12,233)	14.0		4,055	(3,302, 4,808)	-7.3		1,478	(1,301, 1,655)	19.8	
3	35	10,171	(8,894, 11,448)	3.1	0.92	3,680	(2,701, 4,658)	-15.9	0.21	1,733	(1,482, 1,984)	40.4	0.02
Saturated fat, tertiles													
1	34	11,290	(10,202, 12,378)	-		3,612	(2,784, 4,440)	-		1,538	(1,338, 1,738)		
2	35	11,165	(10,179, 12,150)	-1.1		4,614	(3,864, 5,364)	27.8		1,484	(1,300, 1,668)	-3.5	
3	35	8,990	(7,772, 10,208)	-20.4	0.04	3,770	(2844, 4,697)	4.4	0.55	1,341	(1,109, 1,574)	-12.8	0.27
Total fat, tertiles													
1	34	11,116	(10,073, 12,159)	-		4,124	(3,331, 4,916)	-		1,448	(1,239, 1,657)	-	
2	35	10,685	(9,732, 11,638)	-3.9		4,148	(3,424, 4,871)	0.6		1,461	(1,269, 1,653)	0.9	
3	35	9,535	(8,429, 10,641)	-14.2	0.08	3,821	(2,981, 4,661)	-7.3	0.73	1,442	(1,213, 1,671)	-0.4	0.89
Total[e] vitamin E, tertiles													
1	34	10,283	(9,213, 11,352)	-		3,857	(3,053, 4,662)	-		1,471	(1,276, 1,666)	-	
2	35	10,622	(9,580, 11,664)	3.3		3,710	(2,978, 4,443)	-3.8		1,387	(1,205, 1,569)	-5.7	
3	35	10,663	(9,606, 11,721)	3.7	0.23	4,521	(3,727, 5,315)	17.2	0.23	1,502	(1,316, 1,687)	2.1	0.95
Total[e] calcium, tertiles													
1	34	9,927	(8,830, 11,023)	-		3,945	(3,137, 4,752)	-		1,641	(1,448, 1,834)	-	
2	35	10,378	(9329, 11,427)	4.5		4,367	(3,595, 5,140)	10.7		1,342	(1,154, 1,530)	-18.2	
3	35	11,120	(10,094, 12,145)	12.0	0.17	3,776	(3,020, 4,531)	-4.3	0.70	1,371	(1,173, 1,569)	-16.4	0.11
Dietary fiber, tertiles													
1	34	10,920	(9590, 12,249)	-		3,756	(2,779, 4,733)	-		1,476	(1,257, 1,695)	-	
2	35	10,228	(9117, 11,338)	-6.3		4,528	(3,712, 5,344)	20.6		1,378	(1,200, 1,555)	-6.7	
3	35	10,122	(8,739, 11,505)	-7.3	0.73	3,764	(2,747, 4,780)	0.2	0.55	1,556	(1,332, 1,780)	5.4	0.67
Total meat intake, tertiles													
1	34	9,013	(7,682, 10,344)	-		4,042	(3,009, 5,075)	-		1,442	(1,192, 1,691)	-	
2	35	11,030	(10,064, 11,997)	22.4		3,739	(2,989, 4,490)	-7.5		1,518	(1,331, 1,705)	5.3	
3	35	11,461	(10,290, 12,632)	27.2	0.06	4,281	(3,372, 5,190)	5.9	0.67	1,392	(1,170, 1,614)	-3.4	0.42
Total vegetable and fruit intake, tertiles													
1	34	10,479	(9,335, 11,623)	-		3,757	(2,904, 4,610)	-		1,485	(1,279, 1,692)	-	
2	35	10,420	(9,407, 11,432)	-0.6		4,521	(3,766, 5,276)	20.3		1,520	(1,344, 1,696)	2.3	
3	35	10,560	(9,306, 11,814)	0.8	0.94	3,682	(2,747, 4,617)	-2.0	0.87	1,354	(1,148, 1,560)	-8.8	0.40
Serum 25-OH-vitamin D (ng/mL)													
<17.9	34	10,405	(9,302, 11,508)	-		4,234	(3,428, 5,040)	-		1,353	(1,163, 1,543)	-	
17.9 - 26.9	35	10,367	(9,331, 11,403)	-0.4		4,469	(3,712, 5,227)	5.6		1,328	(1,144, 1,512)	-1.8	
>26.9	35	10,662	(9,468, 11,855)	2.5	0.77	3,393	(2,520, 4,265)	-19.9	0.21	1,672	(1,456, 1,889)	23.6	0.09

Abbreviations: CI, confidence interval; NSAID, non-steroidal anti-inflammatory drug; OD, optical density.

[a]Using general linear models, adjusted for staining batch and measured confounding variables listed in Supplementary Table S1.

[b]Measured using automated immunohistochemistry with image analysis.

[c]Calculated as (comparison group mean - reference group mean) / (reference group mean) x 100%.

[d]Take at least once a week.

[e]Dietary plus supplemental intake.

Table 5. Comparisons[a] of adjusted mean E-cadherin expression[b] in the normal-appearing colorectal mucosa of sporadic colorectal adenoma patients (n = 104), by selected participant characteristics

Characteristics	n	Whole crypt, mean (OD)	95% CI	Proportional difference[c] (%)	P-value	Upper 40% of crypts, mean (OD)	95% CI	Proportional difference[c] (%)	P-value	Lower 60% of crypts, mean (OD)	95% CI	Proportional difference[c] (%)	P-value
Age (years)													
47 - 54	34	4,496	(3852, 5,140)	-		3,534	(2,353, 4,714)	-		2,643	(2,241, 3,046)	-	
55 - 62	35	4,840	(4,269, 5,410)	7.6		1,719	(674, 2,765)	-51.3		2,905	(2,549, 3,261)	9.9	
63 - 75	35	4,525	(3,914, 5,136)	0.7	0.83	2,167	(1,048, 3,287)	-38.7	0.25	2,661	(2,279, 3,042)	0.7	0.78
Sex													
Male	48	4,985	(4,433, 5,537)	-		2,541	(1,551, 3,532)	-		2,994	(2,651, 3,336)	-	
Female	56	4,314	(3,805, 4,824)	-13.4	0.08	2,256	(1,341, 3,170)	-11.2	0.27	2,513	(2,197, 2,829)	-16.1	0.05
Regular[d] use of aspirin													
No	64	4,377	(3,852, 4,903)	-		2,730	(1,736, 3,725)	-		2,616	(2,283, 2,949)	-	
Yes	40	4,765	(4,374, 5,156)	8.9	0.90	2,221	(1,481, 2,961)	-18.7	0.88	2,810	(2,562, 3,058)	7.4	0.91
Regular[d] use of other NSAID													
No	69	4,711	(4,108, 5,314)	-		2,235	(1,106, 3,365)	-		2,786	(2,406, 3,167)	-	
Yes	35	4,560	(4,180, 4,940)	-3.2	0.58	2,484	(1,771, 3,196)	11.1	0.66	2,705	(2,465, 2,945)	-2.9	0.62
Currently smoke													
No	96	4,607	(4,308, 4,905)	-		2,345	(1,779, 2,911)	-		2,734	(2,546, 2,922)	-	
Yes	8	4,667	(3,424, 5,911)	1.3	0.89	3,683	(1,326, 6,039)	57.0	0.32	2,726	(1,942, 3,510)	-0.3	0.98
Physical activity													
Low	30	4,713	(4,105, 5,321)	-		2,553	(1,436, 3,670)	-		2,813	(2,432, 3,194)	-	
Moderate	38	4,703	(4,157, 5,248)	-0.2		3,594	(2,592, 4,597)	40.8		2,776	(2,434, 3,118)	-1.3	
High	27	4,419	(3,898, 4,939)	-6.2	0.20	1,247	(291, 2,203)	-51.1	0.20	2,608	(2,282, 2,934)	-7.3	0.18
Body mass index (kg/m^2)													
<25.0	22	4,645	(3,913, 5,376)	-		1,566	(214, 2,918)	-		2,748	(2,285, 3,210)	-	
25.0 - 29.9	43	4,630	(4,133, 5,127)	-0.3		2,616	(1,697, 3,534)	67.0		2,724	(2,410, 3,038)	-0.9	
≥30	39	4,563	(4,001, 5,125)	-1.8	0.87	2,627	(1,588, 3,665)	67.8	0.16	2,728	(2,374, 3,083)	-0.7	0.94
Total energy, tertiles													
1	34	4,691	(4,082, 5,300)	-		1,501	(300, 2,702)	-		2,858	(2,494, 3,222)	-	
2	35	4,226	(3,701, 4,752)	-9.9		2,747	(1,711, 3,783)	83.0		2,523	(2,199, 2,846)	-11.7	
3	35	4,823	(4,244, 5,403)	2.8	0.90	2,750	(1,607, 3,894)	83.2	0.24	2,779	(2,424, 3,134)	-2.8	0.78
Saturated fat, tertiles													
1	34	4,143	(3,563, 4,723)	-		2,708	(1,559, 3,857)	-		2,472	(2,105, 2,838)	-	
2	35	5,055	(4,531, 5,578)	22.0		2,403	(1,366, 3,440)	-11.3		3,001	(2,670, 3,332)	21.4	
3	35	4,609	(3,965, 5,254)	11.3	0.35	2,226	(949, 3,504)	-17.8	0.68	2,712	(2,304, 3,119)	9.7	0.36
Total fat, tertiles													
1	34	4,243	(3,648, 4,839)	-		2,241	(1,081, 3,401)	-		2,488	(2,117, 2,860)	-	
2	35	4,904	(4,361, 5,447)	15.6		2,178	(1,121, 3,235)	-2.8		2,927	(2,588, 3,266)	17.6	
3	35	4,603	(3,989, 5,216)	8.5	0.54	2,593	(1,398, 3,787)	15.7	0.79	2,728	(2,345, 3,111)	9.6	0.41
Total[e] vitamin E, tertiles													
1	34	4,771	(4,156, 5,386)	-		2,525	(1,375, 3,675)	-		2,831	(2,448, 3,215)	-	
2	35	4,509	(3,941, 5,077)	-5.5		2,661	(1,599, 3,723)	5.4		2,649	(2,294, 3,003)	-6.5	
3	35	4,537	(3,866, 5,209)	-4.9	0.93	2,016	(761, 3,272)	-20.2	0.61	2,698	(2,280, 3,117)	-4.7	0.92
Total[e] calcium, tertiles													
1	34	4,346	(3,741, 4,951)	-		2,510	(1,294, 3,725)	-		2,563	(2,180, 2,946)	-	
2	35	4,172	(3,574, 4,769)	-4.0		2,301	(1,101, 3,501)	-8.3		2,455	(2,077, 2,832)	-4.2	
3	35	5,171	(4,558, 5,784)	19.0	0.10	2,048	(816, 3,280)	-18.4	0.69	3,081	(2,693, 3,469)	20.2	0.11
Dietary fiber, tertiles													
1	34	4,512	(3,763, 5,260)	-		2,045	(896, 3,194)	-		2,679	(2,208, 3,151)		
2	35	4,539	(3,935, 5,144)	0.6		2,178	(1,061, 3,295)	6.5		2,668	(2,287, 3,048)	-0.4	
3	35	4,800	(4,096, 5,504)	6.4	0.51	2,768	(1,490, 4,046)	35.4	0.19	2,847	(2,404, 3,290)	6.3	0.54
Total meat intake, tertiles													
1	34	4,430	(3,681, 5,178)	-		1,724	(249, 3,198)	-		2,580	(2,111, 3,049)	-	
2	35	4,485	(3,917, 5,502)	1.2		3,115	(1,996, 4,233)	80.7		2,730	(2,374, 3,086)	5.8	
3	35	4,773	(4,110, 5,435)	7.7	0.84	2,121	(816, 3,426)	23.1	0.78	2,791	(2,376, 3,207)	8.2	0.86
Total vegetable and fruit intake, tertiles													
1	34	3,915	(3,315, 4,516)	-		2,890	(1,761, 4,019)	-		2,309	(1,932, 2,686)	-	
2	35	4,901	(4,361, 5,441)	25.2		1,396	(381, 2,411)	-51.7		2,861	(2,521, 3,200)	23.9	
3	35	5,030	(4,410, 5,651)	28.5	0.02	3,042	(1,875, 4,208)	5.2	0.99	3,035	(2,645, 3,425)	31.4	0.16
Serum 25-OH-vitamin D (ng/mL)													
<17.9	34	5,305	(4,725, 5,885)	-		2,045	(896, 3,194)	-		3,126	(2,764, 3,488)	-	
17.9 - 26.9	35	4,385	(3,821, 4,949)	-17.3		2,178	(1,061, 3,295)	6.5		2,636	(2,284, 2,987)	-15.7	
>26.9	35	4,048	(3,403, 4,693)	-23.7	0.01	2,768	(1,490, 4,046)	35.4	0.48	2,369	(1,967, 2,772)	-24.2	0.01

Abbreviations: CI, confidence interval; NSAID, non-steroidal anti-inflammatory drug; OD, optical density.

[a]Using general linear models, adjusted for staining batch and measured confounding variables listed in Supplementary Table S1.

[b]Measured using automated immunohistochemistry with image analysis.

[c]Calculated as (comparison group mean - reference group mean) / (reference group mean) x 100%.

[d]Take at least once a week.

[e]Dietary plus supplemental intake.

Table 6. Comparisons[a] of adjusted mean MSH2 expression[b] in the normal-appearing colorectal mucosa of sporadic colorectal adenoma patients (n = 104), by selected participant characteristics

Characteristics	n	Whole crypt, mean (OD)	95% CI	Proportional difference[c] (%)	P-value	Upper 40% of crypts, mean (OD)	95% CI	Proportional difference[c] (%)	P-value	Lower 60% of crypts, mean (OD)	95% CI	Proportional difference[c] (%)	P-value
Age (years)													
47 - 54	34	1,918	(1,643, 2,193)	-		353	(-599, 1,305)	-		1,524	(1,311, 1,737)	-	
55 - 62	35	1,939	(1,687, 2,192)	1.1		320	(-556, 1,196)	-9.4		1,555	(1,359, 1,751)	2.1	
63 - 75	35	1,854	(1,609, 2,099)	-3.3	0.79	966	(108, 1,825)	173.7	0.60	1,438	(1,246, 1,630)	-5.6	0.69
Sex													
Male	48	1,914	(1,690, 2,139)	-		925	(235, 1,615)	-		1,525	(1,348, 1,701)	-	
Female	56	1,797	(1,601, 1,992)	-6.2	0.27	167	(-435, 769)	-82.0	0.19	1,400	(1,245, 1,554)	-8.2	0.17
Regular[d] use of aspirin													
No	64	1,998	(1,791, 2,204)	-		459	(-295, 1,214)	-		1,561	(1,402, 1,719)	-	
Yes	40	1,743	(1,586, 1,900)	-12.7	0.03	599	(25, 1,173)	30.5	0.70	1,384	(1,263, 1,505)	-11.3	0.06
Regular[d] use of other NSAID													
No	69	1,995	(1,752, 2,238)	-		811	(0.1, 1,622)	-		1,570	(1,385, 1,755)	-	
Yes	35	1,734	(1,556, 1,911	-13.1	0.29	542	(-52, 1,136)	-33.2	0.39	1,377	(1,241, 1,512)	-12.3	0.30
Currently smoke													
No	96	1,863	(1,729, 1,998)	-		556	(97, 1,014)	-		1,467	(1,363, 1,570)	-	
Yes	8	1,903	(1,335, 2,471)	2.1	0.92	-886	(-2816, 1,044)	-259.4	0.20	1,499	(1,063, 1,936)	2.2	0.96
Physical activity													
Low	30	1,825	(1,522, 2,128)	-		845	(-149, 1,838)	-		1,450	(1,220, 1,680)	-	
Moderate	38	1,878	(1,622, 2,134)	2.9		149	(-690, 987)	-82.4		1,487	(1,292, 1,681)	2.5	
High	27	1,893	(1,659, 2,126)	3.7	0.95	953	(188, 1,719)	12.8	0.65	1,492	(1,315, 1,670)	2.9	0.88
Body mass index (kg/m^2)													
<25.0	22	1,804	(1,511, 2,097)	-		1,527	(509, 2,545)	-		1,448	(1,226, 1,671)	-	
25.0 - 29.9	43	1,989	(1,759, 2,218)	10.2		549	(-247, 1,344)	-64.1		1,552	(1,378, 1,726)	7.2	
≥30	39	1,879	(1,658, 2,099)	4.1	0.82	104	(-661, 870)	-93.2	0.06	1,468	(1,300, 1,635)	1.3	0.99
Total energy, tertiles													
1	34	1,602	(1,306, 1,898)	-		817	(-206, 1,839)	-		1,234	(1,009, 1,459)	-	
2	35	1,866	(1,633, 2,099)	16.5		88	(-717, 893)	-89.2		1,478	(1,301, 1,655)	19.8	
3	35	2,173	(1,843, 2,504)	35.7	0.04	928	(-213, 2,068)	13.6	0.81	1,733	(1,482, 1,984)	40.4	0.02
Saturated fat, tertiles													
1	34	1,942	(1,679, 2,205)	-		260	(-598, 1,119)	-		1,538	(1,338, 1,738)		
2	35	1,860	(1,617, 2,102)	-4.2		822	(31, 1,613)	215.5		1,484	(1,300, 1,668)	-3.5	
3	35	1,736	(1,430, 2,043)	-10.6	0.37	559	(-441, 1,558)	114.5	0.70	1,341	(1,109, 1,574)	-12.8	0.27
Total fat, tertiles													
1	34	1,832	(1,559, 2,105)	-		410	(-487, 1,307)	-		1,448	(1,239, 1,657)	-	
2	35	1,838	(1,588, 2,088)	0.3		1,114	(292, 1,937)	171.7		1,461	(1,269, 1,653)	0.9	
3	35	1,835	(1,536, 2,133)	0.2	0.86	549	(-432, 1,530)	33.9	0.97	1,442	(1,213, 1,671)	-0.4	0.89
Total[e] vitamin E, tertiles													
1	34	1,852	(1,599, 2,105)	-		236	(-589, 1,061)	-		1,471	(1,276, 1,666)	-	
2	35	1,749	(1,513, 1,985)	-5.6		699	(-73, 1,471)	196.7		1,387	(1,205, 1,569)	-5.7	
3	35	1,928	(1,688, 2,168)	4.1	0.81	794	(9, 1,578)	236.8	0.43	1,502	(1,316, 1,687)	2.1	0.95
Total[e] calcium, tertiles													
1	34	2,142	(1,889, 2,394)	-		420	(-478, 1,317)	-		1,641	(1,448, 1,834)	-	
2	35	1,678	(1,433, 1,923)	-21.6		689	(-183, 1,561)	64.1		1,342	(1,154, 1,530)	-18.2	
3	35	1,703	(1,444, 1,961)	-20.5	0.05	562	(-357, 1,481)	34.0	0.91	1,371	(1,173, 1,569)	-16.4	0.11
Dietary fiber, tertiles													
1	34	1,877	(1,592, 2,161)	-		344	(-646, 1,334)	-		1,476	(1,257, 1,695)		
2	35	1,726	(1,495, 1,956)	-8.0		1,115	(313, 1,918)	224.1		1,378	(1,200, 1,555)	-6.7	
3	35	2,002	(1,711, 2,293)	6.7	0.65	14	(-998, 1,027)	-95.8	0.53	1,556	(1,332, 1,780)	5.4	0.67
Total meat intake, tertiles													
1	34	1,841	(1,513, 2,169)	-		1,328	(230, 2,425)	-		1,442	(1,192, 1,691)	-	
2	35	1,914	(1,668, 2,160)	3.9		0	(-822, 823)	-100.0		1,518	(1,331, 1,705)	5.3	
3	35	1,765	(1,473, 2,057)	-4.1	0.38	233	(-744, 1,210)	-82.5	0.38	1,392	(1,170, 1,614)	-3.4	0.42
Total vegetable and fruit intake, tertiles													
1	34	1,877	(1,604, 2,151)	-		166	(-695, 1,027)	-		1,485	(1,279, 1,692)	-	
2	35	1,946	(1,713, 2,180)	3.7		1,219	(486, 1,953)	636.3		1,520	(1,344, 1,696)	2.3	
3	35	1,709	(1,437, 1,982)	-8.9	0.45	338	(-519, 1,196)	104.4	0.68	1,354	(1,148, 1,560)	-8.8	0.40
Serum 25-OH-vitamin D (ng/mL)													
<17.9	34	1,708	(1,460, 1,956)	-		268	(-615, 1,150)	-		1,353	(1,163, 1,543)	-	
17.9 - 26.9	35	1,677	(1,437, 1,916)	-1.8		1,396	(543, 2,250)	422.0		1,328	(1,144, 1,512)	-1.8	
>26.9	35	2,138	(1,855, 2,420)	25.2	0.08	7	(-998, 1,011)	-97.5	0.90	1,672	(1,456, 1,889)	23.6	0.09

Abbreviations: CI, confidence interval; NSAID, non-steroidal anti-inflammatory drug; OD, optical density.

[a]Using general linear models, adjusted for staining batch and measured confounding variables listed in Supplementary Table S1.

[b]Measured using automated immunohistochemistry with image analysis.

[c]Calculated as (comparison group mean - reference group mean) / (reference group mean) x 100%.

[d]Take at least once a week.

[e]Dietary plus supplemental intake.

Table 7. Comparisons[a] of adjusted mean APC/β-catenin expression[b] and MSH2/mib-1 expression in the normal-appearing colorectal mucosa of sporadic colorectal adenoma patients (n = 104), by selected participant characteristics

		APC/β-catenin[c]												MSH2/mib-1[d]			
Characteristics	n	Whole crypt, mean (OD)	95% CI	Proportional difference[e] (%)	P-value	Upper 40% of crypts, mean (OD)	95% CI	Proportional difference[e] (%)	P-value	Lower 60% of crypts, mean (OD)	95% CI	Proportional difference[e] (%)	P-value	Whole crypt, mean (OD)	95% CI	Proportional difference[e] (%)	P-value
Age (years)																	
47 - 54	34	0.22	(0.15, 0.29)	-		0.19	(0.13, 0.26)	-		0.27	(0.18, 0.36)	-		1.72	(1.43, 2.01)	-	
55 - 62	35	0.24	(0.18, 0.31)	10.2		0.21	(0.15, 0.28)	11.6		0.29	(0.20, 0.38)	8.4		1.62	(1.37, 1.86)	-6.1	
63 - 75	35	0.22	(0.16, 0.28)	-1.0	0.92	0.19	(0.14, 0.25)	1.0	0.92	0.25	(0.17, 0.33)	-5.2	0.96	1.53	(1.28, 1.78)	-11.2	0.36
Sex																	
Male	48	0.09	(-0.02, 0.20)	-		0.08	(-0.03, 0.19)	-		0.10	(-0.05, 0.25)	-		1.76	(1.57, 1.94)	-	
Female	56	0.38	(0.29, 0.47)	320.6	0.01	0.33	(0.25, 0.42)	326.4	0.01	0.43	(0.30, 0.55)	323.0	0.02	1.49	(1.33, 1.65)	-15.1	0.05
Regular[f] use of aspirin																	
No	64	0.21	(0.15, 0.28)	-		0.18	(0.11, 0.24)	-		0.27	(0.18, 0.35)	-		1.74	(1.54, 1.94)	-	
Yes	40	0.21	(0.16, 0.26)	-1.3	0.81	0.19	(0.14, 0.24)	8.3	0.71	0.24	(0.17, 0.30)	-11.3	0.38	1.48	(1.32, 1.63)	-15.4	0.04
Regular[f] use of other NSAID																	
No	69	0.23	(0.18, 0.27)	-		0.21	(0.16, 0.25)	-		0.25	(0.19, 0.31)	-		1.66	(1.43, 1.89)	-	
Yes	35	0.25	(0.22, 0.28)	12.1	0.48	0.22	(0.19, 0.25)	6.1	0.97	0.30	(0.26, 0.34)	19.8	0.22	1.53	(1.36, 1.69)	-7.8	0.72
Currently smoke																	
No	96	0.25	(0.23, 0.27)	-		0.22	(0.20, 0.24)	-		0.29	(0.26, 0.32)	-		1.62	(1.50, 1.75)	-	
Yes	8	0.17	(0.07, 0.26)	-33.7	0.18	0.14	(0.04, 0.23)	-36.7	0.20	0.21	(0.07, 0.34)	-29.2	0.34	1.26	(0.73, 1.79)	-22.5	0.38
Physical activity																	
Low	30	0.21	(0.14, 0.27)	-		0.18	(0.12, 0.24)	-		0.24	(0.16, 0.33)	-		1.60	(1.32, 1.89)	-	
Moderate	38	0.21	(0.14, 0.27)	-1.1		0.18	(0.12, 0.24)	-0.3		0.24	(0.15, 0.33)	-2.3		1.40	(1.17, 1.64)	-12.5	
High	27	0.20	(0.15, 0.26)	-1.7	0.81	0.19	(0.13, 0.25)	3.8	0.74	0.23	(0.14, 0.31)	-7.9	0.89	1.70	(1.48, 1.92)	6.1	0.76
Body mass index (kg/m^2)																	
<25.0	22	0.24	(0.18, 0.29)	-		0.21	(0.15, 0.26)	-		0.28	(0.20, 0.35)	-		1.65	(1.37, 1.94)	-	
25.0 - 29.9	43	0.24	(0.21, 0.28)	2.2		0.21	(0.18, 0.25)	2.1		0.28	(0.23, 0.33)	1.4		1.67	(1.46, 1.88)	1.0	
≥30	39	0.25	(0.21, 0.29)	6.0	0.53	0.22	(0.18, 0.26)	7.0	0.51	0.28	(0.23, 0.34)	2.9	0.72	1.55	(1.34, 1.76)	-6.3	0.75
Total energy, tertiles																	
1	34	0.25	(0.20, 0.31)	-		0.22	(0.17, 0.28)	-		0.29	(0.22, 0.37)	-		1.61	(1.33, 1.90)	-	
2	35	0.21	(0.16, 0.25)	-19.2		0.19	(0.15, 0.23)	-16.8		0.23	(0.17, 0.28)	-23.3		1.40	(1.17, 1.62)	-13.6	
3	35	0.29	(0.23, 0.34)	12.4	0.48	0.25	(0.19, 0.30)	9.5	0.67	0.34	(0.26, 0.41)	14.2	0.46	1.75	(1.43, 2.07)	8.4	0.74
Saturated fat, tertiles																	
1	34	0.25	(0.21, 0.30)	-		0.21	(0.17, 0.26)	-		0.30	(0.24, 0.36)	-		1.47	(1.23, 1.71)	-	
2	35	0.23	(0.19, 0.27)	-8.4		0.22	(0.18, 0.26)	1.2		0.24	(0.18, 0.30)	-19.3		1.71	(1.48, 1.93)	16.0	
3	35	0.26	(0.21, 0.30)	1.4	0.94	0.22	(0.17, 0.26)	1.2	0.94	0.31	(0.24, 0.37)	2.3	0.87	1.58	(1.30, 1.86)	7.4	0.51
Total fat, tertiles																	
1	34	0.24	(0.20, 0.29)	-		0.22	(0.18, 0.27)	-		0.26	(0.20, 0.32)	-		1.49	(1.23, 1.75)	-	
2	35	0.26	(0.21, 0.30)	6.1		0.23	(0.19, 0.27)	3.0		0.29	(0.23,0.35)	13.2		1.61	(1.37, 1.85)	7.7	
3	35	0.25	(0.20, 0.30)	2.2	0.97	0.20	(0.15, 0.25)	-9.5	0.50	0.31	(0.24, 0.38)	19.7	0.45	1.63	(1.34, 1.91)	9.0	0.61
Total[g] vitamin E, tertiles																	
1	34	0.22	(0.16, 0.29)	-		0.19	(0.12, 0.26)	-		0.27	(0.18, 0.37)	-		1.54	(1.29, 1.79)	-	
2	35	0.24	(0.18, 0.30)	7.8		0.20	(0.14, 0.26)	5.9		0.30	(0.21, 0.38)	9.7		1.55	(1.32, 1.78)	0.7	
3	35	0.23	(0.17, 0.29)	1.6	0.68	0.21	(0.15, 0.27)	11.1	0.44	0.25	(0.17, 0.33)	-8.7	0.94	1.65	(1.42, 1.89)	7.4	0.51
Total[g] calcium, tertiles																	
1	34	0.27	(0.22, 0.31)	-		0.24	(0.20, 0.29)	-		0.29	(0.23, 0.35)	-		1.75	(1.51, 1.99)	-	
2	35	0.25	(0.20, 0.29)	-6.4		0.22	(0.17, 0.26)	-11.0		0.30	(0.23, 0.36)	2.2		1.49	(1.25, 1.73)	-14.9	
3	35	0.22	(0.18, 0.27)	-16.3	0.38	0.19	(0.14, 0.24)	-21.1	0.25	0.26	(0.20, 0.32)	-10.2	0.73	1.51	(1.26, 1.76)	-13.6	0.17
Dietary fiber, tertiles																	
1	34	0.21	(0.16, 0.27)	-		0.17	(0.12, 0.23)			0.26	(0.19, 0.33)	-		1.66	(1.39, 1.93)	-	
2	35	0.24	(0.20, 0.29)	14.0		0.21	(0.17, 0.25)	19.8		0.29	(0.23, 0.35)	12.2		1.67	(1.45, 1.89)	0.6	
3	35	0.29	(0.23, 0.34)	34.3	0.31	0.27	(0.22, 0.32)	54.6	0.07	0.31	(0.23, 0.38)	18.1	0.96	1.49	(1.22, 1.77)	-10.2	0.36
Total meat intake, tertiles																	
1	34	0.30	(0.25, 0.35)	-		0.27	(0.22, 0.32)	-		0.34	(0.27, 0.41)	-		1.47	(1.17, 1.78)	-	
2	35	0.23	(0.19, 0.27)	-23.2		0.20	(0.16, 0.24)	-26.3		0.27	(0.21, 0.32)	-21.7		1.67	(1.44, 1.89)	13.0	
3	35	0.20	(0.16, 0.25)	-32.8	0.03	0.18	(0.13, 0.22)	-35.2	0.03	0.24	(0.17, 0.30)	-30.7	0.08	1.55	(1.29, 1.82)	5.4	0.75
Total vegetable and fruit intake, tertiles																	
1	34	0.26	(0.21, 0.31)	-		0.24	(0.19, 0.28)			0.29	(0.22, 0.36)	-		1.57	(1.32, 1.83)	-	
2	35	0.23	(0.19, 0.27)	-11.9		0.21	(0.17, 0.25)	-12.1		0.26	(0.20, 0.32)	-10.4		1.71	(1.49, 1.94)	8.9	
3	35	0.24	(0.19, 0.29)	-8.0	0.83	0.20	(0.15, 0.25)	-15.3	0.51	0.29	(0.22, 0.36)	-0.3	0.81	1.46	(1.21, 1.72)	-6.9	0.55
Serum 25-OH-vitamin D (ng/mL)																	
<17.9	34	0.23	(0.19, 0.28)	-		0.20	(0.16, 0.24)	-		0.28	(0.22, 0.34)	-		1.37	(1.13, 1.61)	-	
17.9 - 26.9	35	0.22	(0.17, 0.27)	-4.0		0.20	(0.15, 0.25)	-1.6		0.26	(0.19, 0.32)	-8.0		1.62	(1.38, 1.86)	18.0	
>26.9	35	0.29	(0.24, 0.34)	25.0	0.24	0.26	(0.21, 0.31)	30.8	0.19	0.32	(0.25, 0.40)	16.2	0.57	1.77	(1.49, 2.04)	28.9	0.03

Abbreviations: CI, confidence interval; NSAID, non-steroidal anti-inflammatory drug; OD, optical density.

[a]Using general linear models, adjusted for staining batch and measured confounding variables listed in Supplementary Table S1.

[b]Measured using automated immunohistochemistry with image analysis.

[c]APC expression divided by β-catenin expression.

[d]MSH2 expression divided by mib-1 expression.

[e]Calculated as (comparison group mean - reference group mean) / (reference group mean) x 100%.

[f]Take at least once a week.

[g]Dietary plus supplemental intake.

Table S1. Model covariates[a] for multivariable-adjusted general linear models used for comparisons of mean biomarker expression[b] in the normal-appearing colorectal mucosa of sporadic colorectal adenoma patients (n = 104)

Biomarkers	Characteristics	Model covariates
APC	Age	Aspirin, smoking, saturated fat, dietary fiber
	Sex	Fiber
	Aspirin	Smoking, saturated fat, dietary fiber
	Other NSAID	Physical activity, total energy, saturated fat, vegetables and fruits
	Smoking	Aspirin, physical activity, saturated fat, meat, total energy
	Physical activity	Total energy, saturated fat, dietary fiber, vegetables and fruits
	BMI	Total fat, meat, total energy
	Total energy	Saturated fat, calcium, dietary fiber, meat
	Saturated fat	Total energy, meat
	Total fat	BMI, total energy, vitamin E, meat
	Calcium[e]	Total energy, saturated fat, vitamin D
	Dietary fiber	Total energy, saturated fat, meat
	Vitamin D[f]	Total energy, calcium, meat, vegetables and fruits
	Vitamin E	Aspirin, smoking, total energy, total fat, dietary fiber, meat, vegetables and fruits
	Meat	Total energy, saturated fat
	Vegetables and fruits	Physical activity, total energy, saturated fat, calcium, fiber, meat
β-catenin	Age	Smoking, saturated fat, total energy, calcium
	Sex	Vitamin E, BMI, saturated fat, meat, total energy
	Aspirin	Smoking, calcium, saturated fat, dietary fiber
	Other NSAID	Physical activity, BMI, saturated fat, meat, total energy
	Smoking	Vitamin E, physical activity, total energy
	Physical activity	Vitamin E, smoking, BMI, total fat, meat, total energy
	BMI	Physical activity, total fat, total energy
	Total energy	Saturated fat, vitamin E, calcium, meat
	Saturated fat	Physical activity, dietary fiber, meat total energy
	Total fat	Vitamin E, meat, total energy
	Calcium	Saturated fat, total energy, vegetables and fruits
	Dietary fiber	Saturated fat, calcium, vitamin D, meat, vegetables and fruits, total energy
	Vitamin D	Saturated fat, total energy, calcium
	Vitamin E	Total fat, meat, total energy
	Meat	Total energy, physical activity, total fat
	Vegetables and fruits	Total energy, vitamin E, physical activity, saturated fat, dietary fiber, meat
E-cadherin	Age	Physical activity,total fat, calcium, vegetables and fruits, total energy
	Sex	Age, physical activity
	Aspirin	Physical activity, total energy, calcium, vitamin D, meat, vegetables and fruits
	Other NSAID	Physical activity, saturated fat, total energy, vitamin D, meat, vegetables and fruits
	Smoking	Physical activity, total energy, saturated fat, vitamin D, vegetables and fruits
	Physical activity	Age, saturated fat, vegetables and fruits, total energy
	BMI	Physical activity, saturated fat, total energy, vitamin D, meat, vegetables and fruits
	Total energy	Calcium, vitamin D, vegetables and fruits, total energy
	Saturated fat	Physical activity, calcium, vitamin D, vegetables and fruits, total energy
	Total fat	Vitamin E, vitamin D, vegetables and fruits, total energy
	Calcium	Total energy, saturated fat, vitamin D, meat, vegetables and fruits, total energy
	Dietary fiber	Age, physical activity, total energy, calcium, meat
	Vitamin D	Calcium
	Vitamin E	Physical activity, total fat, vitamin D
	Meat	Total energy, total fat, calcium, vitamin D, vegetables and fruits
	Vegetables and fruits	Total energy, physical activity, saturated fat
MSH2	Age	Aspirin, other NSAID, calcium, meat, vegetables and fruits, total energy
	Sex	Vitamin E, aspirin, physical activity, calcium
	Aspirin	Calcium
	Other NSAID	Age, BMI, total energy, meat, vegetables and fruits
	Smoking	Age, aspirin, physical activity, total energy, calcium, dietary fiber, meat
	Physical activity	Other NSAID, saturated fat, BMI, total energy, dietary fiber, meat, vegetables and fruits
	BMI	Other NSAID, aspirin, saturated fat, total energy
	Total energy	Other NSAID, saturated fat, calcium, vitamin D, meat
	Saturated fat	Vitamin E, age, total energy, dietary fiber, meat, vegetables and fruits
	Total fat	Vitamin E, total energy, BMI, calcium, dietary fiber, meat
	Calcium	Total energy, vitamin D
	Dietary fiber	Aspirin, totla energy, saturated fat, calcium, vitamin D, vegetables and fruits
	Vitamin D	Total energy, calcium
	Vitamin E	Total energy, calcium, vitamin D, dietary fiber, meat, vegetables and fruits
	Meat	Total energy, physical activity, saturated fat, calcium, vegetables and fruits
	Vegetables and fruits	Total energy, saturated fat, vitamin D, dietary fiber, meat
APC/β-catenin[c]	Age	Aspirin, smoking, saturated fat, dietary fiber, calcium
	Sex	Fiber, vitamin E, BMI, saturated fat, meat, total energy
	Aspirin	Smoking, calcium, saturated fat, dietary fiber
	Other NSAID	Physical activity, BMI, total energy, saturated fat, vegetables and fruits
	Smoking	Aspirin, vitamin E, physical activity, saturated fat, meat, total energy
	Physical activity	Total energy, meat, smoking, vitamin E, saturated fat, dietary fiber, vegetables and fruits
	BMI	Total fat, physical activity, meat, total energy
	Total energy	Saturated fat, vitamin E, calcium, dietary fiber, meat
	Saturated fat	Total energy, vitamin E, meat
	Total fat	BMI, total energy, vitamin E, meat
	Calcium	Total energy, vegetables and fruits, saturated fat, vitamin D
	Dietary fiber	Saturated fat, calcium, vitamin D, meat, vegetables and fruits, total energy
	Vitamin D	Total energy, saturated fat, calcium, meat, vegetables and fruits
	Vitamin E	Aspirin, smoking, total energy, total fat, dietary fiber, meat, vegetables and fruits
	Meat	Total energy, physical activity, saturated fat
	Vegetables and fruits	Physical activity, vitamin E, total energy, saturated fat, calcium, dietary fiber, meat
MSH2/mib-1[d]	Age	Aspirin, other NSAID, calcium, meat, vegetables and fruits, total energy
	Sex	Vitamin E, aspirin, physical activity, calcium
	Aspirin	Calcium
	Other NSAID	Age, BMI, total energy, meat, vegetables and fruits
	Smoking	Age, aspirin, physical activity, total energy, calcium, dietary fiber, meat
	Physical activity	Other NSAID, saturated fat, BMI, total energy, dietary fiber, meat, vegetables and fruits
	BMI	Other NSAID, aspirin, saturated fat, total energy
	Total energy	Other NSAID, saturated fat, calcium, vitamin D, meat
	Saturated fat	Vitamin E, age, total energy, dietary fiber, meat, vegetables and fruits
	Total fat	Vitamin E, total energy, BMI, calcium, dietary fiber, meat
	Calcium	Total energy, vitamin D
	Dietary fiber	Aspirin, totla energy, saturated fat, calcium, vitamin D, vegetables and fruits
	Vitamin D	Total energy, calcium
	Vitamin E	Total energy, calcium, vitamin D, dietary fiber, meat, vegetables and fruits
	Meat	Total energy, physical activity, saturated fat, calcium, vegetables and fruits
	Vegetables and fruits	Total energy, saturated fat, vitamin D, dietary fiber, meat

Abbreviations: NSAID, non-steroidal anti-inflammatory drug; BMI, body mass index.

[a]Models also included staining batch.

[b]Measured using automated immunohistochemistry with image analysis.

[c]APC expression divided by β-catenin expression.

[d]MSH2 expression divided by mib-1 expression.

[e]Dietary plus supplemental calcium.

[f]Serum 25-OH-vitamin D.

Table S2. Comparisons[a] of minimally-adjusted mean APC expression[b] in the normal-appearing colorectal mucosa of sporadic colorectal adenoma patients (n = 104), by selected participant characteristics

Characteristics	n	Whole crypt, mean (OD)	95% CI	Proportional difference[c] (%)	P-value	Upper 40% of crypts, mean (OD)	95% CI	Proportional difference[c] (%)	P-value	Lower 60% of crypts, mean (OD)	95% CI	Proportional difference[c] (%)	P-value
Age (years)													
47 - 54	34	2,502	(2,148, 2,856)	-		979	(817, 1,141)	-		1,389	(1,157, 1,621)	-	
55 - 62	35	2,576	(2,224, 2,928)	3.0		1,040	(879, 1,201)	6.3		1,384	(1,153, 1,615)	-0.3	
63 - 75	35	2,457	(2,106, 2,808)	-1.8	0.88	1,026	(866, 1,186)	4.8	0.68	1,275	(1,045, 1,505)	-8.2	0.51
Sex													
Male	48	2,714	(2,338, 3,089)	-		1,074	(905, 1,242)	-		1,483	(1,261, 1,706)	-	
Female	56	2,338	(1,990, 2,685)	-13.9	0.15	966	(810, 1,122)	-10.0	0.36	1,231	(1,026, 1,437)	-17.0	0.10
Regular[d] use of aspirin													
No	64	2,586	(2,267, 2,904)	-		1,078	(933, 1,223)	-		1,350	(1,141, 1,560)	-	
Yes	40	2,465	(2,221, 2,708)	-4.7	0.58	977	(866, 1,088)	-9.4	0.30	1,346	(1,186, 1,506)	-0.3	0.98
Regular[d] use of other NSAID													
No	69	2,488	(2,152, 2,824)	-		953	(801, 1,106)	-		1,395	(1,175, 1,475)	-	
Yes	35	2,523	(2,293, 2,754)	1.4	0.87	1,048	(943, 1,152)	9.9	0.34	1,324	(1,172, 1,475)	-5.2	0.61
Currently smoke													
No	96	2,538	(2,349, 2,726)	-		1,029	(943, 1,115)	-		1,358	(1,234, 1,482)	-	
Yes	8	2,196	(1,480, 2,912)	-13.5	0.37	856	(529, 1,183)	-16.9	0.32	1,226	(754, 1,698)	-9.7	0.60
Physical activity													
Low	30	2,425	(2,051, 2,798)	-		1,012	(841, 1,182)	-		1,260	(1,016, 1,505)	-	
Moderate	38	2,545	(2,204, 2,886)	4.9		1,027	(872, 1,183)	1.6		1,366	(1,143, 1,589)	8.4	
High	27	2,518	(2,206, 2,830)	3.8	0.74	1,002	(859, 1,144)	-1.0	0.91	1,375	(1,171, 1,580)	9.1	0.52
Body mass index (kg/m^2)													
<25.0	22	2,477	(2,047, 2,907)	-		986	(789, 1,182)	-		1,360	(1,077, 1,642)	-	
25.0 - 29.9	43	2,575	(2,270, 2,880)	4.0		1,046	(907, 1,186)	6.1		1,379	(1,179, 1,579)	1.4	
≥30	39	2,461	(2,139, 2,782)	0.1	0.89	999	(852, 1,146)	1.4	0.99	1,307	(1,096, 1,518)	-3.9	0.73
Total energy, tertiles													
1	34	2,813	(2,476, 3,149)	-		1,116	(960, 1,271)	-		1,548	(1,327, 1,768)	-	
2	35	2,355	(2,017, 2,692)	-16.3		937	(781, 1,094)	-16.0		1,279	(1,058, 1,500)	-17.4	
3	35	2,388	(2,057, 2,719)	-15.1	0.08	1,003	(850, 1,156)	-10.1	0.31	1,230	(1,014, 1,447)	-20.5	0.05
Saturated fat, tertiles													
1	34	2,756	(2,424, 3,089)	-		1,116	(961, 1,270)	-		1,482	(1,266, 1,698)	-	
2	35	2,571	(2,238, 2,905	-6.7		974	(820, 1,129)	-12.7		1,461	(1,244, 1,678)	-1.4	
3	35	2,203	(1,844, 2,562)	-20.1	0.04	960	(794, 1,126)	-14.0	0.17	1,094	(861, 1,327)	-26.2	0.03
Total fat, tertiles													
1	34	2,602	(2,267, 2,938)	-		1,047	(891, 1,203)	-		1,406	(1,189, 1,623)	-	
2	35	2,667	(2,335, 2,999)	2.5		1,027	(873, 1,182)	-1.9		1,497	(1,283, 1,711)	6.5	
3	35	2,256	(1,915, 2,596)	-13.3	0.17	972	(814, 1,131)	-7.1	0.52	1,132	(912, 1,352)	-19.5	0.10
Total[e] vitamin E, tertiles													
1	34	2,448	(2,087, 2,810)	-		1,001	(835, 1,166)	-		1,306	(1070, 1,543)	-	
2	35	2,495	(2,156, 2,834)	1.9		1,027	(871, 1,182)	2.6		1,310	(1,088, 1,532)	0.3	
3	35	2,589	(2,230, 2,949)	5.6	0.61	1,020	(855, 1,184)	1.9	0.88	1,425	(1,190, 1,661)	9.1	0.51
Total[e] calcium, tertiles													
1	34	2,521	(2,156, 2,885)	-		1,011	(845, 1,177)	-		1,361	(1,123, 1,600)	-	
2	35	2,509	(2,134, 2,884)	-0.5		990	(819, 1,161)	-2.1		1,377	(1,132, 1,623)	1.2	
3	35	2,504	(2,150, 2,859)	-0.7	0.95	1,046	(884, 1,207)	3.4	0.77	1,305	(1,073, 1,538)	-4.1	0.74
Dietary fiber, tertiles													
1	34	2,297	(1,915, 2,679)	-		993	(817, 1,170)	-		1,145	(899, 1,391)	-	
2	35	2,505	(2,162, 2,847)	9.1		1,027	(869, 1,186)	3.4		1,329	(1,108, 1,550)	16.1	
3	35	2,726	(2,358, 3,094)	18.7	0.15	1,026	(856, 1,197)	3.3	0.81	1,564	(1,326, 1,801)	36.6	0.03
Total meat intake, tertiles													
1	34	2,841	(2,415, 3,267)	-		1,097	(898, 1,296)	-		1,586	(1,307, 1,866)	-	
2	35	2,537	(2,206, 2,868)	-11.6		1,072	(918, 1,226)	-2.3		1,307	(1,090, 1,524)	-17.6	
3	35	2,228	(1,846, 2,610)	-20.9	0.08	902	(724, 1,080)	-17.8	0.23	1,196	(946, 1,446)	-24.6	0.09
Total vegetable and fruit intake, tertiles													
1	34	2,649	(2,288, 3,011)	-		1,088	(921, 1,254)	-		1,406	(1,168, 1,643)	-	
2	35	2,406	(2,067, 2,745)	-9.3		948	(792, 1,104)	-12.8		1,324	(1,101, 1,547)	-5.8	
3	35	2,506	(2,151, 2,861)	-5.1	0.64	1,024	(860, 1,187)	-5.9	0.66	1,328	(1,094, 1,562)	-5.5	0.69
Serum 25-OH-vitamin D (ng/mL)													
<17.9	34	2,548	(2,209, 2,887)	-		1,020	(863, 1,178)	-		1,370	(1,148, 1,593)	-	
17.9 - 26.9	35	2,214	(1,870, 2,559)	-13.1		921	(761, 1,081)	-9.8		1,157	(930, 1,383)	-15.6	
>26.9	35	2,779	(2,425, 3,134)	9.1	0.44	1,109	(945, 1,274)	8.7	0.51	1,521	(1,288, 1,754)	11.0	0.44

Abbreviations: CI, confidence interval; NSAID, non-steroidal anti-inflammatory drug; OD, optical density.

[a]Using general linear models, adjusted for staining batch.

[b]Measured using automated immunohistochemistry with image analysis.

[c]Calculated as (comparison group mean - reference group mean) / (reference group mean) x 100%.

[d]Take at least once a week.

[e]Dietary plus supplemental intake.

Table S3. Comparisons[a] of minimally-adjusted mean β-catenin expression[b] in the normal-appearing colorectal mucosa of sporadic colorectal adenoma patients (n = 104), by selected participant characteristics

Characteristics	n	Whole crypt, mean (OD)	95% CI	Proportional difference[c] (%)	P-value	Upper 40% of crypts, mean (OD)	95% CI	Proportional difference[c] (%)	P-value	Lower 60% of crypts, mean (OD)	95% CI	Proportional difference[c] (%)	P-value
Age (years)													
47 - 54	34	10,364	(9,295, 11,434)	-		3,917	(3,149, 4,686)	-		6,288	(5,643, 6,933)	-	
55 - 62	35	10,516	(9,476, 11,557)	1.5		3,818	(3,071, 4,566)	-2.5%		6,363	(5,736, 6,990)	1.2	
63 - 75	35	10,671	(9,612, 11,730)	3.0	0.70	4,354	(3,594, 5,115)	11.1%	0.46	6,330	(5,692, 6,968)	0.7	0.93
Sex													
Male	48	10,813	(10,034, 11,593)	-		4,380	(3,805, 4,954)			6,524	(6,055, 6,993)	-	
Female	56	10,272	(9,550, 10,993)	-5.0	0.31	3,740	(3,208, 4,272)	-14.6%	0.11	6,159	(5,725, 6,594)	-5.6	0.26
Regular[d] use of aspirin													
No	64	10,496	(9,548, 11,443)	-		4,032	(3,347, 4,564)			6,313	(5,742, 6,883)	-	
Yes	40	10,538	(9,808, 11,266)	0.4	0.95	4,038	(3,511, 4,564)	0.2%	0.99	6,337	(5,898, 6,776)	0.4	0.95
Regular[d] use of other NSAID													
No	69	10,600	(9,591, 11,609)	-		4,377	(3,653, 5,100)			6,371	(5,763, 6,979)	-	
Yes	35	10,481	(9,787, 11,176)	-1.1	0.85	3,862	(3,365, 4,360)	-11.8%	0.27	6,306	(5,888, 6,742)	-1.0	0.87
Currently smoke													
No	96	10,460	(9,890, 11,029)	-		4,007	(3,595, 4,419)			6,298	(5,954, 6,641)	-	
Yes	8	11,264	(9,127, 13,401)	7.7	0.48	4,373	(2,826, 5,919)	9.1%	0.65	6,688	(5,399, 7,977)	6.2	0.57
Physical activity													
Low	30	10,681	(9,549, 11,813)	-		3,913	(3,101, 4,726)			6,430	(5,747, 7,112)	-	
Moderate	38	10,799	(9,763, 11,835)	1.1		3,707	(2,964, 4,450)	-5.3%		6,487	(5,863, 7,112)	0.9	
High	27	10,255	(9,338, 11,171)	-4.0	0.51	4,435	(3,778, 5,093)	13.3%	0.25	6,167	(5,615, 6,719)	-4.1	0.50
Body mass index (kg/m^2)													
<25.0	22	9,965	(8,692, 11,237)	-		4,520	(3,604, 5,437)			5,992	(5,226, 6,759)	-	
25.0 - 29.9	43	10,825	(9,942, 11,708)	8.6		3,801	(3,165, 4,437)	-15.9%		6,524	(5,992, 7,055)	8.9	
≥30	39	10,501	(9,545, 11,457)	5.4	0.62	4,020	(3,331, 4,709)	-11.1%	0.50	6,301	(5,725, 6,877)	5.2	0.65
Total energy, tertiles													
1	34	9,877	(8,859, 10,895)	-		4,438	(3,690, 5,185)			5,980	(5,365, 6,596)	-	
2	35	11,477	(10,481, 12,472)	16.2		4,059	(3,328, 4,791)	-8.5%		6,870	(6,268, 7,472)	14.9	
3	35	10,147	(9,782, 11,111)	2.7	0.80	3,631	(2,923, 4,340)	-18.2%	0.12	6,097	(5,514, 6,680)	2.0	0.88
Saturated fat, tertiles													
1	34	10,808	(9,805, 11,812)	-		3,826	(3,104, 4,547)			6,532	(5,929, 7,135)	-	
2	35	11,076	(10,115, 12,036)	2.5		4,661	(3,971, 5,351)	21.9%		6,665	(6,089, 7,242)	2.0	
3	35	9,648	(8,601, 10,694)	-10.7	0.16	3,582	(2,830, 4,334)	-6.4%	0.78	5,766	(5,138, 6,395)	-11.7	0.12
Total fat, tertiles													
1	34	11,190	(10,188, 12,192)	-		3,973	(3,238, 4,708)			6,717	(6,114, 7,320)	-	
2	35	10,507	(9,554, 11,460)	-6.1		4,283	(3,584, 4,981)	7.8%		6,358	(5,784, 6,931)	-5.4	
3	35	9,868	(8,876, 10,861)	-11.8	0.07	3,836	(3,108, 4,563)	-3.5%	0.79	5,907	(5,310, 6,504)	-12.1	0.07
Total[e] vitamin E, tertiles													
1	34	11,258	(10,181, 12,336)	-		3,871	(3,088, 4,654)			6,794	(6,145, 7,443)	-	
2	35	10,440	(9,453, 11,427)	-7.3		3,772	(3,055, 4,490)	-2.6%		6,235	(5,641 6,830)	-8.2	
3	35	9,888	(8,862, 10,913)	-12.2	0.09	4,458	(3,713, 5,203)	15.2%	0.29	5,967	(5,350, 6,585)	-12.2	0.09
Total[e] calcium, tertiles													
1	34	10,120	(9,051, 11,189)	-		3,694	(2,926, 4,463)			6,068	(5,424, 6,713)	-	
2	35	10,479	(9,418, 11,541)	3.5		4,498	(3,735, 5,261)	21.8%		6,350	(5,710, 6,960)	4.7	
3	35	10,964	(9,953, 11,975)	8.3	0.27	3,927	(3,200, 4,653)	6.3%	0.77	6,566	(5,956, 7,175)	8.2	0.29
Dietary fiber, tertiles													
1	34	10,583	(9,456, 11,711)	-		3,505	(2,704, 4,306)			6,341	(5,662, 7,020)	-	
2	35	10,293	(9,197, 11,390)	-2.7		4,479	(3,700, 5,259)	27.8%		6,200	(5,539, 6,861)	-2.2	
3	35	10,690	(9,521, 11,859)	1.0	0.93	4,106	(3,276, 4,937)	17.2%	0.30	6,442	(5,738, 7,147)	1.6	0.88
Total meat intake, tertiles													
1	34	9,580	(8,316, 10,843)	-		4,142	(3,204, 5,079)			5,763	(5,000, 6,526)	-	
2	35	10,951	(9,964, 11,939)	14.3		3,709	(2,976, 4,442)	-10.4%		6,631	(6,035, 7,228)	15.1	
3	35	10,859	(9,737, 11,981)	13.3	0.31	4,270	(3,437, 5,103)	3.1%	0.80	6,491	(5,813, 7,169)	12.6	0.35
Total vegetable and fruit intake, tertiles													
1	34	10,358	(9,329, 11,387)	-		3,741	(2,995, 4,487)			6,179	(5,558, 6,801)	-	
2	35	10,550	(9,554, 11,547)	1.9		4,373	(3,652, 5,094)	16.9%		6,328	(5,726, 6,930)	2.4	
3	35	10,591	(9,515, 11,666)	2.2	0.72	3,972	(3,196, 4,748)	6.2%	0.64	6,442	(5,793, 7,092)	4.3	0.55
Serum 25-OH-vitamin D (ng/mL)													
<17.9	34	10,424	(9,390, 11,459)	-		4,004	(3,262, 4,746)			6,284	(5,659, 6,908)	-	
17.9 - 26.9	35	10,323	(9,328, 11,317)	-1.0		4,372	(3,658, 5,086)	9.2%		6,254	(5,653, 6,854)	-0.5	
>26.9	35	10,826	(9,781, 11,871)	3.9	0.61	3,721	(2,971, 4,471)	-7.1%	0.62	6,450	(5,819, 7,080)	2.6	0.72

Abbreviations: CI, confidence interval; NSAID, non-steroidal anti-inflammatory drug; OD, optical density.

[a]Using general linear models, adjusted for staining batch.

[b]Measured using automated immunohistochemistry with image analysis.

[c]Calculated as (comparison group mean - reference group mean) / (reference group mean) x 100%.

[d]Take at least once a week.

[e]Dietary plus supplemental intake.

Table S4. Comparisons[a] of minimally-adjusted mean E-cadherin expression[b] in the normal-appearing colorectal mucosa of sporadic colorectal adenoma patients (n = 104), by selected participant characteristics

Characteristics	n	Whole crypt, mean (OD)	95% CI	Proportional difference[c] (%)	P-value	Upper 40% of crypts, mean (OD)	95% CI	Proportional difference[c] (%)	P-value	Lower 60% of crypts, mean (OD)	95% CI	Proportional difference[c] (%)	P-value
Age (years)													
47 - 54	34	4,611	(4,027, 5,195)	-		3,100	(2,014, 4,185)	-		2,721	(2,360, 3,081)	-	
55 - 62	35	4,795	(4,234, 5,355)	4.0		1,963	(922, 3,004)	-36.7		2,880	(2,534, 3,226)	5.9	
63 - 75	35	4,363	(3,793, 4,934)	-5.4	0.58	1,966	(906, 3,027)	-36.6	0.16	2,551	(2,198, 2,903)	-6.3	0.54
Sex													
Male	48	4,924	(4,388, 5,459)	-		2,700	(1,708, 3,693)	-		2,948	(2,615, 3,281)	-	
Female	56	4,299	(3,803, 4,794)	-12.7	0.09	2,003	(1,084, 2,922)	-25.8	0.31	2,516	(2,208, 2,824)	-14.7	0.06
Regular[d] use of aspirin													
No	64	4,619	(4,103, 5,136)	-		2,400	(1,429, 3,371)	-		2,736	(2,415, 3,056)	-	
Yes	40	4,567	(4,171, 4,963)	-1.1	0.88	2,278	(1,534, 3,022)	-5.1	0.85	2,703	(2,457, 2,948)	-1.2	0.88
Regular[d] use of other NSAID													
No	69	4,575	(4,023, 5,126)	-		1,848	(819, 2,876)	-		2,709	(2,368, 3,051)	-	
Yes	35	4,593	(4,216, 4,971)	0.4	0.96	2,567	(1,864, 3,270)	38.9	0.28	2,718	(2,485, 2,952)	0.3	0.97
Currently smoke													
No	96	4,608	(4,300, 4,917)	-		2,233	(1,657, 2,809)	-		2,731	(2,540, 2,922)	-	
Yes	8	4,332	(3,153, 5,511)	-6.0	0.66	3,426	(1,224, 5,629)	53.4	0.31	2,525	(1,795, 3,255)	-7.5	0.59
Physical activity													
Low	30	4,686	(4,093, 5,280)	-		2,361	(1,293, 3,428)	-		2,797	(2,428, 3,165)	-	
Moderate	38	4,741	(4,197, 5,285)	1.2		3,465	(2,486, 4,444)	46.8		2,784	(2,446, 3,122)	-0.5	
High	27	4,438	(3,941, 4,934)	-5.3	0.49	1,395	(502, 2,289)	-40.9	0.11	2,626	(2,317, 2,934)	-6.1	0.47
Body mass index (kg/m^2)													
<25.0	22	4,413	(3,710, 5,116)	-		1,118	(-170, 2,405)	-		2,606	(2,170, 3,041)	-	
25.0 - 29.9	43	4,615	(4,118, 5,112)	4.6		2,667	(1,756, 3,578)	138.6		2,735	(2,427, 3,043)	5.0	
≥30	39	4,654	(4,134, 5,175)	5.5	0.61	2,628	(1,675, 3,581)	135.1	0.11	2,756	(2,434, 3,078)	5.8	0.61
Total energy, tertiles													
1	34	4,543	(3,993, 5,093)	-		1,805	(758, 2,852)	-		2,718	(2,375, 3,062)	-	
2	35	4,245	(3,706, 4,784)	-6.6		2,640	(1,614, 3,666)	46.3		2,538	(2,201, 2,875)	-6.6	
3	35	4,981	(4,445, 5,517)	9.6	0.27	2,490	(1,470, 3,511)	38.0	0.36	2,895	(2,559, 3,230)	6.5	0.47
Saturated fat, tertiles													
1	34	4,262	(3,721, 4,803)	-		2,069	(1,033, 3,105)	-		2,539	(2,203, 2,874)	-	
2	35	4,985	(4,443, 5,526)	16.9		2,581	(1,545, 3,618)	24.8		2,971	(2,636, 3,307)	17.1	
3	35	4,491	(3,902, 5,081)	5.4	0.44	2,308	(1,179, 3,438)	11.6	0.71	2,621	(2,256, 2,987)	3.3	0.58
Total fat, tertiles													
1	34	4,430	(3,880, 4,980)	-		2,369	(1,330, 3,408)	-		2,598	(2,259, 2,938)	-	
2	35	4,855	(4,307, 5,402)	9.6		2,056	(1,021, 3,090)	-13.2		2,907	(2,569, 3,245)	11.9	
3	35	4,461	(3,905, 5,016)	0.7	0.93	2,565	(1,516, 3,615)	8.3	0.80	2,629	(2,286, 2,973)	1.2	0.88
Total[e] vitamin E, tertiles													
1	34	4,716	(4,125, 5,308)	-		2,471	(1,368, 3,573)	-		2,806	(2,440, 3,172)	-	
2	35	4,507	(3,953, 5,061)	-4.4		2,717	(1,684, 3,749)	10.0		2,643	(2,299, 2,986)	-5.8	
3	35	4,541	(3,963, 5,119)	-3.7	0.70	1,791	(714, 2,867)	-27.5	0.41	2,700	(2,343, 3,058)	-3.8	0.71
Total[e] calcium, tertiles													
1	34	4,646	(4,067, 5,224)	-		2,421	(1,329, 3,513)	-		2,737	(2,379, 3,095)	-	
2	35	4,346	(3,748, 4,944)	-6.4		2,403	(1,274, 3,532)	-0.7		2,567	(2,196, 2,937)	-6.2	
3	35	4,762	(4,194, 5,331)	2.5	0.77	2,152	(1,078, 3,225)	-11.1	0.73	2,838	(2,486, 3,191)	3.7	0.68
Dietary fiber, tertiles													
1	34	4,628	(4,003, 5,254)	-		2,635	(1,498, 3,771)	-		2,728	(2,341, 3,116)	-	
2	35	4,454	(3,889, 5,019)	-3.8		1,309	(283, 2,335)	-50.3		2,627	(2,277, 2,977)	-3.7	
3	35	4,680	(4,090, 5,270)	1.1	0.88	3,039	(1968, 4,110)	15.4	0.53	2,791	(2,426, 3,157)	2.3	0.80
Total meat intake, tertiles													
1	34	4,734	(4,023, 5,446)	-		1,626	(288, 2,964)	-		2,759	(2,311, 3,206)	-	
2	35	4,315	(3,760, 4,870)	-8.9		3,068	(2,024, 4,112)	88.7		2,617	(2,268, 2,966)	-5.1	
3	35	4,722	(4,084, 5,361)	-0.3	0.89	2,189	(988, 3,389)	34.6	0.70	2,775	(2,373, 3,177)	0.6	0.87
Total vegetable and fruit intake, tertiles													
1	34	4,084	(3,507, 4,662)	-		3,028	(1,931, 4,124)	-		2,412	(2,051, 2,773)	-	
2	35	4,816	(4,267, 5,365)	17.9		1,400	(357, 2,442)	-53.8		2,810	(2,467, 3,154)	16.5	
3	35	4,861	(4,294, 5,429)	19.0	0.09	2,559	(1,481, 3,638)	-15.5	0.64	2,928	(2,573, 3,284)	21.4	0.07
Serum 25-OH-vitamin D (ng/mL)													
<17.9	34	5,146	(4,600, 5,691)	-		2,191	(1,129, 3,253)	-		3,021	(2,680, 3,362)	-	
17.9 - 26.9	35	4,298	(3,738, 4,858)	-16.5		2,246	(1,157, 3,335)	2.5		2,579	(2,230, 2,929)	-14.6	
>26.9	35	4,309	(3,738, 4,880)	-16.3	0.04	2,543	(1,433, 3,654)	16.1	0.67	2,540	(2,184, 2,897)	-15.9	0.06

Abbreviations: CI, confidence interval; NSAID, non-steroidal anti-inflammatory drug; OD, optical density.

[a]Using general linear models, adjusted for staining batch.

[b]Measured using automated immunohistochemistry with image analysis.

[c]Calculated as (comparison group mean - reference group mean) / (reference group mean) x 100%.

[d]Take at least once a week.

[e]Dietary plus supplemental intake.

Table S5. Comparisons[a] of minimally-adjusted mean MSH2 expression[b] in the normal-appearing colorectal mucosa of sporadic colorectal adenoma patients (n = 104), by selected participant characteristics

Characteristics	n	Whole crypt, mean (OD)	95% CI	Proportional difference[c] (%)	P-value	Upper 40% of crypts, mean (OD)	95% CI	Proportional difference[c] (%)	P-value	Lower 60% of crypts, mean (OD)	95% CI	Proportional difference[c] (%)	P-value
Age (years)													
47 - 54	34	1,947	(1,709, 2,184)	-		335	(-483, 1,154)	-		1,521	(1,340, 1,702)	-	
55 - 62	35	1,806	(1,574, 2,037)	-7.2		283	(-516, 1,081)	-15.8		1,449	(1,273, 1,625)	-4.7	
63 - 75	35	1,783	(1,548, 2,018)	-8.4	0.35	988	(179, 1,797)	194.7	0.30	1,394	(1,216, 1,573)	-8.3	0.34
Sex													
Male	48	1,962	(1,763, 2,161)	-		803	(193, 1,412)	-		1,562	(1,406, 1,718)	-	
Female	56	1,740	(1,556, 1,924)	-11.3	0.11	322	(-243, 886)	-60.0	0.25	1,359	(1,215, 1,504)	-13.0	0.06
Regular[d] use of aspirin													
No	64	2,030	(1,825, 2,235)	-		422	(-310, 1,153)	-		1,582	(1,425, 1,739)	-	
Yes	40	1,725	(1,567, 1,883)	-15.0	0.03	620	(58, 1,182)	47.0	0.69	1,372	(1,252, 1,493)	-13.2	0.05
Regular[d] use of other NSAID													
No	69	1,898	(1,675, 2,120)	-		937	(173, 1,700)	-		1,492	(1,324, 1,661)	-	
Yes	35	1,814	(1,661, 1,968)	-4.4	0.56	344	(-183, 871)	-63.3	0.23	1,433	(1,316, 1,549)	-4.0	0.58
Currently smoke													
No	96	1,850	(1,723, 1,977)	-		613	(177, 1,050)	-		1,460	(1,364, 1,557)	-	
Yes	8	1,753	(1,276, 2,230)	-5.2	0.70	-293	(-1,932, 1,346)	-147.8	0.30	1,363	(1,001, 1,724)	-6.7	0.61
Physical activity													
Low	30	1,782	(1,533, 2,031)	-		523	(-334, 1,380)	-		1,403	(1,215, 1,592)	-	
Moderate	38	1,912	(1,685, 2,140)	7.3		180	(-604, 963)	-65.7		1,509	(1,337, 1,681)	7.5	
High	27	1,845	(1,636, 2,054)	3.5	0.77	867	(147, 1,586)	65.5	0.48	1,456	(1,298, 1,614)	3.7	0.74
Body mass index (kg/m^2)													
<25.0	22	1,767	(1,479, 2,055)	-		1,441	(471, 2,412)	-		1,422	(1,203, 1,640)	-	
25.0 - 29.9	43	1,884	(1,682, 2,086)	6.6		405	(-276, 1,087)	-71.9		1,482	(1,328, 1,635)	4.2	
≥30	39	1,839	(1,624, 2,054)	4.1	0.77	190	(-536, 915)	-86.8	0.06	1,439	(1,275, 1,602)	1.2	0.97
Total energy, tertiles													
1	34	1,752	(1,522, 1,983)	-		968	(173, 1,763)	-		1,369	(1,194, 1,544)	-	
2	35	1,820	(1,591, 2,049)	3.9		104	(-686, 893)	-89.3		1,448	(1,275, 1,622)	5.8	
3	35	1,950	(1,726, 2,174)	11.3	0.22	596	(-176, 1,367)	-38.5	0.52	1,537	(1,367, 1,706)	12.2	0.18
Saturated fat, tertiles													
1	34	1,886	(1,654, 2,119)	-		350	(-452, 1,152)	-		1,492	(1,315, 1,668)	-	
2	35	1,823	(1,597, 2,050)	-3.3		721	(-60, 1,502)	105.9		1,451	(1,280, 1,623)	-2.7	
3	35	1,819	(1,575, 2,062)	-3.6	0.69	550	(-292, 1,392)	57.1	0.71	1,416	(1,231, 1,601)	-5.1	0.57
Total fat, tertiles													
1	34	1,839	(1,607, 2,071)	-		322	(-462, 1,107)	-		1,460	(1,284, 1,636)	-	
2	35	1,812	(1,584, 2,040)	-1.5		1,149	(376, 1,921)	256.5		1,430	(1,256, 1,636)	-2.1	
3	35	1,878	(1,644, 2,111)	2.2	0.82	124	(-665, 914)	-61.4	0.75	1,470	(1,293, 1,647)	0.7	0.94
Total[e] vitamin E, tertiles													
1	34	1,908	(1,665, 2,152)	-		300	(-547, 1,146)	-		1,511	(1,325, 1,696)	-	
2	35	1,730	(1,502, 1,957)	-9.3		513	(-277, 1,304)	71.4		1,377	(1,204, 1,550)	-8.8	
3	35	1,891	(1,651, 2,131)	-0.9	0.93	811	(-24, 1,645)	170.7	0.43	1,473	(1,290, 1,655)	-2.5	0.79
Total[e] calcium, tertiles													
1	34	2,085	(1,853, 2,317)	-		376	(-455, 1,207)	-		1,607	(1,429, 1,785)	-	
2	35	1,660	(1,414, 1,906)	-20.4		760	(-121, 1,642)	102.3		1,330	(1,141, 1,518)	-17.3	
3	35	1,776	(1,548, 2,007)	-14.8	0.08	501	(-321, 1,323)	33.4	0.84	1,418	(1,242, 1,594)	-11.7	0.16
Dietary fiber, tertiles													
1	34	1,972	(1,716, 2,228)	-		365	(-513, 1,244)	-		1,539	(1,344, 1,734)	-	
2	35	1,717	(1,487, 1,947)	-12.9		1,139	(351, 1,927)	211.7		1,371	(1,196, 1,546)	-10.9	
3	35	1,842	(1,597, 2,086)	-6.6	0.56	122	(-716, 959)	-66.7	0.65	1,452	(1,266, 1,638)	-5.7	0.60
Total meat intake, tertiles													
1	34	1,963	(1,668, 2,258)	-		1,312	(310, 2,314)	-		1,536	(1,313, 1,759)	-	
2	35	1,914	(1,683, 2,145)	-2.5		-27	(-810, 756)	-102.1		1,515	(1,341, 1,690)	-1.4	
3	35	1,669	(1,405, 1,933)	-15.0	0.20	459	(-439, 1,357)	-65.0	0.41	1,320	(1,120, 1,520)	-14.1	0.21
Total vegetable and fruit intake, tertiles													
1	34	1,828	(1584, 2,072)	-		237	(-589, 1,063)	-		1,444	(1,258, 1,629)	-	
2	35	1,955	(1,726, 2,184)	7.0		1,237	(463, 2,012)	422.4		1,526	(1,353, 1,700)	5.7	
3	35	1,733	(1,494, 1,971)	-5.2	0.57	155	(-653, 962)	-34.7	0.83	1,380	(1,198, 1,561)	-4.4	0.61
Serum 25-OH-vitamin D (ng/mL)													
<17.9	34	1,863	(1,629, 2,097)	-		103	(-691, 897)	-		1,464	(1,286, 1,641)	-	
17.9 - 26.9	35	1,741	(1,501, 1,982)	-6.5		1,220	(403, 2,037)	108.6		1,380	(1,198, 1,563)	-5.7	
>26.9	35	1,925	(1,984, 2,166)	3.3	0.75	301	(-518, 1,121)	192.8	0.70	1,516	(1,333, 1,699)	3.6	0.72

Abbreviations: CI, confidence interval; NSAID, non-steroidal anti-inflammatory drug; OD, optical density.

[a]Using general linear models, adjusted for staining batch.

[b]Measured using automated immunohistochemistry with image analysis.

[c]Calculated as (comparison group mean - reference group mean) / (reference group mean) x 100%.

[d]Take at least once a week.

[e]Dietary plus supplemental intake.

Table S6. Comparisons[a] of minimally-adjusted mean APC/β-catenin expression[b] and MSH2/mib-1 expression in the normal-appearing colorectal mucosa of sporadic colorectal adenoma patients (n = 104), by selected participant character

		APC/β-catenin[c]												MSH2/mib-1[d]			
Characteristics	n	Whole crypt, mean (OD)	95% CI	Proportional difference[e] (%)	P-value	Upper 40% of crypts, mean (OD)	95% CI	Proportional difference[e] (%)	P-value	Lower 60% of crypts, mean (OD)	95% CI	Proportional difference[e] (%)	P-value	Whole crypt, mean (OD)	95% CI	Proportional difference[e] (%)	P-value
Age (years)																	
47 - 54	34	0.25	(0.20, 0.29)	-		0.22	(0.18, 0.26)	-		0.28	(0.22, 0.34)	-		1.68	(1.46, 1.91)	-	
55 - 62	35	0.26	(0.22, 0.30)	6.8		0.23	(0.19, 0.27)	6.8		0.30	(0.24, 0.36)	7.2		1.53	(1.31, 1.75)	-9.3	
63 - 75	35	0.23	(0.18, 0.27)	-8.0	0.56	0.20	(0.16, 0.24)	-8.7	0.56	0.26	(0.20, 0.31)	-9.0	0.57	1.54	(1.32, 1.77)	-8.4	0.39
Sex																	
Male	48	0.26	(0.22, 0.30)	-		0.23	(0.19, 0.27)	-		0.30	(0.24, 0.35)	-		1.69	(1.52, 1.85)	-	
Female	56	0.23	(0.20, 0.27)	-10.0	0.35	0.20	(0.17, 0.24)	-11.1	0.33	0.27	(0.22, 0.32)	-10.0	0.43	1.49	(1.34, 1.64)	-11.5	0.09
Regular[f] use of aspirin																	
No	64	0.25	(0.21, 0.29)	-		0.21	(0.17, 0.25)	-		0.30	(0.25, 0.35)	-		1.76	(1.56, 1.95)	-	
Yes	40	0.24	(0.21, 0.27)	-3.0	0.77	0.22	(0.19, 0.25)	5.4	0.66	0.27	(0.23, 0.31)	-11.9	0.30	1.47	(1.32, 1.62)	-16.2	0.03
Regular[f] use of other NSAID																	
No	69	0.24	(0.20, 0.28)	-		0.23	(0.19, 0.26)	-		0.26	(0.21, 0.31)	-		1.60	(1.38, 1.81)	-	
Yes	35	0.25	(0.22, 0.27)	1.5	0.89	0.21	(0.18, 0.24)	-6.6	0.56	0.29	(0.25, 0.33)	11.2	0.40	1.57	(1.43, 1.72)	-1.3	0.88
Currently smoke																	
No	96	0.25	(0.23, 0.27)	-		0.22	(0.20, 0.24)	-		0.28	(0.25, 0.31)	-		1.60	(1.48, 1.72)	-	
Yes	8	0.20	(0.12, 0.29)	-17.6	0.34	0.19	(0.10, 0.27)	-13.6	0.51	0.23	(0.11, 0.34)	-20.3	0.36	1.30	(0.85, 1.75)	-18.8	0.21
Physical activity																	
Low	30	0.23	(0.18, 0.27)	-		0.20	(0.15, 0.24)	-		0.26	(0.20, 0.32)	-		1.66	(1.43, 1.90)	-	
Moderate	38	0.25	(0.21, 0.29)	10.0		0.22	(0.18, 0.26)	11.9		0.28	(0.23, 0.34)	8.3		1.49	(1.27, 1.70)	-10.7	
High	27	0.25	(0.21, 0.28)	9.3	0.52	0.22	(0.18, 0.26)	12.1	0.44	0.28	(0.23, 0.33)	7.4	0.66	1.60	(1.40, 1.80)	-3.9	0.77
Body mass index (kg/m^2)																	
<25.0	22	0.25	(0.20, 0.31)	-		0.23	(0.18, 0.28)	-		0.29	(0.21, 0.36)	-		1.54	(1.26, 1.79)	-	
25.0 - 29.9	43	0.24	(0.20, 0.28)	-5.5		0.21	(0.18, 0.25)	-8.5		0.28	(0.23, 0.33)	-3.0		1.60	(1.41, 1.79)	3.9	
≥30	39	0.24	(0.20, 0.28)	-4.7	0.76	0.21	(0.17, 0.25)	-8.1	0.62	0.28	(0.23, 0.33)	-2.2	0.91	1.58	(1.38, 1.79)	2.6	0.86
Total energy, tertiles																	
1	34	0.28	(0.24, 0.32)	-		0.25	(0.21, 0.29)	-		0.32	(0.27, 0.38)	-		1.65	(1.43, 1.87)	-	
2	35	0.21	(0.17, 0.25)	-25.8		0.19	(0.15, 0.23)	-24.1		0.23	(0.17, 0.28)	-29.3		1.39	(1.18, 1.61)	-15.7	
3	35	0.25	(0.21, 0.29)	-12.3	0.24	0.21	(0.17, 0.25)	-16.7	0.14	0.29	(0.24, 0.35)	-8.9	0.47	1.71	(1.50, 1.92)	3.3	0.68
Saturated fat, tertiles																	
1	34	0.27	(0.23, 0.31)	-		0.24	(0.20, 0.28)	-		0.31	(0.26, 0.37)	-		1.44	(1.22, 1.65)	-	
2	35	0.23	(0.19, 0.27)	-13.3		0.22	(0.18, 0.26)	-6.9		0.25	(0.19, 0.30)	-21.5		1.66	(1.45, 1.87)	15.7	
3	35	0.23	(0.19, 0.27)	-14.5	0.19	0.19	(0.15, 0.23)	-19.7	0.14	0.28	(0.22, 0.34)	-9.8	0.38	1.64	(1.42, 1.87)	14.5	0.19
Total fat, tertiles																	
1	34	0.24	(0.20, 0.28)	-		0.22	(0.18, 0.26)	-		0.27	(0.21, 0.32)	-		1.49	(1.27, 1.70)	-	
2	35	0.26	(0.22, 0.30)	7.7		0.24	(0.20, 0.28)	7.9		0.29	(0.24, 0.35)	10.5		1.60	(1.38, 1.82)	7.4	
3	35	0.23	(0.19, 0.27)	-6.0	0.64	0.19	(0.15, 0.23)	-14.5	0.29	0.28	(0.22, 0.34)	5.0	0.73	1.66	(1.44, 1.88)	11.7	0.28
Total[g] vitamin E, tertiles																	
1	34	0.23	(0.18, 0.27)	-		0.20	(0.16, 0.24)	-		0.26	(0.20, 0.31)	-		1.62	(1.39, 1.85)		
2	35	0.25	(0.21, 0.29)	12.0		0.22	(0.18, 0.26)	10.5		0.30	(0.24, 0.35)	15.2		1.49	(1.28, 1.71)	-7.9	
3	35	0.25	(0.21, 0.30)	12.9	0.38	0.23	(0.19, 0.27)	15.7	0.34	0.29	(0.23, 0.34)	11.5	0.51	1.63	(1.40, 1.86)	0.7	0.95
Total[g] calcium, tertiles																	
1	34	0.25	(0.21, 0.29)	-		0.22	(0.18, 0.27)	-		0.28	(0.22, 0.34)	-		1.68	(1.45, 1.91)	-	
2	35	0.24	(0.19, 0.28)	-4.8		0.21	(0.16, 0.25)	-7.7		0.28	(0.22, 0.34)	1.3		1.49	(1.24, 1.73)	-11.5	
3	35	0.25	(0.20, 0.29)	-1.9	0.90	0.22	(0.17, 0.26)	-3.6	0.81	0.28	(0.22, 0.34)	0.7	0.96	1.57	(1.35, 1.79)	-6.4	0.53
Dietary fiber, tertiles																	
1	34	0.21	(0.17, 0.26)	-		0.17	(0.13, 0.22)	-		0.25	(0.19, 0.35)	-		1.74	(1.50, 1.98)	-	
2	35	0.25	(0.21, 0.29)	17.3		0.21	(0.17, 0.25)	22.3		0.29	(0.24, 0.35)	16.7		1.63	(1.41, 1.85)	-6.1	
3	35	0.27	(0.23, 0.32)	29.7	0.08	0.26	(0.22, 0.30)	48.0	0.01	0.29	(0.23, 0.35)	15.4	0.43	1.38	(1.15, 1.61)	-20.8	0.05
Total meat intake, tertiles																	
1	34	0.30	(0.25, 0.36)	-		0.28	(0.23, 0.32)	-		0.34	(0.27, 0.41)	-		1.51	(1.23, 1.79)	-	
2	35	0.23	(0.20, 0.27)	-23.2		0.20	(0.16, 0.24)	-27.5		0.27	(0.22, 0.33)	-20.2		1.68	(1.46, 1.89)	10.8	
3	35	0.21	(0.16, 0.25)	-32.0	0.03	0.18	(0.14, 0.23)	-33.1	0.04	0.24	(0.18, 0.30)	-31.2	0.09	1.56	(1.31, 1.81)	3.1	0.73
Total vegetable and fruit intake, tertiles																	
1	34	0.25	(0.20, 0.29)	-		0.22	(0.17, 0.26)	-		0.28	(0.22, 0.34)	-		1.65	(1.43, 1.87)	-	
2	35	0.24	(0.20, 0.28)	-3.3		0.21	(0.17, 0.25)	-2.1		0.27	(0.22, 0.33)	-2.6		1.69	(1.47, 1.90)	2.3	
3	35	0.25	(0.21, 0.30)	3.0	0.77	0.22	(0.18, 0.26)	2.0	0.85	0.29	(0.24, 0.35)	4.7	0.71	1.41	(1.19, 1.63)	-14.4	0.16
Serum 25-OH-vitamin D (ng/mL)																	
<17.9	34	0.25	(0.21, 0.29)	-		0.21	(0.18, 0.25)	-		0.29	(0.23, 0.34)	-		1.46	(1.23, 1.68)	-	
17.9 - 26.9	35	0.21	(0.16, 0.25)	-16.0		0.18	(0.14, 0.22)	-17.0		0.24	(0.18, 0.30)	-17.0		1.62	(1.39, 1.85)	11.4	
>26.9	35	0.28	(0.24, 0.32)	13.9	0.33	0.25	(0.21, 0.29)	17.8	0.26	0.31	(0.25, 0.37)	8.8	0.62	1.67	I1.44, 1.90)	14.8	0.19

Abbreviations: CI, confidence interval; NSAID, non-steroidal anti-inflammatory drug; OD, optical density.

[a]Using general linear models, adjusted for staining batch.

[b]Measured using automated immunohistochemistry with image analysis.

[c]APC expression divided by β-catenin expression.

[d]MSH2 expression divided by mib-1 expression.

[e]Calculated as (comparison group mean - reference group mean) / (reference group mean) x 100%.

[f]Take at least once a week.

[g]Dietary plus supplemental intake.

Table S7. Comparisons[a] of mean APC ϕ_h (distribution index[b]) in the normal-appearing colorectal mucosa of sporadic colorectal adenoma patients (n = 104), by selected participant characteristics

Characteristics	n	Minimally-adjusted ϕh (OD)	95% CI	Proportional difference[c] (%)	P-value	Adjusted ϕh (OD)	95% CI	Proportional difference[c] (%)	P-value
Age (years)									
47 - 54	34	0.38	(0.34, 0.41)	-		0.37	(0.32, 0.43)	-	
55 - 62	35	0.39	(0.35, 0.43)	4.5		0.39	(0.33, 0.45)	4.5	
63 - 75	35	0.41	(0.37, 0.45)	8.5	0.26	0.40	(0.35, 0.45)	7.1	0.26
Sex									
Male	48	0.38	(0.35, 0.42)	-		0.38	(0.35, 0.41)	-	
Female	56	0.40	(0.37, 0.43)	3.9	0.49	0.40	(0.37, 0.43)	5.5	0.38
Regular[d] use of aspirin									
No	64	0.40	(0.37, 0.44)	-		0.40	(0.34, 0.45)	-	
Yes	40	0.39	(0.36, 0.41)	3.5	0.54	0.38	(0.34, 0.43)	-3.0	0.65
Regular[d] use of other NSAID									
No	69	0.37	(0.33, 0.40)	-		0.38	(0.34, 0.42)	-	
Yes	35	0.41	(0.38, 0.43)	10.2	0.11	0.40	(0.38, 0.43)	6.3	0.20
Currently smoke									
No	96	0.39	(0.37, 0.41)	-		0.40	(0.38, 0.42)	-	
Yes	8	0.38	(0.30, 0.46)	-3.3	0.76	0.39	(0.30, 0.48)	-2.3	0.83
Physical activity									
Low	30	0.40	(0.36, 0.44)	-		0.41	(0.37, 0.45)	-	
Moderate	38	0.40	(0.36, 0.44)	0.0		0.41	(0.37, 0.44)	-0.9	
High	27	0.38	(0.35, 0.41)	-5.1	0.42	0.37	(0.34, 0.41)	-9.0	0.44
Body mass index (kg/m^2)									
<25.0	22	0.39	(0.34, 0.43)	-		0.40	(0.35, 0.45)	-	
25.0 - 29.9	43	0.39	(0.35, 0.42)	-0.3		0.39	(0.35, 0.42)	-2.9	
≥30	39	0.40	(0.37, 0.44)	3.4	0.61	0.40	(0.36, 0.43)	-0.1	0.95
Total energy, tertiles									
1	34	0.38	(0.34, 0.42)	-		0.39	(0.35, 0.43)	-	
2	35	0.38	(0.34, 0.42)	-0.2		0.38	(0.34, 0.42)	-1.6	
3	35	0.42	(0.38, 0.45)	9.1	0.19	0.41	(0.37, 0.45)	5.0	0.39
Saturated fat, tertiles									
1	34	0.39	(0.35, 0.43)	-		0.39	(0.36, 0.43)	-	
2	35	0.36	(0.33, 0.40)	-7.3		0.37	(0.33, 0.43)	-6.8	
3	35	0.43	(0.39, 0.46)	9.1	0.31	0.42	(0.38, 0.46)	6.6	0.57
Total fat, tertiles									
1	34	0.39	(0.36, 0.43)	-		0.39	(0.35, 0.43)	-	
2	35	0.36	(0.33, 0.40)	-7.7		0.37	(0.33, 0.41)	-6.5	
3	35	0.42	(0.39, 0.46)	7.8	0.28	0.42	(0.38, 0.47)	7.1	0.39
Total[e] vitamin E, tertiles									
1	34	0.40	(0.36, 0.44)	-		0.39	(0.33, 0.45)	-	
2	35	0.39	(0.35, 0.43)	-2.3		0.39	(0.33, 0.45)	0.4	
3	35	0.39	(0.35, 0.43)	-1.6	0.83	0.38	(0.32, 0.44)	-2.6	0.70
Total[e] calcium, tertiles									
1	34	0.40	(0.36, 0.44)	-		0.39	(0.35, 0.44)	-	
2	35	0.38	(0.34, 0.42)	-5.6		0.38	(0.34, 0.42)	-3.7	
3	35	0.39	(0.36, 0.43)	-1.9	0.81	0.40	(0.36, 0.45)	2.1	0.84
Dietary fiber, tertiles									
1	34	0.42	(0.37, 0.46)	-		0.40	(0.35, 0.45)	-	
2	35	0.39	(0.35, 0.43)	-6.5		0.38	(0.35, 0.42)	-3.5	
3	35	0.37	(0.33, 0.41)	-9.8	0.21	0.40	(0.35, 0.44)	-0.8	0.51
Total meat intake, tertiles									
1	34	0.38	(0.33, 0.43)	-		0.38	(0.34, 0.43)	-	
2	35	0.41	(0.34, 0.45)	7.2		0.41	(0.37, 0.44)	5.3	
3	35	0.39	(0.34, 0.43)	1.3	0.91	0.39	(0.35, 0.43)	0.9	0.97
Total vegetable and fruit intake, tertiles									
1	34	0.39	(0.35, 0.43)	-		0.38	(0.33, 0.43)	-	
2	35	0.39	(0.36, 0.43)	0.4		0.39	(0.36, 0.43)	3.9	
3	35	0.39	(0.35, 0.43)	0.5	0.93	0.41	(0.36, 0.45)	7.4	0.55
Serum 25-OH-vitamin D (ng/mL)									
<17.9	34	0.39	(0.35, 0.43)	-		0.39	(0.34, 0.43)	-	
17.9 - 26.9	35	0.40	(0.37, 0.44)	3.2		0.40	(0.36, 0.44)	3.4	
>26.9	35	0.38	(0.34, 0.42)	-3.1	0.70	0.39	(0.34, 0.44)	-0.5	0.91

Abbreviations: CI, confidence interval; NSAID, non-steroidal anti-inflammatory drug; OD, optical density.

[a]Using general linear models: adjusted for staining batch for minimally-adjusted ϕh, adjusted for staining batch and measured confounding variables (listed in Supplementary Table S1) for adjusted ϕh.

[b]APC expression in the upper 40% of the crypt divided by expression in the whole crypt, measured using automated immunohistochemistry with image analysis.

[c]Calculated as (comparison group mean - reference group mean) / (reference group mean) x 100%.

[d]Take at least once a week.

[e]Dietary plus supplemental intake.