MutL-Homolog 1 Expression and Risk of Incident, Sporadic Colorectal Adenoma: Search for Prospective Biomarkers of Risk for Colorectal Cancer

Eduard Sidelnikov,[1,2] Roberd M. Bostick,[1,2] W. Dana Flanders,[1,2,3] Qi Long,[2,3] Vaunita L. Cohen,[1,2] Chiranjeev Dash,[1] March E. Seabrook,[4] and Veronika Fedirko[1,2]

[1]Department of Epidemiology, Rollins School of Public Health, [2]Winship Cancer Institute, and [3]Department of Biostatistics and Bioinformatics, Rollins School of Public Health, Emory University, Atlanta, Georgia; [4]Consultants in Gastroenterology, Columbia, South Carolina

Abstract

To characterize the expression of the mismatch repair gene *MutL-homolog 1* (*MLH1*) in normal colorectal crypts in humans, and assess parameters of its expression as a potential biomarker of risk for colorectal neoplasms, we conducted a pilot, colonoscopy-based case-control study (51 cases, 154 controls) of incident, sporadic colorectal adenoma. Biopsies of normal-appearing rectal, sigmoid, and ascending colon mucosa were procured, immunohistochemically processed for MLH1 protein, and analyzed using custom quantitative image analysis procedures. MLH1 expression in the ascending colon was, on average, 49% proportionally lower in cases than controls (P = 0.03), but there was little evidence for case-control differences in the rectum and sigmoid colon. In cases and controls, average MLH1 expression in the ascending colon tended to be lower with increased age [by 56% (P = 0.02) and 25% (P = 0.16), respectively, for those ≥55 years], and with a history of colorectal cancer in a first-degree relative (by 22% [P = 0.56] and 34% [P = 0.16], respectively). Among cases, but not controls, average MLH1 expression tended to be higher with current alcohol consumption, regular aspirin use, and higher total intakes of calcium, vitamin D, and folate. There was little indication of similar differences in the rectum. These preliminary data suggest that lower MLH1 expression in the normal colonic mucosa, at least in the ascending colon, may be associated with increased risk of incident, sporadic colorectal adenoma, as well as with modifiable risk factors for colorectal neoplasms, thus supporting further investigation of MLH1 expression as a potential ''treatable'' biomarker of risk for colorectal neoplasms. (Cancer Epidemiol Biomarkers Prev 2009;18(5):1599–609)

Introduction

Colorectal cancer, the second leading cause of cancer mortality in the United States (1), is a multifactorial disease that appears to be the result of life-style factors interacting with genetic ones (2, 3). The vast majority of so-called ''sporadic'' colorectal cancer develops in the adenomatous polyp, a benign intestinal tumor that is the only accepted biomarker of risk for colorectal cancer (2, 3).

The adenoma is a fairly reliable biomarker of colorectal cancer risk, and removal of this polyp reduces risk of cancer development, but screening procedures for adenoma are costly, labor intensive, require highly qualified personnel, and are not well-accepted by physicians or patients. This prompts the need for discovery of preneoplastic biomarkers or profiles of biomarkers of risk for colorectal neoplasms (*a*) to identify persons most at risk, and (*b*) that could be treatable and thus used to monitor the efficacy of preventive interventions.

The mismatch repair pathway is one of the two main molecular pathways of colorectal cancer development, accounting for ~15% of colorectal neoplasms (4). The DNA mismatch repair system involves a complex set of proteins that identifies and repairs mismatch errors that occur during DNA replication (2, 5).

The *MutL homolog 1*, colon cancer, nonpolyposis type 2 (*Escherichia coli*) *Homo sapiens* (*MLH1*) gene is located at chromosome 3p21-23 (6). The protein product of the *MLH1* gene, an important part of the mismatch repair system, has no known enzymatic activity but probably recruits other DNA repair proteins to the mismatch repair complex (6, 7).

Because of its crucial role in the mismatch repair pathway, the MLH1 protein is one of the potential biomarkers that we chose to investigate for possible incorporation into a biomarker profile. To the authors' knowledge, there is no literature addressing the distribution of MLH1 protein in normal-appearing colorectal mucosa and its potential as a biomarker of risk for colorectal cancer.

This article addresses the distribution of MLH1 protein within the colorectal crypts of the normal-appearing colorectal mucosa and its association with colorectal adenoma as a first step in evaluating this potential prospective biomarker.

Received 8/27/08; revised 2/27/09; accepted 3/9/09; published online 5/7/09.

Grant support: The Fullerton Foundation, the Franklin Foundation, and a Georgia Cancer Coalition Distinguished Scholar Award (R.M. Bostick). The Fullerton Foundation, the Franklin Foundation, and Georgia Cancer Coalition had no influence on the design of the study; the collection, analysis, and interpretation of the data; the decision to submit the manuscript for publication; or the writing of the manuscript.

Requests for reprints: Roberd M. Bostick, Department of Epidemiology, 1518 Clifton Road North East Atlanta, GA 30322. Phone: 404-727-2671; Fax: 404-727-8737. E-mail: rmbosti@sph.emory.edu

doi:10.1158/1055-9965.EPI-08-0800

Participants and Methods

Study Design and Population. As reported previously (8), the Markers of Adenomatous Polyps II study is a pilot case-control study (51 cases and 154 controls) designed to investigate potential biomarkers of risk for incident, sporadic colorectal adenomas. Participants were recruited from people scheduled for elective outpatient colonoscopy at Consultants in Gastroenterology, a large gastroenterology practice in Columbia, SC. To be eligible for the study, participants must have been age 30 to 74 y, English speaking, and capable of providing informed consent. Persons of both sexes and all races were eligible to participate in the study.

Specific exclusion criteria were history of previous colorectal adenomas or inflammatory bowel disease, bowel resection, history of cancer other than nonmelanoma skin cancer, and medical contraindication to colorectal mucosal biopsies (medically unstable, bleeding disorders, cannot stop warfarin, or aspirin) or a polyethylene glycol colon cleansing preparation.

Over a 5-mo period, 351 patients were identified for recruitment; of these, 232 (76%) agreed to participate in the study; and of these, 205 (51 cases and 154 controls) met final eligibility criteria and were included in the study. Due to limited resources, only biopsies from all cases and a random sample of an equal number of controls were processed for MLH1 expression; from these there was adequate tissue for analysis on 46 cases and 43 controls.

Data Collection. Before the colonoscopy visit, patients completed mailed questionnaires, including a modified Willett Food Frequency Questionnaire. The questionnaires were used to obtain information on medical history, family history of cancer, diet, life-style, and anthropometrics.

The colon site and *in vivo* size and shape of all polyps found were recorded, and all polyps were removed and placed in separate containers. All polyps were examined by one study index pathologist who identified polyp type, subtype, and degree of atypia according to criteria established by the National Polyp Study (9).

After a 12-h fast and polyethylene glycol bowel cleansing preparation, biopsies of normal-appearing mucosa were collected according to a standard protocol by gastroenterologists using standard-cup flexible endoscopy forceps during usual care colonoscopies. Six sextant pinch biopsies, ~1-mm-thick, were obtained from the rectum (10 cm above the anus) on all participants, and from the midsigmoid and proximal ascending (immediately distal to the cecum) colon on 20% of participants, for a total of up to 18 biopsies. No biopsies were taken within 4.0 cm of a polypoid lesion.

Biopsies specimens were fixed by 10% normal buffered formalin for 24 h, and then transferred to 70% ethanol. Within a week, the biopsies were processed and embedded in paraffin blocks with three biopsies per colon site per participant per block.

Immunohistochemistry. Within 7 d of being embedded in paraffin blocks, 3.0-μm-thick sections taken 30 μm apart were cut from each block with a microtome such that 5 slides with 4 levels each (yielding a total of 20 levels) were prepared per colon site per person. The immunohistochemistry protocol was calibrated to get the darkest biomarker labeling staining possible short of yielding nonspecific background staining (10). The slides were immunohistochemically processed using a DAKO Automated Immunostainer (DAKO Corp.) and Leica H&E Autostainer (Leica Microsystems, Inc.). First, MLH1 antigen was unmasked through a heat-induced epitope retrieval procedure by placing the slides in a preheated Pretreatment Module (Lab Vision Corp.) with 100× Citrate Buffer (pH 6.0; DAKO S1699) and steamed for 40 min. Then the slides were immunohistochemically processed using an anti-MLH1 antibody (BD Pharmingen 554072) in a 1:15 dilution, a DAKO LSAB2 detection kit (DAKO K0675), and 3,3′ diaminobenzidine (DAKO K3466) as the chromogen. No counterstaining was used and all stained slides were glass coverslipped with a Leica Automated Coverslipper (Leica Microsystems, Inc.). All 5 slides per colon site per person were included in 1 staining batch of up to 48 slides that also included negative and positive control slides. A surgical specimen of normal colon was used for the control slides; the negative and the positive control slides were treated identically to study participant slides except that antibody diluent was used rather than primary antibody on the negative control slide.

Image Analysis. Because MSH1 is expressed in a density gradient along the crypt (Fig. 1) that is not quantifiable by eye (e.g., by counting cells), its expression density, detected by immunohistochemical staining, was quantified in the stained slides using image analysis densitometry methods (8, 11). The procedure was conducted by one trained ''scorer'' using a light microscope (Olympus BX40; Olympus Corporation), digital camera (Polaroid DMC Digital Light Microscope Camera; Polaroid Corporation), digital drawing tablet, and a custom-developed plug-in to ImagePro Plus (Media Cybernetics, Inc.) image

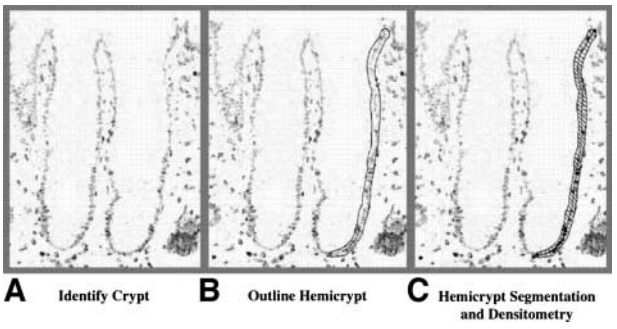

Figure 1. Image analysis of normal colorectal crypt immunohistochemically processed for MLH1. In the figure, there is no counterstain; *all dark areas,* immunohistochemically detected MLH1. Note that MLH1 localizes in the nuclei of the crypt epithelium and is expressed in a declining density gradient from the lower to the upper portions of crypts. To quantify this: (**A**) a full-length crypt is identified by light microscopy at ×200 and the image digitally captured with a digital camera, (**B**) the nuclear zone of the selected hemicrypt is outlined manually using a digital drawing tablet, and then (**C**) the image analysis program divides the hemicrypt into 6.59-μm width segments, conducts morphometry and measures the optical density of the staining in the entire hemicrypt as well as within each individual segment, and enters the data into a database.

analysis software. The scorer was blinded to case-control status and colon site.

The imaging and analysis unit was a ''hemicrypt,'' defined as one side of a colonic crypt bisected from base to colon lumen surface. Intact (at most two contiguous cells missing) hemicrypts extending from the muscularis mucosae to the colon lumen were considered eligible for quantitative image analysis (''scorable'').

For each patient, the two of the three biopsies from each colon site with the greatest number of scorable hemicrypts were selected for quantitative image analysis (''scoring''). Intact hemicrypts were ''scored'' in order from the first hemicrypt on the first biopsy from left to right. The goal was to find at least 16 scorable hemicrypts per biopsy (32 per patient; ref. 10). If the 16th hemicrypt was reached before the level was finished, the scorer continued scoring until either the level was finished or the 20th hemicrypt was scored, whichever came first. No more than 20 hemicrypts per biopsy were scored. If the two best biopsies from a colon site on a patient had <32 scorable hemicrypts, an attempt was made to cut more slides. If that did not solve the issue, scoring was completed if the two best biopsies had 16 or more scorable hemicrypts between them. All three biopsies harvested from the same colon site were scored only if there was less than a total of 16 scorable hemicrypts between the 2 best biopsies.

To ensure adherence, a scorer was guided through the scoring protocol by the computer software. For each scored slide background correction images were obtained and automatically used by the computer program to yield background-corrected densitometries for all hemicrypts analyzed on that slide. All images were taken at $\times$200 magnification (the maximum magnification at which full-length colorectal crypts can be completely included in a single visual field) and stored and analyzed as 16-bit grayscale 1,600 $\times$ 1,200 pixel images.

As shown in Fig. 1, using a digital drawing tablet, hemicrypts were manually traced by the scorer from the crypt base center cell up along the crypt basement membrane to the beginning of the turn of the crypt onto the colonic mucosal surface and then back down along the crypt luminal surface of the epithelial nuclei (8, 11). The software program divided the traced hemicrypt into segments corresponding in width to that of an average normal crypt epithelial cell (6.59 μ; ref. 10), and then calculated overall hemicrypt- and segment-specific optical densities and entered these data into a Microsoft Access database along with various dimensional parameters of the hemicrypt (8, 11).

For quality assurance, slide sets from 10% of the participants were randomly selected by the statistical team, blinded, and resubmitted to the scorer for rescoring (10).

Statistical Analysis. Statistical analyses were done using SAS 9.1.3 statistical software (Copyright 2002-2003 by SAS Institute, Inc.). The entire Markers of Adenomatous Polyps II Study population (51 cases and 154 controls) as well as a subset of participants for whom slides were immunohistochemically processed for MLH1 protein (46 cases and 43 controls) were assessed for comparability using the t test for continuous variables, and the Fisher's exact test or χ^2 test for categorical variables as appropriate. Biopsy scoring reliability was assessed with intraclass correlation coefficients. All optical density means were calculated using linear mixed models. Potential confounders as well as staining batch were included in the models as fixed effects, and correlation among multiple optical density measurements was accounted for by including a patient variable as a random effect. Mean proportional differences were calculated as the model-predicted mean optical density for cases minus that for controls divided by the mean for cases. Statistical significance of these measurement differences was evaluated by t test.

The distribution of MLH1 protein within a colonic crypt was evaluated graphically with the Loess procedure as implemented in SAS 9.1.3 statistical software (12). First, the number of cells within a hemicrypt was standardized to 50 cells (the average number of cells within a column of colonic crypt cells). Then, average colon site-specific levels of MLH1 for cases and controls predicted by the Loess model were plotted in the graphs (Fig. 2) along with smoothing lines (using a smoothing parameter of 0.5) to make graphical evaluation easier.

Potential confounders were evaluated on the basis of biological plausibility and whether the variable of interest was associated with the exposure based on existing epidemiologic, medical, and basic science literature. As an additional method of selecting potential confounders, previously identified variables were added into the regression model and their ability to substantially change regression coefficients was evaluated. None of the variables changed the odds ratio (OR) by >10%, and the adjustment was based on *a priori* considerations. Potential confounders considered in this analysis included the following: age, sex, physical activity, body mass index (BMI), family history of colorectal cancer in a first-degree relative, smoking, alcohol consumption, aspirin and nonsteroidal anti-inflammatory drug (NSAID) use, and total intakes of energy, fat, fiber, folate, calcium, and vitamin D. All nutrient values were adjusted for total energy according to the residual regression method (13). Continuous variables were dichotomized based on their distributions in the controls. A staining batch variable was included in the models to control for potential variability between staining batches.

The association between MLH1 expression and risk of incident sporadic colorectal adenoma was assessed by calculating ORs from the generalized linear mixed (logistic) models containing potential confounders and staining batch as fixed effects. The models accounted for lack of independence among hemicrypt optical density measurements within a patient by having a patient ID variable as a random effect. A 95% C.I. was calculated for each OR.

Generalized linear mixed (logistic) models were also used to model associations between the level of MLH1 expression in the rectum and adenoma location, shape, size, multiplicity, histologic type, and degree of dysplasia. For this analysis, the MLH1 expression variable was batch standardized by dividing a patient-specific optical density measurement by the batch-specific mean optical density, and then dichotomized based on the colon site-specific mean of the standardized variable in the controls. The models also contained a random intercept for each patient to account for correlations due to repeated measurements (i.e., optical densities of multiple crypts per colon site per patient).

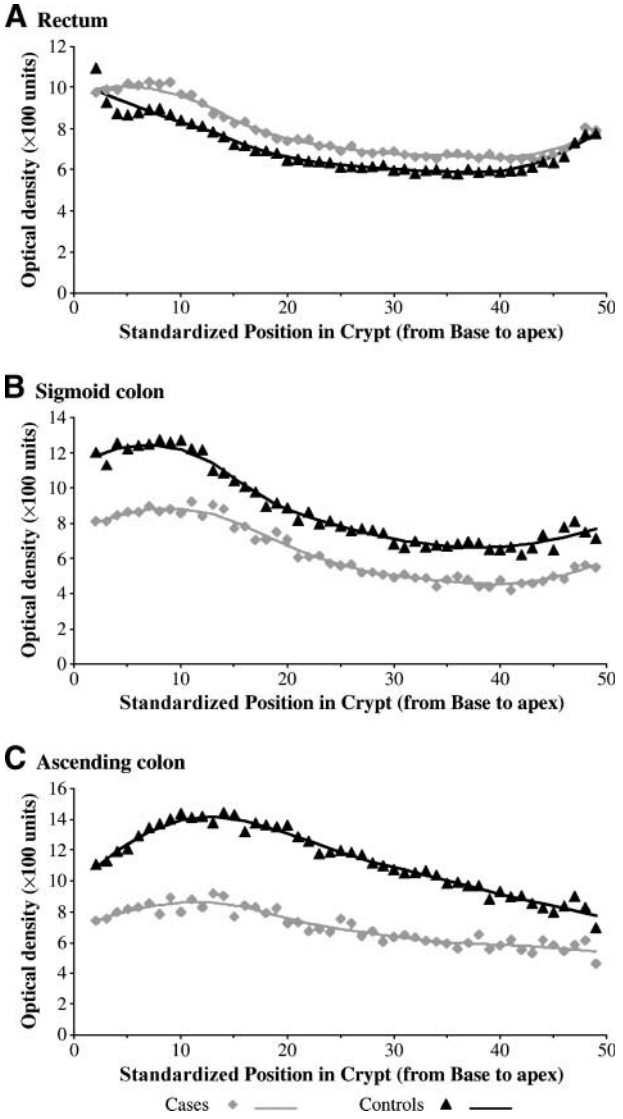

Figure 2. Expression of MLH1 protein at standardized positions within the crypts of normal-appearing mucosa in cases and controls for three colon sites: (**A**) rectum, (**B**) mid sigmoid colon, (**C**) proximal ascending colon. The Markers of Adenomatous Polyps II Study. *Data points,* average optical density for all cases or all controls at a particular standardized position in the crypt; *curves,* Loess smoothing curves (smoothing parameter 0.5).

The associations of MLH1 expression within a colon site with various demographic, life-style, and dietary characteristics were assessed by linear mixed models stratified by colon site controlling for each of the characteristics one at a time. Each model also included a fixed effect variable to control for staining batch. Because optical density was measured on multiple hemicrypts on each patient, each model accounted for that by a random effect variable.

In sensitivity analyses, we also analyzed data without standardization for batch, as well as by using different mathematical transformations; the results from these analyses did not differ materially from those reported.

Results

The subpopulation of subjects whose biopsies were stained for MLH1 and analyzed (46 cases and 43 controls) was compared with the entire MAPII study population (51 cases and 154 controls) and found completely comparable with respect to all considered characteristics (data not shown). Selected characteristics of cases and controls of the population considered in this analysis are shown in Table 1. On average, relative to controls, cases tended to be older and more likely to be male, a current smoker, currently consume alcohol, regularly take an NSAID, and to have a higher total energy intake and lower intakes of calcium, vitamin D, and folate, although only the difference for total energy intake was statistically significant. Physical activity, BMI, aspirin use, and fat and fiber intakes did not differ substantially between cases and controls.

Among cases, 48% had multiple adenomas, 7% had an adenoma that was 1.0 cm or greater in diameter, 89% had a mild degree of atypia in their largest or most advanced adenoma, and in 41% of cases the largest or most advanced adenoma was located in the right colon (data not shown). Biopsy scoring reliability (intraclass correlation coefficient) was r = 0.97.

Figure 2 depicts the distribution of MLH1 protein within colonic crypts in the rectum, sigmoid, and ascending colon. For each of the three colon sites, the MLH1 expression curves for cases and controls closely paralleled each other. Levels of MLH1 in the rectum were slightly higher in cases than in controls, but in the sigmoid and ascending colon, the relationship shifted such that MLH1 levels in controls were consistently higher than those in cases. The difference in MLH1 expression levels between cases and controls was greater in the ascending than the sigmoid colon. Because case-control differences appeared uniform throughout the lengths of the colon crypts, only analyses of overall crypt MLH1 expression data are presented in Tables 2 to 5.

Table 2 presents ''crude'' (controlled for staining batch only), age- and sex-adjusted, and multivariable-adjusted MLH1 expression in all cases and controls stratified by colon site, as well as the combined ORs for the associations of MLH1 expression with incident, sporadic colorectal adenoma. Although on average, expression of MLH1 protein in the rectum in adenoma cases tended to be slightly higher, in the other colon sites cases tended to have lower expression of the protein than did the controls. The proportional difference in expression between cases and controls widened from the distal to the proximal colon sites, reaching a statistically significant 49% after multivariable adjustment (model 3; Table 2). MLH1 expression in colonic crypts was nonstatistically significantly inversely associated with risk of incident, sporadic colorectal adenomas (OR = 0.89, 95% C.I., 0.45-1.76).

Although estimated differences between all cases and controls at the rectum were negligible (Table 2), to assess whether MLH1 expression at the rectum may be associated with a subset of cases (especially those with right-sided adenomas), we investigated associations of MLH1 expression in the rectum with various adenoma characteristics (Table 3). MLH1 expression in the rectum tended to be more strongly associated with adenomas in the right colon (OR 1.81; 95% C.I., 0.86-3.80) than in the left colon (OR 1.19; 95% C.I., 0.62-2.28). Rectal mucosal

Table 1. Selected characteristics of incident, sporadic colorectal adenoma cases and controls; the Markers of Adenomatous Polyps II Study

Characteristic*	*n* (cases/controls)	Adenoma cases	Controls	*P* †
Demographics				
Age (y.)	46/43	56.8 (7.7)	55.7 (8.4)	0.52
Male (%)	46/43	54	44	0.40
White race (%)	46/42	96	98	1.00
Family history				
First-degree relative with colorectal cancer (%)	46/43	17	14	0.77
Lifestyle				
Physical activity (METs/d)	44/42	29.5 (23.5)	27.1 (20.9)	0.33 ‡
BMI (kg/m^2)	46/42	30.8 (7.3)	30.4 (7.0)	0.79 ‡
Take aspirin at least once per week (%)	46/42	39	38	1.00
Take NSAID § at least once per week (%)	46/42	35	43	0.51
Smoking status (%)				
Never	46/42	41	52	0.30
Former		41	41	
Current		18	7	
Alcohol consumption (%)				
Never	46/42	11	14	0.74
Former		22	26	
Current		67	60	
Dietary intakes				
Total energy (kcal/d)	44/41	1,939.5 (780.0)	1,509.2 (405.5)	0.002 ‡
Total fat ‖ (grams/d)	44/42	65.7 (16.5)	65.8 (15.2)	0.99
Total dietary fiber ‖ (grams/d)	44/42	15.4 (5.7)	15.4 (5.9)	0.98
Total ¶ calcium ‖ (mg/d)	44/42	882.7 (487.3)	995.0 (505.4)	0.24 ‡
Total ¶ vitamin D ‖ (IU/d)	44/42	323.1 (289.9)	373.4 (277.8)	0.20 ‡
Total ¶ folate ‖ (mcg/d)	44/42	480.2 (235.0)	522.4 (266.3)	0.44

*Continuous variables presented as mean (± SD), categorical variables as proportions in percent.
†Based on *t* test for continuous variables, Fisher's exact test for dichotomous variables, and χ^2 test for multilevel categorical variables.
‡Variables that were not normally distributed were normalized by natural log transformation.
§NSAID, not including aspirin.
‖Energy adjusted using residual method.
¶Total = diet + supplements.

MLH1 expression also tended to be more strongly positively associated with pedunculated (OR 2.18; 95% C.I., 0.67-7.09) and single adenomas (OR 1.81; 95% C.I., 0.95-3.44).

We also assessed the potential of MLH1 expression as a modifiable biomarker of risk by evaluating associations of MLH1 expression with various risk factors for colorectal neoplasms. The associations tended to vary between the adenoma cases (Table 4) and controls (Table 5). In cases and controls, average MLH1 expression in the ascending colon tended to be lower with increased age [by 56% (P = 0.02) and 25% (P = 0.16), respectively, for

Table 2. Differences in full crypt MLH1 protein expression in normal-appearing mucosa between incident sporadic colorectal adenoma cases and controls, by colon site; the Markers of Adenomatous Polyps II Study

Colon site	*n*	Optical density mean (SD)		Proportional difference (%)*	*P* †
		Cases	Controls		
Model 1: controls for staining batch only					
Rectum	84	496.07 (18.66)	464.74 (20.46)	7%	0.22
Sigmoid	32	313.82 (27.44)	330.64 (29.04)	−5%	0.68
Ascending	27	380.97 (58.69)	500.30 (51.38)	−24%	0.13
Combined OR ‡ (95% C.I.)	89	0.84		(0.47-1.49)	
Model 2: controls for age, sex, and staining batch					
Rectum	84	495.63 (18.88)	464.49 (20.79)	7%	0.22
Sigmoid	32	310.23 (28.15)	335.68 (30.13)	−8%	0.55
Ascending	27	375.92 (50.90)	481.17 (45.03)	−22%	0.10
Combined OR (95% C.I.)	89	0.87		(0.49-1.55)	
Model 3: controls for age, sex, history of colorectal cancer in a first-degree relative, physical activity, BMI, aspirin use, total energy intake, calcium, vitamin D, and folate intakes, and staining batch					
Rectum	80	505.23 (27.48)	465.30 (28.05)	9%	0.18
Sigmoid	31	345.18 (39.71)	301.52 (34.53)	14%	0.41
Ascending	26	257.77 (84.74)	510.12 (53.19)	−49%	0.03
Combined OR (95% C.I.)	85	0.89		(0.45-1.76)	

*[(cases − controls)/controls] × 100%.
†Based on *t* test for comparing the two means.
‡Combined OR (cases versus controls) controlling for all three colon sites and the covariates indicated in the model specification. The optical density (MLH1 expression) variable was dichotomized using the mean of the colon site specific distributions in the controls.

Table 3. Crude associations of batch-standardized full crypt MLH1 expression in the normal-appearing rectal mucosa with risk of incident sporadic colorectal adenomas overall and according to adenoma characteristics; the Markers of Adenomatous Polyps II Study

Adenoma characteristic	*n* (cases/controls)	MLH1 expression		95% C.I.
		Low (OR)	High (OR)	
All Adenomas	44/43	1.0	1.35	(0.77-2.38)
Location				
Right colon*	18/43	1.0	1.81	(0.86-3.80)
Left colon†	26/43	1.0	1.19	(0.62-2.28)
Multiplicity				
Single adenoma	23/43	1.0	1.81	(0.95-3.44)
Multiple adenomas	21/43	1.0	1.01	(0.48-2.14)
Dysplasia				
Mild	39/43	1.0	1.43	(0.79-2.59)
Moderate/severe	5/43	1.0	1.26	(0.35-4.57)
Histologic type				
Tubular	31/43	1.0	1.37	(0.75-2.51)
Tubulovillous/villous	13/43	1.0	1.51	(0.63-3.62)
Shape				
Pedunculated	6/43	1.0	2.18	(0.67-7.09)
Sessile	38/43	1.0	1.31	(0.74-2.34)

*Right colon includes cecum, ascending colon, hepatic flexure and transverse colon.
†Left colon includes splenic flexure, descending colon, sigmoid colon and rectum.

those ≥55 years] and a history of colorectal cancer in a first-degree relative [by 22% (P = 0.56) and 34% (P = 0.16), respectively], as well as among smokers [by 38% (P = 0.26) and 25% (P = 0.31), respectively]. MLH1 expression in the ascending colon also tended to be lower in those with higher physical activity [by 45% (P = 0.42) and 15% (P = 0.56), respectively]; however, for those on whom ascending colon tissue was available for MLH1 evaluation, only one case and three controls were categorized as having high physical activity. Among cases, but not controls, average MLH1 expression tended to be higher with current alcohol consumption (by 58%), regular aspirin use (by 46%), and higher total intakes of calcium (by 32%), vitamin D (by 22%), and folate (by 37%), but none of these findings were statistically significant. There was little indication of similar differences in the rectum.

Discussion

To our knowledge, this is the first study to report on the distribution of the MLH1 protein within normal human colorectal crypts or on associations of MLH1 expression in normal-appearing colorectal mucosa with risk for incident, sporadic colorectal neoplasms, or with risk factors for colorectal cancer. Our preliminary data support the hypothesis that MLH1 expression in the normal colonic mucosa—especially in the more proximal sites of the colon—may be associated with risk of incident, sporadic colorectal adenoma. The data also suggest the possibility that MLH1 expression in the normal colon, especially in the more proximal part of it, may be associated with modifiable risk factors for colorectal neoplasms.

The expression curves for MLH1 appear to mirror the cell proliferation pattern within a colonic crypt (Fig. 2) with high expression of the protein in the lower 60% of crypts (proliferation zone), and lower expression in the upper 40%. This suggests that MLH1 expression may be correlated with the proliferative activity of colonic cells.

For chemoprevention trials or other potential outpatient applications, the most practical colon site for obtaining colorectal tissue is the rectum (10). The procedures for obtaining rectal biopsies 10 cm above the anus are minimally invasive and do not require fasting or bowel cleansing preparations (10). Although the estimated differences between cases and controls in the rectum in this study were negligible (slightly higher in cases but not statistically significant), to assess whether MLH1 expression in the rectum may be associated with a subset of cases (especially those with right-sided adenomas), we investigated associations of MLH1 expression in the rectum with various adenoma characteristics (Table 3). There was some suggestion that higher MLH1 expression in the rectum was associated with higher risk for adenomas that were right sided, pedunculated, or single. These findings were not statistically significant and may have been due to chance. On the other hand, if such findings are confirmed in a full-scale study, they could suggest that there is a reciprocal relationship between MLH1 expression in the rectum and right colon; a possible explanation for this could be that MLH1 expression in the ascending colon may be more influenced by reduced expression through DNA methylation, and that MLH1 expression in the rectum may be less influenced by DNA methylation and simply reflects higher levels of proliferation (i.e., higher mismatch repair activity follows higher proliferation). It remains possible that rectal expression of MLH1 in combination with other biomarkers may increase the predictive value of such a panel of biomarkers, a subject of ongoing work. Should our findings regarding MLH1 expression in the rectum not be confirmed, assessing MLH1 expression in colonoscopic biopsies from the ascending colon may still be useful; for example, for helping assess if and when someone with a normal screening colonsocopy may need a subsequent one.

Two of the most noncontroversial risk factors for colorectal cancer are increasing age and a family history of colorectal cancer in a first-degree relative (2, 3, 14).

Table 4. Associations of full crypt MLH1 expression in normal-appearing colorectal mucosa with potential risk factors of colorectal cancer in incident, sporadic colorectal adenoma cases, by colon site; the Markers of Adenomatous Polyps II Study

Characteristic*	Rectum			Sigmoid			Ascending		
	n	MLH1 Expression† (SE)	*P*‡	*n*	MLH1 Expression (SE)	*P*	*n*	MLH1 Expression (SE)	*P*
Age (y)									
35-54	21	522.75 (27.77)		8	278.76 (43.38)		6	535.26 (56.12)	
≥55	22	470.63 (26.90)	0.20	9	349.19 (43.14)	0.30	7	234.96 (72.46)	0.02
% Difference		−10%			24%			−56%	
Sex									
Male	23	478.65 (27.04)		10	297.04 (43.12)		7	406.41 (82.91)	
Female	20	513.36 (26.44)	0.36	7	342.35 (40.34)	0.42	6	351.95 (87.41)	0.64
% Difference	7%				15%			−13%	
Family history of colorectal cancer‖									
No	37	501.02 (20.07)		14	313.09 (30.37)		10	381.85 (66.09)	
Yes	6	469.77 (49.83)	0.56	3	316.84 (76.04)	0.96	3	297.98 (127.22)	0.56
% Difference	−6%				1%			−22%	
Physical activity (METs/d)									
Low	25	505.02 (25.06)		13	324.25 (34.42)		12	365.40 (55.61)	
High	18	484.59 (30.49)	0.61	4	283.72 (61.54)	0.58	1	201.08 (197.00)	0.42
% Difference		−4%			−12%			−45%	
BMI¶ (kg/m^2)									
<30	21	488.87 (29.43)		9	334.19 (43.86)		8	370.13 (78.24)	
≥30	22	501.40 (25.84)	0.75	8	290.69 (43.22)	0.53	5	352.39 (97.79)	0.90
% Difference		3%			−13%			−5%	
Smoking**									
Never	19	533.08 (28.18)		9	295.99 (38.62)		7	473.75 (97.69)	
Ever	24	475.55 (25.16)	0.12	8	332.08 (44.45)	0.57	6	296.09 (97.00)	0.26
% Difference		−11%			12%			−38%	
Alcohol consumption††									
Former/never	14	529.62 (33.69)		7	326.21 (44.46)		5	249.65 (77.77)	
Current	29	483.03 (23.84)	0.28	10	305.41 (38.73)	0.74	8	393.92 (69.52)	0.17
% Difference		−9%			−6%			58%	
Aspirin intake‡‡									
No	27	492.16 (23.09)		13	333.96 (32.25)		10	328.69 (65.58)	
Yes	16	504.48 (31.58)	0.75	4	236.54 (64.74)	0.21	3	478.93 (106.30)	0.26
% Difference	3%				−29%			46%	
NSAID§§ intake‡‡									
No	27	524.14 (24.13)		10	348.16 (44.06)		8	358.55 (75.42)	
Yes	16	456.25 (28.53)	0.07	7	262.83 (57.10)	0.32	5	372.33 (95.74)	0.92
% Difference	−13%				−25%			4%	
Total energy intake (kcal/d)‖‖									
Low¶¶	9	532.93 (43.70)		7	390.31 (42.36)		7	336.05 (91.78)	
High	32	487.79 (22.17)	0.37	10	257.23 (37.65)	0.04	6	381.19 (104.69)	0.74
% Difference	−8%				−34%			13%	
Total calcium intake (mg/d)*§									
Low	26	504.80 (25.00)		11	296.56 (34.68)		7	312.18 (71.24)	
High	15	484.50 (33.91)	0.64	6	344.09 (51.04)	0.47	6	412.44 (78.08)	0.34
% Difference	−4%				16%			32%	
Total vitamin D intake (IU/d)									
Low	24	507.78 (26.10)		8	357.48 (40.93)		5	318.04 (89.30)	
High	17	482.68 (30.96)	0.55	9	258.89 (44.56)	0.15	8	388.04 (71.81)	0.56
% Difference	−5%				−28%			22%	
Total folate intake (mcg/d)									
Low	20	518.23 (28.16)		5	351.59 (57.40)		4	284.87 (99.11)	
High	21	477.92 (27.40)	0.31	12	294.42 (38.07)	0.46	9	389.65 (62.50)	0.39
% Difference		−8%			−16%			37%	

*All variables except age, sex, family history of colorectal cancer, and total energy intake adjusted for age and sex; also smoking status variable adjusted for alcohol consumption and alcohol consumption variable adjusted for smoking status.
†Mean optical density adjusted for staining batch.
‡Based on the F-test for significance of fixed effects in a linear mixed model.
§From diet and supplements.
‖Family history of colorectal cancer in a first-degree relative.
¶BMI, kg/m^2.
**Categories ''Current smoker'' and ''Former smoker'' were combined into the ''Ever smoker'' category due to extremely small sample size of the Current smoker category.
††Categories ''Never consumed'' and ''Former consumer'' were combined due to extremely small sample size of the Never consumed category.
‡‡Yes defined as regularly taking this medication at least weekly.
§§Not including aspirin.
‖‖Throughout the table: ''Low,'' below the 50th percentile of the sex-specific distribution in controls; ''High,'' at or above the 50th percentile of the sex-specific distribution in controls.
¶¶Adjusted for physical activity.

Table 5. Associations of full crypt MLH1 expression in normal-appearing colorectal mucosa with potential risk factors of colorectal cancer in controls, by colon site; the Markers of Adenomatous Polyps II Study

Characteristic*	Rectum			Sigmoid			Ascending		
	n	MLH1 Expression† (SE)	*P*‡	*n*	MLH1 Expression (SE)	*P*	*n*	MLH1 Expression (SE)	*P*
Age (y)									
35-54	21	432.34 (26.42)		9	345.20 (39.19)		8	563.59 (56.12)	
≥55	20	500.60 (27.28)	0.06	6	304.87 (50.95)	0.56	6	423.11 (73.52)	0.16
% Difference		16%			12%			−25%	
Sex									
Male	19	472.74 (28.38)		7	413.16 (42.18)		6	500.99 (89.02)	
Female	22	456.24 (26.26)	0.65	8	254.94 (39.60)	0.02	8	499.87 (76.87)	0.99
% Difference		−3%			−38%			0%	
Family history of colorectal cancer§									
No	36	457.54 (21.80)		11	312.96 (36.75)		10	558.17 (63.16)	
Yes	5	510.74 (52.92)	0.34	4	378.11 (65.12)	0.42	4	367.89 (106.36)	0.16
% Difference		12%			21%			−34%	
Physical activity (METs/d)									
Low	25	450.66 (26.05)		10	315.14 (42.08)		10	537.37 (59.00)	
High	15	494.30 (33.71)	0.30	4	342.06 (68.38)	0.76	3	456.21 (113.06)	0.56
% Difference		10%			9%			−15%	
BMI‖ (kg/m^2)									
<30	22	466.40 (27.90)		8	371.56 (39.60)		7	528.88 (74.62)	
≥30	18	464.66 (29.68)	0.96	6	264.46 (44.61)	0.09	6	501.39 (71.25)	0.80
% Difference		0%			−29%			−5%	
Smoking¶									
Never	21	474.31 (28.06)		6	250.08 (52.46)		6	591.08 (97.23)	
Ever	19	470.84 (29.14)	0.93	8	384.56 (44.11)	0.09	7	444.18 (79.87)	0.31
% Difference		−1%			54%			−25%	
Alcohol consumption**									
Former/Never	16	469.52 (30.80)		6	366.07 (49.80)		5	604.07 (87.28)	
Current	24	469.39 (25.83)	1.00	8	282.16 (43.08)	0.24	8	460.08 (71.89)	0.29
% Difference		0%			−23%			−24%	
Aspirin intake††									
No	24	478.85 (26.03)		9	314.01 (37.87)		8	536.98 (65.50)	
Yes	16	447.20 (32.58)	0.43	5	344.32 (54.23)	0.65	5	479.66 (76.55)	0.60
% Difference		−7%			10%			−11%	
NSAID‡‡ intake§§									
No	23	470.83 (27.32)		9	329.50 (40.43)		8	543.89 (76.35)	
Yes	17	470.86 (30.09)	1.00	5	316.50 (59.65)	0.88	5	477.62 (90.34)	0.63
% Difference		0%			−4%			−12%	
Total energy intake (kcal/d)‖‖									
Low‡‡	19	464.45 (29.85)		8	303.97 (39.87)		7	546.72 (82.97)	
High	20	472.32 (28.42)	0.84	6	319.05 (48.54)	0.82	6	493.16 (97.65)	0.69
% Difference		2%			5%			−10%	
Total¶¶ calcium intake (mg/d)									
Low	20	473.18 (27.91)		8	371.85 (42.30)		7	532.49 (64.64)	
High	20	459.04 (29.21)	0.71	6	265.73 (47.96)	0.13	6	490.24 (76.26)	0.69
% Difference		−3%			−29%			−8%	
Total vitamin D intake (IU/d)									
Low	19	461.05 (29.68)		9	354.76 (39.13)		9	515.87 (59.18)	
High	21	471.18 (28.86)	0.80	5	285.51 (52.90)	0.32	4	501.15 (100.34)	0.90
% Difference		2%			−20%			−3%	
Total folate intake (mcg/d)									
Low	19	464.52 (28.73)		9	351.62 (37.71)		9	540.55 (54.96)	
High	21	466.77 (27.82)	0.95	5	269.07 (54.11)	0.23	4	430.77 (96.94)	0.35
% Difference		0%			−23%			−20%	

*All variables except age, sex, family history of colorectal cancer, and total energy intake adjusted for age and sex; also smoking status variable adjusted for alcohol consumption and alcohol consumption variable adjusted for smoking status.
†Mean optical density adjusted for staining batch.
‡Based on the F-test for significance of fixed effects in a linear mixed model.
§Family history of colorectal cancer in a first-degree relative.
‖BMI, kg/m^2.
¶Categories Current smoker and Former smoker were combined into the Ever smoker category due to extremely small sample size of the Current smoker category.
**Categories Never consumed and Former consumer were combined due to extremely small sample size of the Never consumed category.
††Yes defined as regularly taking this medication at least weekly.
‡‡Not including aspirin.
§§Adjusted for physical activity.
‖‖Throughout the table: Low, below the 50th percentile of the sex-specific distribution in controls; High, at or above the 50th percentile of the sex-specific distribution in controls.
¶¶From diet and supplements.

Consistent with this and our findings of lower MLH1 expression in the ascending colon in cases, we found that MLH1 expression in the ascending colon was lower in those who were older or had a first-degree relative with colorectal cancer, regardless of case-control status.

Some of the most strongly supported modifiable risk factors for colorectal neoplasms are physical activity, aspirin, and other NSAID use, and calcium and vitamin D intakes (2, 3, 14-23). Folate intake has also been a subject of investigation and has been linked to DNA methylation and thus may influence MLH1 expression (2, 16, 17, 24-27). The results of our small study suggest that MLH1 expression in the ascending colon may be higher with aspirin use and higher total intakes of calcium, vitamin D, or folate but only in persons who have developed a sporadic adenoma. MLH1 expression in the ascending colon also tended to be lower in those with higher physical activity; however, for those on whom ascending colon tissue was available for MLH1 evaluation, only one case and three controls were categorized as having high physical activity. At odds with the findings for aspirin, there was no substantial indication that use of other NSAIDs was associated with MLH1 expression in the ascending colon. Again, these results—perhaps related to the small sample size—were not statistically significant and thus may have simply been due to chance. However, if confirmed in a subsequent larger study, they suggest that aspirin, calcium, and vitamin D may be most effective as chemopreventive agents in persons already at increased risk for colorectal neoplasms.

Important strengths of this study included the following: (*a*) all participants underwent colonoscopy, which ensured accurate classification of cases and controls; (*b*) all self-reported information (including dietary data) was collected before colonoscopies and thus determination of case-control status, thus minimizing possible recall bias; (*c*) detailed information on potential confounders, such as anthropometrics, diet, vitamin and mineral supplements, and medications, used was collected; and (*d*) the rigorous procedures for biopsy collection, processing, and quantitative assessment of the density of immunohistochemically detected MLH1 expression using our custom-developed software, which minimized possible measurement error.

Because this study was a pilot study, its main limitation was the small sample size. Due to limited resources, the biopsies on only a subset of the patients were evaluated for MLH1, which further reduced the sample size. The same reasons explain why biopsies for all three colon sites were often not available. Using an automated immunostainer did not completely eliminate staining variability between staining batches, which introduced an additional source of variability into the analysis that had to be accounted for. The participants of this study were drawn from people who underwent a colonoscopy, and so the results of this study may not be directly applicable to the general population. Data collected by food frequency questionnaires and self-reported data have shortcomings that are well described in the literature, but because these data were collected before case-control status was determined, any possible bias is likely nondifferential.

Basic science and epidemiologic literature (28-31) describes the central role of MLH1 in the function of the mismatch repair machinery and the inability of this machinery to function when the *MLH1* gene is damaged or silenced. The MLH1-based protein complex participates in repairing all known kinds of DNA mismatches, and its concentration increases when cell proliferation activity increases. Changes in MLH1 expression and its distribution within a colonic crypt may indicate changes in the cell proliferation pattern and give some information about cancer risk in a patient. Analytic epidemiologic studies (2, 3, 32, 33) have not been convincingly consistent with respect to the importance of various dietary factors as risk factors for colorectal adenoma and cancer. The literature about relationships of these factors with MLH1 expression is very limited (33-39). It was hypothesized that certain dietary components, such as folate, alcohol, and others may play a role in carcinogenesis because of their involvement in DNA methylation. Hypermethylation of CpG islands near promoter regions and subsequent transcriptional silencing of the *MLH1* gene is a common pathway for inactivating this gene.

Slattery et al. (37) investigated associations between dietary (fiber, folate, alcohol, methionine, and vitamins B_6 and B_{12}) and life-style (BMI, physical activity, and use of aspirin and NSAIDs) factors, and colon cancer. The investigators found that high folate and fiber intakes were inversely associated with risk of incident carcinomas, irrespective of *MLH1* promoter methylation; however, in those with the high-methylator phenotype, the association appeared stronger, suggesting involvement of these compounds in the DNA methylation process. On the other hand, a prospective cohort study done in the Netherlands found no association between folate or fiber and MLH1 protein deficient colorectal cancer (38). Our data support a possible inverse association between folate and MLH1 expression in the sigmoid and ascending colon in adenoma free controls but suggest a positive association in incident, sporadic colorectal adenoma patients. These findings suggest that the role of folate role in DNA methylation may also be important in the development of colorectal adenomas.

Consistent with evidence that folate and methionine may influence methyl group availability, Giovannucci et al. (25) found that methyl-deficient diets might be associated with early stages of colorectal neoplasia. The hypothesis that folate may be inversely associated with DNA hypomethylation was supported by the results of a clinical trial (24) and several observational studies (26, 27). Our findings are also consistent with this hypothesis. On the other hand, several observational studies that specifically investigated the association between folate and MLH1 did not find significant evidence of such an association (38, 40). Thus far, the evidence for a role for folate in DNA methylation is inconclusive and further investigation by more definitive studies is needed.

Several recent clinical trials (18-20, 22, 23) found that calcium reduced colorectal adenoma recurrence. Associations of calcium and vitamin D with incident adenomas have been investigated only in case-control studies (21, 41-44) and are consistent with reduced risk. As discussed above, the results of our small study suggest that MLH1 expression in the ascending colon may be higher with higher total intakes of calcium or vitamin

D but only in persons who have developed a sporadic adenoma. To our knowledge, this is the first study to investigate this association specifically.

In summary, we developed a reliable procedure for detecting and describing MLH1 expression in normal colorectal crypts, and report, to our knowledge, the first study of the distribution of the MLH1 protein within normal colorectal crypts or associations of MLH1 expression in normal-appearing colorectal mucosa with risk for incident, sporadic colorectal neoplasms or with risk factors for colorectal cancer. We found that the distribution of the MLH1 protein within normal colonic crypts parallels that of the normal proliferation zone of normal crypts. The data from this preliminary study suggest that lower MLH1 expression in the normal colonic mucosa, at least in the ascending colon, may be associated with increased risk of incident, sporadic colorectal adenoma as well as with modifiable risk factors for colorectal neoplasms, and thus support further investigation of MLH1 expression, alone or in combination with other biomarkers, as a potential ''treatable'' biomarker of risk for colorectal neoplasms.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank the physicians and staff of Consultants in Gastroenterology, 131 Summerplace Dr., Columbia, SC 29169 for work on biopsy procurement; and all study participants for their time and dedication to the study.

References

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2008. CA Cancer J Clin 2008;58:71–96.
2. Potter JD. Colorectal cancer: molecules and populations. J Natl Cancer Inst 1999;91:916–32.
3. Potter JD, Slattery ML, Bostick RM, Gapstur SM. Colon cancer: a review of the epidemiology. Epidemiol Rev 1993;15:499–545.
4. Haydon AM, Jass JR. Emerging pathways in colorectal-cancer development. Lancet Oncol 2002;3:83–8.
5. Kolodner RD. Mismatch repair: mechanisms and relationship to cancer susceptibility. Trends Biochem Sci 1995;20:397–401.
6. Mitchell RJ, Farrington SM, Dunlop MG, Campbell H. Mismatch repair genes hMLH1 and hMSH2 and colorectal cancer: a HuGE review. Am J Epidemiol 2002;156:885–902.
7. Modrich P. Mechanisms and biological effects of mismatch repair. Annu Rev Genet 1991;25:229–53.
8. Daniel CR, Bostick RM, Flanders WD, et al. TGF-α expression as a potential biomarker of risk within the normal-appearing colorectal mucosa of patients with and without incident sporadic adenoma. Cancer Epidemiol Biomarkers Prev 2009;18:65–73.
9. Winawer SJ, Zauber AG, O'Brien MJ, et al. Randomized comparison of surveillance intervals after colonoscopic removal of newly diagnosed adenomatous polyps. The National Polyp Study Workgroup. N Engl J Med 1993;328:901–6.
10. Bostick RM, Fosdick L, Lillemoe TJ, et al. Methodologic findings and considerations for measuring colorectal epithelial cell proliferation in humans. Cancer Epidemiol Biomarkers Prev 1997;6:931–42.
11. Fedirko V, Bostick RM, Flanders WD, et al. Effects of vitamin D and calcium supplementation on markers of apoptosis in normal colon mucosa: A randomized, double-blind, placebo-controlled clinical trial. J Ca Prev Res. In press 2008.
12. Clark V, SAS Institute. The MIXED Procedure. SAS/STAT 91: user's guide. Cary (NC): SAS Pub.; 2004. p. 2659–853.
13. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 1986;124:17–27.
14. Boyle P, Langman JS. ABC of colorectal cancer: epidemiology. Br Med J 2000;321:805–8.
15. Bostick RM, Fosdick L, Wood JR, et al. Calcium and colorectal epithelial cell proliferation in sporadic adenoma patients: a randomized, double-blinded, placebo-controlled clinical trial. J Natl Cancer Inst 1995;87:1307–15.
16. Johnson IT, Lund EK. Review article: nutrition, obesity and colorectal cancer. Aliment Pharmacol Ther 2007;26:161–81.
17. Lipkin M, Reddy B, Newmark H, Lamprecht SA. Dietary factors in human colorectal cancer. Annu Rev Nutr 1999;19:545–86.
18. Bonithon-Kopp C, Kronborg O, Giacosa A, Rath U, Faivre J. Calcium and fibre supplementation in prevention of colorectal adenoma recurrence: a randomised intervention trial. European Cancer Prevention Organisation Study Group. Lancet 2000;356:1300–6.
19. Grau MV, Baron JA, Sandler RS, et al. Prolonged effect of calcium supplementation on risk of colorectal adenomas in a randomized trial. J Natl Cancer Inst 2007;99:129–36.
20. Hartman TJ, Albert PS, Snyder K, et al. The association of calcium and vitamin D with risk of colorectal adenomas. J Nutr 2005;135:252–9.
21. Levine AJ, Harper JM, Ervin CM, et al. Serum 25-hydroxyvitamin D, dietary calcium intake, and distal colorectal adenoma risk. Nutr Cancer 2001;39:35–41.
22. Shaukat A, Scouras N, Schunemann HJ. Role of supplemental calcium in the recurrence of colorectal adenomas: a metaanalysis of randomized controlled trials. Am J Gastroenterol 2005;100:390–4.
23. Wallace K, Baron JA, Cole BF, et al. Effect of calcium supplementation on the risk of large bowel polyps. J Natl Cancer Inst 2004;96:921–5.
24. Cravo ML, Pinto AG, Chaves P, et al. Effect of folate supplementation on DNA methylation of rectal mucosa in patients with colonic adenomas: correlation with nutrient intake. Clin Nutr 1998;17:45–9.
25. Giovannucci E, Stampfer MJ, Colditz GA, et al. Folate, methionine, and alcohol intake and risk of colorectal adenoma. J Natl Cancer Inst 1993;85:875–84.
26. Kawakami K, Ruszkiewicz A, Bennett G, Moore J, Watanabe G, Iacopetta B. The folate pool in colorectal cancers is associated with DNA hypermethylation and with a polymorphism in methylenetetrahydrofolate reductase. Clin Cancer Res 2003;9:5860–5.
27. van Engeland M, Weijenberg MP, Roemen GM, et al. Effects of dietary folate and alcohol intake on promoter methylation in sporadic colorectal cancer: the Netherlands cohort study on diet and cancer. Cancer Res 2003;63:3133–7.
28. Bronner CE, Baker SM, Morrison PT, et al. Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. Nature 1994;368:258–61.
29. Kunkel TA, Erie DA. DNA mismatch repair. Annu Rev Biochem 2005;74:681–710.
30. Plotz G, Piiper A, Wormek M, Zeuzem S, Raedle J. Analysis of the human MutLα.MutSα complex. Biochem Biophys Res Commun 2006;340:852–9.
31. Shih IM, Zhou W, Goodman SN, Lengauer C, Kinzler KW, Vogelstein B. Evidence that genetic instability occurs at an early stage of colorectal tumorigenesis. Cancer Res 2001;61:818–22.
32. Smith-Warner SA, Elmer PJ, Fosdick L, et al. Fruits, vegetables, and adenomatous polyps: the Minnesota Cancer Prevention Research Unit case-control study. Am J Epidemiol 2002;155:1104–13.
33. Satia JA, Keku T, Galanko JA, et al. Diet, lifestyle, and genomic instability in the North Carolina Colon Cancer Study. Cancer Epidemiol Biomarkers Prev 2005;14:429–36.
34. Diergaarde B, Braam H, van Muijen GN, Ligtenberg MJ, Kok FJ, Kampman E. Dietary factors and microsatellite instability in sporadic colon carcinomas. Cancer Epidemiol Biomarkers Prev 2003;12:1130–6.
35. Luchtenborg M, Weijenberg MP, de Goeij AF, et al. Meat and fish consumption, APC gene mutations and hMLH1 expression in colon and rectal cancer: a prospective cohort study (The Netherlands). Cancer Causes Control 2005;16:041–54.
36. Luchtenborg M, Weijenberg MP, Kampman E, et al. Cigarette smoking and colorectal cancer: APC mutations, hMLH1 expression, and GSTM1 and GSTT1 polymorphisms. Am J Epidemiol 2005;161:806–15.
37. Slattery ML, Curtin K, Sweeney C, et al. Diet and lifestyle factor associations with CpG island methylator phenotype and BRAF mutations in colon cancer. Int J Cancer 2007;120:656–63.
38. Wark PA, Weijenberg MP, van 't Veer P, et al. Fruits, vegetables, and

hMLH1 protein-deficient and -proficient colon cancer: The Netherlands cohort study. Cancer Epidemiol Biomarkers Prev 2005;14:1619–25.
39. Weijenberg MP, Luchtenborg M, de Goeij AF, et al. Dietary fat and risk of colon and rectal cancer with aberrant MLH1 expression, APC or KRAS genes. Cancer Causes Control 2007;18:865–79.
40. Mas S, Lafuente MJ, Crescenti A, et al. Lower specific micronutrient intake in colorectal cancer patients with tumors presenting promoter hypermethylation in p16(INK4a), p4(ARF) and hMLH1. Anticancer Res 2007;27:1151–6.
41. Boyapati SM, Bostick RM, McGlynn KA, et al. Calcium, vitamin D, and risk for colorectal adenoma: dependency on vitamin D receptor BsmI polymorphism and nonsteroidal anti-inflammatory drug use? Cancer Epidemiol Biomarkers Prev 2003;12:631–7.
42. Breuer-Katschinski B, Nemes K, Marr A, et al. Colorectal adenomas and diet: a case-control study. Colorectal Adenoma Study Group. Dig Dis Sci 2001;46:86–95.
43. Morimoto LM, Newcomb PA, Ulrich CM, Bostick RM, Lais CJ, Potter JD. Risk factors for hyperplastic and adenomatous polyps: evidence for malignant potential? Cancer Epidemiol Biomarkers Prev 2002;11:1012–8.
44. Peters U, Chatterjee N, Yeager M, et al. Association of genetic variants in the calcium-sensing receptor with risk of colorectal adenoma. Cancer Epidemiol Biomarkers Prev 2004;13:2181–6.

MutL-Homolog 1 Expression and Risk of Incident, Sporadic Colorectal Adenoma: Search for Prospective Biomarkers of Risk for Colorectal Cancer

Eduard Sidelnikov, Roberd M. Bostick, W. Dana Flanders, et al.

Cancer Epidemiol Biomarkers Prev 2009;18:1599-1609.