Published in final edited form as:

Int J Colorectal Dis. 2017 February ; 32(2): 183–192. doi:10.1007/s00384-016-2688-1.

Case-Control Study of Candidate Gene Methylation and Adenomatous Polyp Formation

Alexander M^{1,2}, Burch JB^{1,2,3}, Steck SE^{1,2,*}, Chen C-F⁴, Hurley TG¹, Cavicchia P^{1,2,5}, Shivappa N^{1,2}, Guess J^{1,2}, Zhang H⁶, Youngstedt SD⁷, Creek KE⁸, Lloyd S⁹, Jones K⁴, and Hébert JR^{1,2,10,*}

¹South Carolina Statewide Cancer Prevention & Control Program, University of South Carolina, Columbia, SC

²Department of Epidemiology and Biostatistics, Arnold School of Public Health, University of South Carolina, Columbia, SC

³William Jennings Bryant Dorn Department of Veterans Affairs Medical Center, Columbia, SC

⁴Center for Molecular Studies, Greenwood Genetic Center, Greenwood, SC

⁵Bureau of Epidemiology, Division of Disease Control and Health Protection, Florida Department of Health

⁶Division of Epidemiology, Biostatistics, and Environmental Health, School of Public Health, University of Memphis, Memphis, TN

⁷College of Nursing and Health Innovation, College of Health Solutions, Arizona State University, Phoenix, AZ

⁸Department of Drug Discovery and Biomedical Sciences, South Carolina College of Pharmacy, University of South Carolina

⁹South Carolina Medical Endoscopy Center, and Department of Family Medicine, University of South Carolina School of Medicine

¹⁰Department of Family and Preventive Medicine, School of Medicine, University of South Carolina, Columbia, SC

Abstract

Purpose—Colorectal cancer (CRC) is one of the most common and preventable forms of cancer, but remains the second leading cause of cancer-related death. Colorectal adenomas are precursor lesions that develop in 70–90% of CRC cases. Identification of peripheral biomarkers for adenomas would help to enhance screening efforts. This exploratory study examined the

Corresponding author: James Burch, Department of Epidemiology and Biostatistics, Arnold School of Public Health, Cancer Prevention & Control Program, 915 Greene St, Room 228 University of South Carolina, Columbia, SC 29209 burch@mailbox.sc.edu
Tel.: +1 803 576-5659, Fax: +1 803 576-5624.

*Co-Senior authors

Statement of Author Contributions: Drs. Burch, Steck, and Hébert designed the study and applied for Research Ethics Board approval. Dr. Stephen Lloyd, Ms. Alexander, and Ms. Guess recruited the patients and collected the data. Drs. Chen and Creek and Mr. Jones carried out all appropriate laboratory assays. Ms. Alexander prepared the manuscript draft with important intellectual input from all authors. All authors approved the final manuscript.

methylation status of 20 candidate markers in peripheral blood leukocytes and their association with adenoma formation.

Methods—Patients recruited from a local endoscopy clinic provided informed consent, and completed an interview to ascertain demographic, lifestyle, and adenoma risk factors. Cases were individuals with a histopathologically confirmed adenoma, and controls included patients with a normal colonoscopy, or those with histopathological findings not requiring heightened surveillance (normal biopsy, hyperplastic polyp). Methylation-specific polymerase chain reaction was used to characterize candidate gene promoter methylation. Odds ratios and 95% confidence intervals (OR, 95% CI) were calculated using unconditional multivariable logistic regression to test the hypothesis that candidate gene methylation differed between cases and controls, after adjustment for confounders.

Results—Complete data were available for 107 participants; 36% had adenomas (men: 40%, women: 31%). Hypomethylation of the *MINT1* locus (OR: 5.3, 95% CI: 1.0–28.2), and the *PER1* (OR: 2.9, 95% CI: 1.1–7.7) and *PER3* (OR: 11.6, 95% CI: 1.6–78.5) clock gene promoters was more common among adenoma cases. While specificity was moderate to high for the three markers (71–97%), sensitivity was relatively low (18–45%).

Conclusion—Follow-up of these epigenetic markers is suggested to further evaluate their utility for adenoma screening or surveillance.

Keywords

adenomatous polyp; circadian rhythm; colorectal cancer; screening tests

INTRODUCTION

Despite recent decreases in colorectal cancer (CRC) incidence and associated mortality, CRC remains one of the most common and deadly forms of cancer in the United States, with a lifetime risk for diagnosis of 5%.¹ Screening via colonoscopy presents an opportunity to interrupt the disease process (via polypectomy), thus facilitating primary CRC prevention. However, in 2012, the Centers for Disease Control and Prevention reported that CRC screening rates among eligible men and women fell far short of the *Healthy People 2020* target of 70.5%.² Indeed, only ~25–60% of eligible adults undergo CRC screening due to socioeconomic, racial, geographic, or other barriers that contribute to a lack of compliance.^{3–6} This suggests a need for valid and effective CRC screening methods that are more accessible to patients.

Some procedures that are less invasive than colonoscopy, such as stool-based tests (fecal occult blood test [FOBT] or the fecal immunochemical test [FIT]), may have slightly higher adherence (uptake is around one-third among eligible adults who are not under an intervention designed to improve compliance),⁷ but they are limited by reduced adenoma detection sensitivity (FOBT: 7–23%; FIT: 13–26%) compared to a colonoscopy, which has the ability to detect 73–94% of adenomas.^{8–11} A recent meta-analysis of stool-based DNA, an alternative CRC screening strategy, found that these tests may provide a diagnostic benefit for CRC or advanced adenoma detection in high-risk, but not average-risk, populations,¹² plus cost remains a potential barrier.¹³ Interestingly, methylation markers

provided better diagnostic performance for adenoma detection than did mutations (67% sensitivity vs. 10% sensitivity, respectively),¹² FOBT, or FIT (26%).^{10,11} Because blood-based measures can increase compliance with CRC screening and surveillance, genetic and epigenetic biomarkers in circulation are under active investigation.^{14,15}

The polyp→carcinoma sequence within the colon has been widely studied, and both genetic and epigenetic alterations have been observed at different stages of CRC development. An extensive literature describes the genetic lesions involved in this process, and an estimated 30% of CRC cases can be attributed to heritable factors.¹⁶ However, highly penetrant genetic markers account for only ~5% of CRC cases.¹⁶ Epigenetic modification represents an important and early event in the adenoma→CRC sequence. Methylation within gene promoter regions can lead to gene silencing and reduced gene expression.^{17,18} Promoter methylation of genes responsible for tumor growth suppression, DNA repair, or other critical pathways may reduce their expression and thereby promote tumor development. Aberrant methylation of several CRC-related loci (e.g., *MLH1*, *CDKN2A* [*p16*], *MINT1*, *MINT2*, *MINT31*) corresponds to a specific CRC subtype, the CpG island methylator phenotype (CIMP),¹⁹ which occurs in 0–44% of adenomas,^{20,21} and up to 50% of CRCs.²² However, associations between aberrant gene methylation and cancer have not always been consistent, possibly due to differences in the various molecular paths leading to CRC development.²³ For example, *MINT1*, *MINT2*, and *MINT12* are putative tumor suppressors, but were less methylated in adenomas from individuals with multiple polyps relative to those with at least one polyp and high microsatellite instability (MSI-H) in their adenomas.²³ Also, hypermethylation of *MINT31* has been associated with increased CRC risk,²⁴ but also has been associated with longer disease-free survival among CRC patients.²⁵ These findings highlight the need for more research on the relationship between promoter gene methylation and adenoma development or progression.

Despite the inherent advantages in terms of specimen accessibility and patient compliance relative to endoscopy, only a few studies have examined epigenetic markers in peripheral blood leukocytes (PBLs) for early detection of adenomas or CRC. Global hypomethylation of retrotransposable (LINE-1) elements in PBLs has been associated with increased odds of adenoma formation in some studies,^{26–29} and other studies targeting candidate genes also have had some success in identifying patients with adenomas or CRC, although results among these investigations have been mixed.^{15,3–34} Epigenetic changes in PBLs may reflect those observed in target tissues³⁵ or may serve as biologically relevant indicators of behavioral, environmental, psychosocial, or lifestyle factors that contribute to adenoma formation.^{18,36}

This exploratory case-control study tested the hypothesis that adenoma case status was associated with aberrant methylation of candidate gene promoters in PBL DNA relative to control colonoscopy patients. The objective of this study was to identify candidate epigenetic markers that can be used to improve adenoma detection and prevention. A panel of twenty candidate gene markers was selected for evaluation following a literature review based on their potential role in adenoma formation or CRC risk: *APC*, *BRCA1*, *CDKN2AP16*, *CYP24A*, *CYP27B1*, *ER-alpha*, *IGF2*, *MGMT*, *MINT1*, *MLH1*, *NGFR*, *PER1*, *PER2*, *PER3*, *SEPT9*, *SFRP4*, *SFRP5*, *TIMP3*, *TMEFF2*, and *WIF1*.^{15,18,37–43} Two

candidate genes associated with adenomas in this study (*PER1*, *PER3*) are involved in the maintenance of endogenous circadian rhythms, as well as biochemical pathways that are important in carcinogenesis (cell cycle control, DNA damage response, apoptosis).^{44–46}

MATERIALS AND METHODS

The Epigenetics and Diet in the Carcinogenesis Process (EDCaP) study was performed among patients undergoing a colonoscopy procedure at a community endoscopy center in Columbia, SC.⁴⁷ Prior to their clinic visit, 138 participants provided informed consent, including permission to access medical records, in accordance with the University of South Carolina's Institutional Review Board approval process. Participants completed a questionnaire to provide information on: demographic (sex, marital status, income, race/ethnicity), lifestyle (smoking history, diet, physical activity), and occupational (employment status, job industry, type of shift, history of shiftwork) factors, as well as personal and family medical history (ever being diagnosed with other diseases, personal cancer history, family CRC history).

A total of 256 subjects scheduled for a screening or surveillance colonoscopy were eligible to participate, and 154 were enrolled. Among those subjects, 138 had complete questionnaire data, and 107 of those participants provided biospecimens for epigenetic analysis (Figure 1). Information on cecal intubation, visualization of the appendix, and bowel preparation were abstracted from the patient's medical record to evaluate colonoscopy quality. Cases were defined as individuals with a complete colonoscopy procedure and at least one histologically confirmed adenoma based on review by a consulting clinical pathology laboratory. Controls included patients with a complete procedure and normal findings, or histopathological findings not requiring heightened surveillance (normal biopsy, hyperplastic polyp).⁴⁸

A peripheral whole blood sample was collected from each participant using vacutainers containing EDTA anticoagulant; and genomic DNA was isolated using the PUREGENE genomic DNA purification kit (Gentra Systems, Minneapolis, MN) according to the manufacturer's protocol. Sodium bisulfite conversion of DNA was performed using EpiTect kits (QIAGEN, Germantown, MD, USA). Briefly, DNA (2 µg) was added to the reaction buffer and bisulfite DNA conversion was conducted in a Bio-Rad S1000 thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA) using the manufacturer's suggested program for the conversion reaction. Converted DNA was eluted from a spin column in TE buffer and either used immediately or stored at –20°C. DNA oligonucleotides for primer pairs were purchased from Integrated DNA Technologies (Coralville, IA, USA, Table S1). To ensure that the polymerase chain reaction (PCR) primers were specific for the detection of methylated DNA, a control human DNA set (EpiTect Control DNA, QIAGEN Sciences, Germantown, MD) containing positive (bisulfite converted, 100% methylated) and negative (bisulfite converted, 0% methylated) control DNA was used. To quantify methylation specific PCR (MSP) results, the QIAxcel system was used (QIAGEN Sciences, Germantown, MD), which utilizes a multiplexed fluorescence detection method with a resolution of 3–5 bp for DNA fragments. The detection sensitivity of QIAxcel system was 0.1 ng/µl DNA in undiluted amplification reactions. The QIAxcel system was equipped with

the BioCalculator software that produces a tabular display of a variety of peak properties, including number of peaks as well as the height, width, and area of each peak. The height of the peak was used for separation of the results into three methylation categories: high (peak height >0.30), medium (peak height 0.29-0.05), and low (peak height <0.05), or none (no methylation detected). Results were categorized as either unmethylated or methylated (combination of medium/low, and high methylation categories). The methylation status of each sample was normalized via a two-step process. First, a NanoDrop™ Lite Spectrophotometer (Thermo Fisher Scientific, Waltham, MA) was used to measure concentrations of the DNA templates. Subsequent experiments used the same amount of DNA template for each MSP reaction. To facilitate quantification, equal volumes of the MSP products were loaded on the QIAxcel system for analysis. Second, BRCA1 gene methylation was used as an internal standard since the same amount of DNA template had extremely high methylation in each sample. The measured peak height of BRCA1 methylation was then used to normalize the methylation of the genes of interest for each sample. All methylation assays were performed blinded to case status.

Statistical analyses were performed using SAS® version 9.2 (Cary, NC). Frequency distributions of study variables by case status were examined using the chi-squared test for differences between proportions. Student's t-test was used for comparisons among cases and controls for normally distributed continuous variables. In instances of non-normally distributed continuous variables, the Wilcoxon–Mann–Whitney test was used to compare cases and controls. Unconditional multiple logistic regression was used to estimate odds ratios (OR) with 95% confidence intervals (CIs), comparing candidate gene methylation among adenoma cases relative to controls, after adjustment for the effects of potential confounding factors. Variables considered for inclusion in adjusted models were known or suspected adenoma or CRC risk factors (age, sex, race, body mass index [BMI], CRC family history, smoking history, work-related factors, diet, vitamin and supplement use, physical activity, sociodemographic characteristics [income, education], personal history of inflammatory bowel disease), and variables that differed among participants and non-participants (fruit and vegetable intake). Final models included variables that were statistically significant ($p < 0.05$) in the saturated model, or whose inclusion produced a 10% change in the parameter estimate for the gene of interest. Each candidate gene was selected *a priori*, thus no correction for multiple testing was performed.⁴⁹ Due to sparse data, individuals who received a colonoscopy due to symptoms (presence of gastrointestinal bleeding, fecal occult blood, iron deficiency, or constipation, $n=3$) were grouped with patients undergoing a screening colonoscopy. Sensitivity analyses were conducted to determine whether associations were driven by data from people with a right-sided polyp, as right-sided hyperplastic polyps have been linked with the serrated adenoma, MSI-H CRC pathway.⁵⁰ To ensure that precision was due to reduced sample size after exclusion of hyperplastics from the control series, sample-size-adjusted 95% CIs were produced using a method as described by Hébert et al,⁵¹ using the formula $\text{antilog}[b \pm 1.96 \times \text{SE}_b / \sqrt{n_{\text{all controls}} / n_{\text{hyperplastics}}}]$, where b = log odds ratio, SE_b = standard error of b , $n_{\text{all controls}}$ = number of all controls, and $n_{\text{hyperplastics}}$ = number of hyperplastic controls. Sensitivity and specificity, and positive and negative predictive values (PPV, NPV, respectively) were calculated for methylation markers with a statistically significant association with adenoma case status.

RESULTS

Complete data were obtained from 107 of the 154 patients (70%) who consented to participate (Figure 1). The mean age ($\pm$ SD) among participants was 60 ± 9 years; European Americans (EAs) comprised the majority of subjects (73%), and 46% of the population was female (Table 1). Most participants (96%) had a bowel preparation that was rated as fair or better, and the cecal intubation rate was 100%. No cases of advanced adenoma (>10 mm in size in any dimension, high-grade dysplasia, villous histology), serrated adenomatous polyps, or CRC were identified. The detection rate for any polyp was 65%, and the adenoma detection rate was 36% (male: 40%, female: 31%), with a mean of 0.7 ± 0.5 adenomas per person. All procedures with a right-sided hyperplastic polyp ($n=7$) were synchronous with adenoma detection in the same patient. Cases were more likely to be smokers compared to controls (68% vs. 45%, $p=0.02$), and were less likely to ever regularly take vitamin D supplements (5% vs. 29%, $p<0.01$). Though not statistically significant, cases also had a tendency to be 65 years of age ($p=0.07$), married ($p=0.16$), to currently drink alcohol ($p=0.13$), or to ever regularly take a multivitamin ($p=0.07$), or vitamin C ($p=0.15$) compared to controls.

After adjustment for potential confounding factors, hypomethylation (i.e., no methylation detected) in the *MINT1* (methylated in tumor1; OR: 5.3, 95% CI: 1.0–28.2) locus, and the *PER1* (Period 1; OR: 2.9, 95% CI: 1.1–7.7), and *PER3* (Period 3; OR: 11.1, 95% CI: 1.6–78.5) clock gene promoters was associated with an increased odds of adenoma detection (Table 2). Figures S1 and S2 (Appendix) present agarose gel images of MSP products for *PER1*, *PER2*, *PER3* and *MINT1*, respectively, in a subsample of participants. Sensitivity analyses that were conducted by excluding seven subjects with right-sided hyperplastic polyps yielded the following results for hypomethylation of *MINT1* (OR: 5.0, 95% CI: 0.9–27.9), *PER1* (OR: 2.8, 95% CI: 1.1–7.5) and *PER3* (OR: 9.8, 95% CI: 1.4–70.6). The time of day of sample collection or working at least one year of shift work did not modify the relationship between clock gene (*PER1*, *PER3*) promoter methylation and adenoma case status (data not shown). The ORs and corresponding sample-size-adjusted 95% CIs are provided in Table S2. Adjusted ORs for *PER1* and *PER3* increased in magnitude (OR: 4.7, 95% CI: 1.9–11.6 and OR: 19.9, 95% CI: 2.6–152.1 respectively) and remained significant after exclusion of hyperplastic polyps ($n=32$) from the control group; however, *MINT1* was no longer statistically significant (OR: 3.1, 95% CI: 0.5–18.0).

The sensitivity, specificity, PPV, and NPV for hypomethylation of *MINT1*, *PER1*, *PER3*, and their combinations are presented in Table S3 (Appendix). *PER1*, *PER3*, or *MINT1* hypomethylation correctly identified 16% to 45% of individuals with adenomas. Among patients without adenomas, specificity ranged from 71% to 97%. When evaluated in combination, hypomethylation of these genes yielded sensitivities ranging from 53% to 74%, and specificities ranging from 9% to 36% (Table S3). Among the possible combinations, *PER3/MINT1* yielded the highest sensitivity (74%), and the *PER1/PER3/MINT1* combination had the highest specificity (36%).

DISCUSSION

Identification of novel genes associated with adenoma risk may lead to a better understanding of carcinogenesis pathways and subsequently improve CRC screening and prevention efforts. Several studies have targeted blood-based epigenetic biomarkers to identify CRC cases, and only a few have used this approach to evaluate the formation of colorectal adenomas.^{14,15} In this exploratory case-control study, hypomethylation in the *MINT1* locus and the *PER1*, and *PER3* clock gene promoters was more common among adenoma cases relative to controls. Furthermore, associations for *PER1* and *PER3* strengthened in magnitude and remained significant after adjustment of the 95% CIs for reduced sample size following exclusion of patients with hyperplastic polyps from the control group.

The *MINT1* locus is one of several markers used to characterize CIMP status among CRC cases.^{19,52} Aberrant DNA methylation is considered an early event in the adenoma→carcinoma sequence,¹⁸ and changes in CIMP markers have been identified in aberrant crypt foci and in colorectal adenomas.^{21,53–55} MINT methylation in adenomas or associated CRC tissue has been linked with down regulation of mismatch repair (MLH1) protein expression, microsatellite instability, and BRAF (V600E) mutation relative to normal tissue.⁵⁶ However, prevalence of the CIMP phenotype is variable in adenomas (0–44%)^{20,21} as well as normal gastrointestinal tissues.⁵⁷ To our knowledge, the *MINT1* locus has not been examined as a circulatory marker for adenoma risk and thus results obtained in the present study require confirmation.

Two of the three candidate genes associated with adenomas in this study (*PER1*, *PER3*) are members of the clock gene family. Clock gene expression is involved in the maintenance of endogenous circadian rhythms, as well as the regulation of cellular processes that are considered hallmarks of carcinogenesis (cell cycle control, DNA damage response, apoptosis).^{44–46} In colon and other cancer cell lines, *PER1* insertion can activate cell cycle checkpoint proteins, sensitize cells to ionizing radiation-induced apoptosis, and exert an antiproliferative effect.⁵⁸ A hypomethylation zone in the *PER1* promoter has been associated with uncoupling of *PER1* transcription from promoter methylation in cervical cancer cells.¹⁷ Structural or epigenetic variability in E-boxes, particularly the E-box closest to the promoter, may play a role in these processes, and mutations within this element may lead to a reduction of *PER1* promoter activity.^{17,59} *PER1* and *PER2* mutations occur in human colorectal tumors,⁶⁰ and *PER* expression is reduced in colorectal adenomas or tumors relative to normal tissue.^{61–66} Polymorphisms in the *PER3* gene have been associated with inflammatory bowel disease, an established CRC risk factor,⁶⁷ and with increased odds of adenoma formation.⁶⁶ Aberrant methylation of *PER* genes has been observed among leukemia and other cancer patients.^{68–72} However, to the authors' knowledge, no studies have examined epigenetic modifications in the *PER* or other clock genes in relation to adenoma formation.^{46,66}

Clock genes facilitate the circadian expression of ~5–10% of the mammalian transcriptome, and clock-controlled genes include known tumor suppressors (e.g., *p21*, *Chk2*, *XPA*, *ATM*) and oncogenes (e.g., β -*catenin*, *c-Myc*, *WEE-1*).⁴⁵ Circadian patterns of clock-controlled

gene expression occur via rhythmic epigenetic modification of histones and chromatin.^{45,46,73} Histone modification may play a role in tumorigenesis,⁷⁴ although few studies have examined the role of circadian histone modifications in relation to adenoma or CRC development.⁴⁶ A significant proportion of clock-controlled gene regulation in mouse liver is mediated by clock gene interactions with the histone methyltransferase, MLL3 (mixed lineage leukemia 3), a tumor suppressor that has been associated with CRC and other cancers.^{73,75} *PER* complexes recruit histone-modifying proteins that help orchestrate the circadian expression of core clock genes, although it is not known whether this function extends to clock-controlled, cancer-related genes.^{46,76}

DNA hypomethylation within the context of CRC has been associated with dysregulation of retrotransposable elements (e.g., LINE-1, Alu, SVA), which is considered an early event in CRC development that can facilitate genomic instability, loss of imprinting, oncogene activation, aberrant splicing, and mutagenesis.^{18,77} For example, seminal studies identified a somatic LINE-1 insertion in the *APC* gene, as well as global DNA hypomethylation, as processes associated with CRC.^{78,79} More recent studies using PBLs have identified increased odds of global DNA hypomethylation among adenoma or CRC cases relative to controls, although not consistently.^{26–29} The *PER3* gene contains a variable-number tandem repeat (VNTR) sequence (a component of SVA retrotransposons),^{80,81} and individuals with the 5-repeat *PER3* VNTR sequence had an increased odds of adenoma formation.⁶⁶ However, the *PER3* VNTR did not modify the results obtained for hypomethylation of the *PER3* promoter in the present study; thus it is uncertain whether these processes are related. A growing body of evidence indicates that dysregulation of the *PERs* or other clock genes may have a role in adenoma formation and CRC risk, possibly via epigenetic or retrotransposon pathways, however the specific mechanisms remain to be elucidated.

This study had several noteworthy strengths and limitations. Strengths included the valid ascertainment of adenoma cases and statistical adjustment for known and suspected adenoma risk factors. The sample size limited power and precluded examination of the relationship between methylation patterns and adenoma status within strata of race, reason for colonoscopy, or factors that may influence epigenetic processes. Although each candidate gene was selected *a priori* following a literature review, this study was exploratory in nature and the potential for spurious or false positive findings cannot be eliminated. Also, methylation among specific white blood cell subsets was not quantified. Methylation patterns do not always vary among T-cell subsets, and the extent to which methylation differs among PBL subtypes for *PER1*, *PER3*, or *MINT1* is not known. Nonetheless, a lack of standardization for these potential differences represents a study uncertainty.^{36,82,83} Other studies that targeted clock genes in PBLs indicated that DNA hypomethylation was associated with breast cancer diagnoses (*CLOCK*),⁸⁴ or with advanced stage (II–IV) breast cancer (*TIMELESS*),⁸⁵ whereas promoter hypermethylation was associated with postmenopausal breast cancer (*CRY2*),⁸⁶ and with chronic myeloid leukemia (*PER3*)⁶⁸ relative to controls. The mechanism through which *PER1* or *PER3* gene methylation may impact adenoma formation is unknown and may depend on tissue context, timing of expression relative to other cellular processes, location of epigenetic changes within the gene, or other factors that may disrupt clock gene expression, such as shift work.^{45,46} Time of day of sample collection in the present study did not modify the relationship between

PER1 or *PER3* promoter methylation and adenoma case status (data not shown). However, more detailed genetic and epigenetic studies of the duration, phase and amplitude of *PER1* and *PER3* expression in conjunction with cancer-related, clock-controlled genes are suggested to help elucidate their role in adenoma formation. Methylation of non-target tissue (i.e., PBLs) can be influenced by psychosocial or environmental stressors, or lifestyle risk factors (e.g., diet, smoking). For example, cancer risk factors that can alter clock gene expression, such as shiftwork or sleep disturbances, also may exert epigenetic effects.^{87–91} Global DNA hypomethylation in PBLs was recently observed among night workers relative to those working days, and among the clock genes examined, the largest differences in hypomethylation were observed within *PER3* loci.⁸⁹ In the present study, neither the proportion of current shiftworkers nor those who engaged in at least one year of shiftwork differed by adenoma case status (Table 1), or by *MINT1*, *PER1*, or *PER3* methylation status (data not shown).

In summary, the identification of peripheral epigenetic markers that are associated with adenoma risk may lead to a deeper understanding of colorectal carcinogenesis and facilitate CRC prevention. Results from this study suggest follow-up blood-based monitoring of *MINT1*, *PER1*, and *PER3* methylation to examine their role in adenoma formation and CRC risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors gratefully acknowledge the management, staff, and participating patients of the South Carolina Medical Endoscopy Center (Columbia, SC). Ms. Susannah Kassler and Ms. Amy Messersmith provided technical support with sample processing.

Funding: This work was supported by three grants from the National Cancer Institute (NCI) – an Administrative Supplement to the South Carolina Cancer Disparities Community Network (SCDCN - 3 U01 CA114601-03S5.PI: JR Hébert; Co-Project Leaders: JB Burch, SE Steck), SCDCN-II (1U54 CA153461-01, JR Hébert, PI), and an Established Investigator Award in Cancer Prevention and Control from the Cancer Training Branch of the National Cancer Institute (K05 CA136975; JR Hébert, PI); a USC Research Opportunity Award (PI: SE Steck); the University of South Carolina Behavioral-Biomedical Interface Program with a grant from the National Institute of General Medical Sciences (T32-GM081740), which funded Melannie Alexander's effort; and a grant from the National Center for Research Resources to the USC Center for Colon Cancer Research (COBRE 5P20RR017698), which supported part of Dr. Steck's effort.

Literature Cited

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin*. 2012 Jan-Feb;62(1):10–29. [PubMed: 22237781]
2. Cancer screening - United States, 2010. *MMWR Morb Mortal Wkly Rep*. 2012 Jan 27; 61(3):41–45. [PubMed: 22278157]
3. Semrad TJ, Tancredi DJ, Baldwin LM, Green P, Fenton JJ. Geographic variation of racial/ethnic disparities in colorectal cancer testing among medicare enrollees. *Cancer*. 2011 Apr 15; 117(8):1755–1763. [PubMed: 21472723]
4. Klabunde CN, Schenck AP, Davis WW. Barriers to colorectal cancer screening among Medicare consumers. *Am J Prev Med*. 2006 Apr; 30(4):313–319. [PubMed: 16530618]

5. Halbert CH, Barg FK, Guerra CE, et al. Cultural, economic, and psychological predictors of colonoscopy in a national sample. *J Gen Intern Med*. 2011 Nov; 26(11):1311–1316. [PubMed: 21732197]
6. Nelson DE, Bolen J, Marcus S, Wells HE, Meissner H. Cancer screening estimates for U.S. metropolitan areas. *Am J Prev Med*. 2003; 24:301–309. [PubMed: 12726867]
7. Baker DW, Brown T, Buchanan DR, et al. Comparative effectiveness of a multifaceted intervention to improve adherence to annual colorectal cancer screening in community health centers: a randomized clinical trial. *JAMA Intern Med*. 2014 Aug; 174(8):1235–1241. [PubMed: 24934845]
8. Ahn SB, Han DS, Bae JH, Byun TJ, Kim JP, Eun CS. The Miss Rate for Colorectal Adenoma Determined by Quality-Adjusted, Back-to-Back Colonoscopies. *Gut and liver*. 2012 Jan; 6(1):64–70. [PubMed: 22375173]
9. Hassan C, Giorgi Rossi P, Camilloni L, et al. Meta-analysis: adherence to colorectal cancer screening and the detection rate for advanced neoplasia, according to the type of screening test. *Aliment Pharmacol Ther*. 2012 Nov; 36(10):929–940. [PubMed: 23035890]
10. Morikawa T, Kato J, Yamaji Y, et al. Sensitivity of immunochemical fecal occult blood test to small colorectal adenomas. *Am J Gastroenterol*. 2007 Oct; 102(10):2259–2264. [PubMed: 17617203]
11. Wong CK, Fedorak RN, Prosser CI, Stewart ME, van Zanten SV, Sadowski DC. The sensitivity and specificity of guaiac and immunochemical fecal occult blood tests for the detection of advanced colonic adenomas and cancer. *Int J Colorectal Dis*. 2012 Dec; 27(12):1657–1664. [PubMed: 22696204]
12. Yang H, Xia BQ, Jiang B, et al. Diagnostic value of stool DNA testing for multiple markers of colorectal cancer and advanced adenoma: a meta-analysis. *Can J Gastroenterol*. 2013 Aug; 27(8):467–475. [PubMed: 23936877]
13. Jones RM, Woolf SH, Cunningham TD, et al. The relative importance of patient-reported barriers to colorectal cancer screening. *Am J Prev Med*. 2010 May; 38(5):499–507. [PubMed: 20347555]
14. Ganepola GA, Nizin J, Rutledge JR, Chang DH. Use of blood-based biomarkers for early diagnosis and surveillance of colorectal cancer. *World journal of gastrointestinal oncology*. 2014 Apr 15; 6(4):83–97. [PubMed: 24734154]
15. Hong L, Ahuja N. DNA methylation biomarkers of stool and blood for early detection of colon cancer. *Genetic testing and molecular biomarkers*. 2013 May; 17(5):401–406. [PubMed: 23406208]
16. Jasperson KW, Tuohy TM, Neklason DW, Burt RW. Hereditary and familial colon cancer. *Gastroenterology*. 2010 Jun; 138(6):2044–2058. [PubMed: 20420945]
17. Hsu MC, Huang CC, Choo KB, Huang CJ. Uncoupling of promoter methylation and expression of *Period1* in cervical cancer cells. *Biochem Biophys Res Commun*. 2007 Aug 17; 360(1):257–262. [PubMed: 17592726]
18. Lao VV, Grady WM. Epigenetics and colorectal cancer. *Nat Rev Gastroenterol Hepatol*. 2011 Dec; 8(12):686–700. [PubMed: 22009203]
19. Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, Issa JP. CpG island methylator phenotype in colorectal cancer. *Proc Natl Acad Sci U S A*. 1999 Jul 20; 96(15):8681–8686. [PubMed: 10411935]
20. O'Brien MJ, Yang S, Mack C, et al. Comparison of microsatellite instability, CpG island methylation phenotype, BRAF and KRAS status in serrated polyps and traditional adenomas indicates separate pathways to distinct colorectal carcinoma end points. *Am J Surg Pathol*. 2006 Dec; 30(12):1491–1501. [PubMed: 17122504]
21. Rashid A, Shen L, Morris JS, Issa JP, Hamilton SR. CpG island methylation in colorectal adenomas. *The American journal of pathology*. 2001 Sep; 159(3):1129–1135. [PubMed: 11549606]
22. Conteduca V, Sansonno D, Russi S, Dammacco F. Precancerous colorectal lesions (Review). *Int J Oncol*. 2013 Oct; 43(4):973–984. [PubMed: 23900573]
23. Wynter CV, Kambara T, Walsh MD, Leggett BA, Young J, Jass JR. DNA methylation patterns in adenomas from FAP, multiple adenoma and sporadic colorectal carcinoma patients. *Int J Cancer*. 2006 Feb 15; 118(4):907–915. [PubMed: 16152625]

24. Garrity-Park MM, Loftus EV Jr, Sandborn WJ, Bryant SC, Smyrk TC. Methylation status of genes in non-neoplastic mucosa from patients with ulcerative colitis-associated colorectal cancer. *Am J Gastroenterol.* 2010 Jul; 105(7):1610–1619. [PubMed: 20160714]
25. Kim JC, Choi JS, Roh SA, Cho DH, Kim TW, Kim YS. Promoter methylation of specific genes is associated with the phenotype and progression of colorectal adenocarcinomas. *Ann Surg Oncol.* 2010 Jul; 17(7):1767–1776. [PubMed: 20077021]
26. Pufulete M, Al-Ghnam R, Leather AJ, et al. Folate status, genomic DNA hypomethylation, and risk of colorectal adenoma and cancer: a case control study. *Gastroenterology.* 2003 May; 124(5): 1240–1248. [PubMed: 12730865]
27. Lim U, Flood A, Choi SW, et al. Genomic methylation of leukocyte DNA in relation to colorectal adenoma among asymptomatic women. *Gastroenterology.* 2008 Jan; 134(1):47–55. [PubMed: 18166347]
28. King WD, Ashbury JE, Taylor SA, et al. A cross-sectional study of global DNA methylation and risk of colorectal adenoma. *BMC Cancer.* 2014; 14:488. [PubMed: 24998982]
29. Cravo M, Fidalgo P, Pereira AD, et al. DNA methylation as an intermediate biomarker in colorectal cancer: modulation by folic acid supplementation. *European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation.* 1994 Nov; 3(6):473–479.
30. Ally MS, Al-Ghnam R, Pufulete M. The relationship between gene-specific DNA methylation in leukocytes and normal colorectal mucosa in subjects with and without colorectal tumors. *Cancer Epidemiol Biomarkers Prev.* 2009 Mar; 18(3):922–928. [PubMed: 19258481]
31. Gao Y, Killian K, Zhang H, et al. Leukocyte DNA methylation and colorectal cancer among male smokers. *World J Gastrointest Oncol.* 2012 Aug 15; 4(8):193–201. [PubMed: 22912915]
32. Kaaks R, Stattin P, Villar S, et al. Insulin-like growth factor-II methylation status in lymphocyte DNA and colon cancer risk in the Northern Sweden Health and Disease cohort. *Cancer Res.* 2009 Jul 1; 69(13):5400–5405. [PubMed: 19549920]
33. Mitchell SM, Ross JP, Drew HR, et al. A panel of genes methylated with high frequency in colorectal cancer. *BMC Cancer.* 2014; 14:54. [PubMed: 24485021]
34. Cui H, Cruz-Correa M, Giardiello FM, et al. Loss of IGF2 imprinting: a potential marker of colorectal cancer risk. *Science.* 2003 Mar 14; 299(5613):1753–1755. [PubMed: 12637750]
35. McKay JA, Xie L, Harris S, Wong YK, Ford D, Mathers JC. Blood as a surrogate marker for tissue-specific DNA methylation and changes due to folate depletion in post-partum female mice. *Mol Nutr Food Res.* 2011 Jul; 55(7):1026–1035. [PubMed: 21520493]
36. Terry MB, Delgado-Cruzata L, Vin-Raviv N, Wu HC, Santella RM. DNA methylation in white blood cells: association with risk factors in epidemiologic studies. *Epigenetics : official journal of the DNA Methylation Society.* 2011 Jul; 6(7):828–837.
37. Binefa G, Rodriguez-Moranta F, Teule A, Medina-Hayas M. Colorectal cancer: From prevention to personalized medicine. *World J Gastroenterol.* 2014 Jun 14; 20(22):6786–6808. [PubMed: 24944469]
38. Coppede F, Lopomo A, Spisni R, Migliore L. Genetic and epigenetic biomarkers for diagnosis, prognosis and treatment of colorectal cancer. *World J Gastroenterol.* 2014 Jan 28; 20(4):943–956. [PubMed: 24574767]
39. Sakai E, Nakajima A, Kaneda A. Accumulation of aberrant DNA methylation during colorectal cancer development. *World J Gastroenterol.* 2014 Jan 28; 20(4):978–987. [PubMed: 24574770]
40. Jacobs ET, Van Pelt C, Forster RE, et al. CYP24A1 and CYP27B1 polymorphisms modulate vitamin D metabolism in colon cancer cells. *Cancer Res.* 2013 Apr 15; 73(8):2563–2573. [PubMed: 23423976]
41. Shen R, Tao L, Xu Y, Chang S, Van Brocklyn J, Gao JX. Reversibility of aberrant global DNA and estrogen receptor-alpha gene methylation distinguishes colorectal precancer from cancer. *Int J Clin Exp Pathol.* 2009; 2(1):21–33. [PubMed: 18830381]
42. Mazzocchi G, Panza A, Valvano MR, et al. Clock gene expression levels and relationship with clinical and pathological features in colorectal cancer patients. *Chronobiol Int.* 2011 Dec; 28(10): 841–851. [PubMed: 22080729]
43. Silva TD, Vidigal VM, Felipe AV, et al. DNA methylation as an epigenetic biomarker in colorectal cancer. *Oncol Lett.* 2013 Dec; 6(6):1687–1692. [PubMed: 24260063]

44. Haus EL, Smolensky MH. Shift work and cancer risk: potential mechanistic roles of circadian disruption, light at night, and sleep deprivation. *Sleep Med Rev.* 2013 Aug; 17(4):273–284. [PubMed: 23137527]
45. Mazzocchi G, Vinciguerra M, Papa G, Piepoli A. Circadian clock circuitry in colorectal cancer. *World journal of gastroenterology : WJG.* 2014 Apr 21; 20(15):4197–4207. [PubMed: 24764658]
46. Joska TM, Zaman R, Belden WJ. Regulated DNA methylation and the circadian clock: implications in cancer. *Biology (Basel).* 2014; 3(3):560–577. [PubMed: 25198253]
47. Tabung FK, Steck SE, Burch JB, et al. A Healthy Lifestyle Index Is Associated With Reduced Risk of Colorectal Adenomatous Polyps Among Non-Users of Non-Steroidal Anti-Inflammatory Drugs. *The journal of primary prevention.* 2014 Oct 21.
48. Winawer S, Fletcher R, Rex D, et al. Colorectal cancer screening and surveillance: clinical guidelines and rationale-Update based on new evidence. *Gastroenterology.* 2003 Feb; 124(2):544–560. [PubMed: 12557158]
49. Bender R, Lange S. Adjusting for multiple testing--when and how? *J Clin Epidemiol.* 2001 Apr; 54(4):343–349. [PubMed: 11297884]
50. Hawkins NJ, Ward RL. Sporadic colorectal cancers with microsatellite instability and their possible origin in hyperplastic polyps and serrated adenomas. *J Natl Cancer Inst.* 2001 Sep 5; 93(17):1307–1313. [PubMed: 11535705]
51. Hebert JR, Gupta PC, Bhonsle RB, et al. Dietary exposures and oral precancerous lesions in Srikakulam District, Andhra Pradesh, India. *Public health nutrition.* 2002 Apr; 5(2):303–312. [PubMed: 12020382]
52. Suter CM, Norrie M, Ku SL, Cheong KF, Tomlinson I, Ward RL. CpG island methylation is a common finding in colorectal cancer cell lines. *Br J Cancer.* 2003 Feb 10; 88(3):413–419. [PubMed: 12569385]
53. Kim HC, Roh SA, Ga IH, Kim JS, Yu CS, Kim JC. CpG island methylation as an early event during adenoma progression in carcinogenesis of sporadic colorectal cancer. *J Gastroenterol Hepatol.* 2005 Dec; 20(12):1920–1926. [PubMed: 16336454]
54. Kim YH, Petko Z, Dzieciatkowski S, et al. CpG island methylation of genes accumulates during the adenoma progression step of the multistep pathogenesis of colorectal cancer. *Genes, chromosomes & cancer.* 2006 Aug; 45(8):781–789. [PubMed: 16708352]
55. Chan AO, Broaddus RR, Houlihan PS, Issa JP, Hamilton SR, Rashid A. CpG island methylation in aberrant crypt foci of the colorectum. *The American journal of pathology.* 2002 May; 160(5):1823–1830. [PubMed: 12000733]
56. de Maat MF, Narita N, Benard A, et al. Development of sporadic microsatellite instability in colorectal tumors involves hypermethylation at methylated-in-tumor loci in adenoma. *The American journal of pathology.* 2010 Nov; 177(5):2347–2356. [PubMed: 20952593]
57. Worthley DL, Whitehall VL, Buttenshaw RL, et al. DNA methylation within the normal colorectal mucosa is associated with pathway-specific predisposition to cancer. *Oncogene.* 2010 Mar 18; 29(11):1653–1662. [PubMed: 19966864]
58. Gery S, Komatsu N, Baldjyan L, Yu A, Koo D, Koeffler HP. The circadian gene *per1* plays an important role in cell growth and DNA damage control in human cancer cells. *Mol Cell.* 2006 May 5; 22(3):375–382. [PubMed: 16678109]
59. Brown SA, Ripberger J, Kadener S, et al. PERIOD1-associated proteins modulate the negative limb of the mammalian circadian oscillator. *Science.* 2005 Apr 29; 308(5722):693–696. [PubMed: 15860628]
60. Sjoblom T, Jones S, Wood LD, et al. The consensus coding sequences of human breast and colorectal cancers. *Science.* 2006 Oct 13; 314(5797):268–274. [PubMed: 16959974]
61. Oshima T, Takenoshita S, Akaike M, et al. Expression of circadian genes correlates with liver metastasis and outcomes in colorectal cancer. *Oncol Rep.* 2011 Mar 4.
62. Wang X, Yan D, Teng M, et al. Reduced expression of PER3 is associated with incidence and development of colon cancer. *Ann Surg Oncol.* 2012 Feb 17.
63. Mostafaie N, Kallay E, Sauerzapf E, et al. Correlated downregulation of estrogen receptor beta and the circadian clock gene *Per1* in human colorectal cancer. *Mol Carcinog.* 2009 Jul; 48(7):642–647. [PubMed: 19148895]

64. Krugluger W, Brandstaetter A, Kallay E, et al. Regulation of genes of the circadian clock in human colon cancer: reduced period-1 and dihydropyrimidine dehydrogenase transcription correlates in high-grade tumors. *Cancer research*. 2007 Aug 15; 67(16):7917–7922. [PubMed: 17699798]
65. Karantanos T, Theodoropoulos G, Gazouli M, et al. Expression of clock genes in patients with colorectal cancer. *The International journal of biological markers*. 2013 May 27; 28(3):280–285. [PubMed: 23712462]
66. Alexander M, Burch JB, Steck SE, et al. Case-control study of the PERIOD3 clock gene length polymorphism and colorectal adenoma formation. *Oncology Reports*. 2015; 33:935–941. [PubMed: 25501848]
67. Mazzocchi G, Palmieri O, Corritore G, et al. Association study of a polymorphism in clock gene PERIOD3 and risk of inflammatory bowel disease. *Chronobiology International*. 2012 Oct; 29(8): 994–1003. [PubMed: 22881285]
68. Yang MY, Chang JG, Lin PM, et al. Downregulation of circadian clock genes in chronic myeloid leukemia: alternative methylation pattern of hPER3. *Cancer Sci*. 2006 Dec; 97(12):1298–1307. [PubMed: 16999817]
69. Kuo SJ, Chen ST, Yeh KT, et al. Disturbance of circadian gene expression in breast cancer. *Virchows Arch*. 2009 Apr; 454(4):467–474. [PubMed: 19296127]
70. Chen ST, Choo KB, Hou MF, Yeh KT, Kuo SJ, Chang JG. Deregulated expression of the PER1, PER2 and PER3 genes in breast cancers. *Carcinogenesis*. 2005 Jul; 26(7):1241–1246. [PubMed: 15790588]
71. Shih MC, Yeh KT, Tang KP, Chen JC, Chang JG. Promoter methylation in circadian genes of endometrial cancers detected by methylation-specific PCR. *Mol Carcinog*. 2006 Oct; 45(10):732–740. [PubMed: 16683245]
72. Gery S, Komatsu N, Kawamata N, et al. Epigenetic silencing of the candidate tumor suppressor gene Per1 in non-small cell lung cancer. *Clin Cancer Res*. 2007 Mar 1; 13(5):1399–1404. [PubMed: 17332281]
73. Valekunjia UK, Edgar RS, Oklejewicz M, et al. Histone methyltransferase MLL3 contributes to genome-scale circadian transcription. *Proceedings of the National Academy of Sciences of the United States of America*. 2013 Jan 22; 110(4):1554–1559. [PubMed: 23297224]
74. Waldmann T, Schneider R. Targeting histone modifications-epigenetics in cancer. *Curr Opin Cell Biol*. 2013 Apr; 25(2):184–189. [PubMed: 23347561]
75. Watanabe Y, Castoro RJ, Kim HS, et al. Frequent alteration of MLL3 frameshift mutations in microsatellite deficient colorectal cancer. *PLoS One*. 2011; 6(8):e23320. [PubMed: 21853109]
76. Duong HA, Weitz CJ. Temporal orchestration of repressive chromatin modifiers by circadian clock Period complexes. *Nat Struct Mol Biol*. 2014 Feb; 21(2):126–132. [PubMed: 24413057]
77. Hancks DC, Kazazian HH Jr. Active human retrotransposons: variation and disease. *Curr Opin Genet Dev*. 2012 Jun; 22(3):191–203. [PubMed: 22406018]
78. Miki Y, Nishisho I, Horii A, et al. Disruption of the APC gene by a retrotransposal insertion of L1 sequence in a colon cancer. *Cancer Research*. 1992 Feb 1; 52(3):643–645. [PubMed: 1310068]
79. Feinberg AP, Vogelstein B. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature*. 1983 Jan 6; 301(5895):89–92. [PubMed: 6185846]
80. Hancks DC, Kazazian HH Jr. SVA retrotransposons: Evolution and genetic instability. *Seminars in cancer biology*. 2010 Aug; 20(4):234–245. [PubMed: 20416380]
81. Sabino FC, Ribeiro AO, Tufik S, et al. Evolutionary History of the PER3 Variable Number of Tandem Repeats (VNTR): Idiosyncratic Aspect of Primate Molecular Circadian Clock. *PLoS One*. 2014; 9(9):e107198. [PubMed: 25222750]
82. Cassinotti E, Melson J, Liggett T, et al. DNA methylation patterns in blood of patients with colorectal cancer and adenomatous colorectal polyps. *Int J Cancer*. 2012 Sep 1; 131(5):1153–1157. [PubMed: 22020530]
83. Adalsteinsson BT, Gudnason H, Aspelund T, et al. Heterogeneity in white blood cells has potential to confound DNA methylation measurements. *PLoS One*. 2012; 7(10):e46705. [PubMed: 23071618]

84. Hoffman AE, Yi CH, Zheng T, et al. CLOCK in breast tumorigenesis: genetic, epigenetic, and transcriptional profiling analyses. *Cancer research*. 2010 Feb 15; 70(4):1459–1468. [PubMed: 20124474]
85. Fu A, Leaderer D, Zheng T, Hoffman AE, Stevens RG, Zhu Y. Genetic and epigenetic associations of circadian gene TIMELESS and breast cancer risk. *Molecular carcinogenesis*. 2011 Oct 17.
86. Hoffman AE, Zheng T, Yi CH, et al. The core circadian gene Cryptochrome 2 influences breast cancer risk, possibly by mediating hormone signaling. *Cancer prevention research*. 2010 Apr; 3(4): 539–548. [PubMed: 20233903]
87. Lahti T, Merikanto I, Partonen T. Circadian clock disruptions and the risk of cancer. *Annals of medicine*. 2012 Dec; 44(8):847–853. [PubMed: 23072403]
88. Bollati V, Baccarelli A, Sartori S, et al. Epigenetic effects of shiftwork on blood DNA methylation. *Chronobiol Int*. 2010 Jul; 27(5):1093–1104. [PubMed: 20636218]
89. Bhatti P, Zhang Y, Song X, et al. Nightshift work and genome-wide DNA methylation. *Chronobiology International*. 2014 Sep 4.:1–10.
90. Zhu Y, Stevens RG, Hoffman AE, et al. Epigenetic impact of long-term shiftwork: pilot evidence from circadian genes and whole-genome methylation analysis. *Chronobiology International*. 2011 Dec; 28(10):852–861. [PubMed: 22080730]
91. Qureshi IA, Mehler MF. Epigenetics of sleep and chronobiology. *Curr Neurol Neurosci Rep*. 2014 Mar.14(3):432. [PubMed: 24477387]

Figure 1.

Flow chart of participant recruitment and participation in the Epigenetics and Diet in the Carcinogenesis Process (EDCaP) study.

Table 1

Characteristics of EDCaP Study Participants

Variable	Total population (n = 107)	Controls (n = 69)	Cases (n = 38)	Controls vs. Cases p-value ^I
	n (%)	n (%)	n (%)	
Gender				0.33
Male	58 (54)	35 (51)	23 (61)	
Female	49 (46)	34 (49)	15 (39)	
Race				0.55
European American	78 (73)	49 (71)	29 (76)	
African American	29 (27)	20 (29)	9 (24)	
Married				0.16
Yes	85 (79)	52 (75)	33 (87)	
No	22 (21)	17 (25)	5 (13)	
Education				0.62
Up to High School	32 (30)	21 (30)	11 (29)	
Some College	28 (26)	16 (23)	12 (32)	
College Undergraduate or Post-Graduate Degree	47 (44)	32 (46)	15 (40)	
Income Level				0.86
Under \$50,000	33 (33)	21 (33)	12 (33)	
\$50,000 to \$100,000	44 (44)	29 (46)	15 (42)	
\$100,000	22 (22)	13 (21)	9 (25)	
Age Group (Years)				0.07
<54	28 (26)	20 (29)	8 (21)	
55–64	46 (43)	33 (48)	13 (34)	
65	33 (31)	16 (23)	17 (45)	
Body Mass Index (kg/m ²)				0.57
Underweight or normal	20 (19)	14 (20)	6 (16)	
Overweight or obese	87 (81)	55 (80)	32 (84)	
History of Smoking				0.02
Ever	57 (53)	31 (45)	26 (68)	
Never	50 (47)	38 (55)	12 (32)	
Current alcohol consumption				0.13
Yes	46 (43)	26 (38)	20 (53)	
No	61 (57)	43 (62)	18 (47)	
Ever regularly taken a multivitamin				0.07
Yes	68 (64)	40 (58)	28 (76)	
No	38 (36)	29 (42)	9 (24)	

Variable	Total population (n = 107)	Controls (n = 69)	Cases (n = 38)	Controls vs. Cases p-value ¹
	n (%)	n (%)	n (%)	
Ever regularly taken a vitamin C supplement				0.15
Yes	31 (29)	17 (25)	14 (38)	
No	75 (71)	21 (75)	23 (62)	
Ever regularly taken a vitamin D supplement				<0.01
Yes	22 (21)	20 (29)	2 (5)	
No	84 (79)	49 (71)	35 (95)	
Hemorrhoids				0.18
Yes	67 (63)	40 (58)	27 (71)	
No	40 (37)	29 (42)	11 (29)	
Procedure reason				<0.01
Routine screening/Symptoms ²	34 (32)	30 (43)	4 (11)	
Elevated Risk ³	73 (68)	39 (57)	34 (89)	
Time of day blood was collected				0.23
Morning	83 (78)	56 (81)	27 (71)	
Afternoon	24 (22)	13 (19)	11 (29)	
Worked >1 year of shift work over lifetime				0.99
Yes	45 (42)	29 (42)	16 (42)	
No	62 (58)	40 (58)	22 (58)	
Current Work Shift				0.31
Days	53 (47)	33 (48)	20 (53)	
Other	4 (3)	4 (6)	0 (0)	
Unemployed/Retired	50 (50)	32 (46)	18 (47)	

¹ χ^2 test for differences in proportions between cases and controls.;

² Three subjects exhibited symptoms (presence of gastrointestinal bleeding, hematochezia, melena, fecal occult blood, iron deficiency, or constipation) and were collapsed into screening category;

³ Subject had a previous adenomatous polyp detected (n = 59) and/or a family history of colorectal cancer (n = 20)

Table 2
Unadjusted and Adjusted Odds Ratios for Leukocyte DNA Promoter Methylation Relative to Adenoma Case Status

Candidate Gene	Controls (n = 69) n (%)	Cases (n = 38) n (%)	Crude OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Hypomethylation *						
<i>APC^a</i>	44 (64)	23 (61)	0.9 (0.4 – 2.0)	0.74	1.0 (0.4 – 2.5)	0.97
<i>BRCA1^b</i>	31 (44)	18 (47)	1.1 (0.5 – 2.4)	0.81	1.5 (0.6 – 3.9)	0.36
<i>MLH1^d</i>	4 (6)	7 (18)	3.7 (1.0 – 13.5)	0.05	5.3 (1.0 – 28.2)	0.05
<i>PER1^e</i>	20 (29)	17 (45)	2.0 (0.9 – 4.5)	0.10	2.9 (1.1 – 7.7)	0.03
<i>PER3^f</i>	2 (3)	6 (16)	6.3 (1.2 – 32.9)	0.03	11.1 (1.6 – 78.5)	0.02
<i>SFRP4^e</i>	12 (17)	5 (29)	0.7 (0.2 – 2.2)	0.57	1.1 (0.3 – 3.8)	0.89
<i>SFRP5^b</i>	5 (7)	5 (29)	1.9 (0.5 – 7.2)	0.32	3.7 (0.8 – 17.1)	0.09
<i>TIMP3^g</i>	42 (61)	25 (66)	1.2 (0.5 – 2.8)	0.61	1.2 (0.4 – 3.0)	0.78
<i>TMEFF2^h</i>	33 (48)	21 (55)	1.4 (0.6 – 3.0)	0.46	1.5 (0.6 – 3.4)	0.36
<i>WIF1ⁱ</i>	24 (35)	15 (39)	1.2 (0.5 – 2.8)	0.63	1.3 (0.5 – 3.2)	0.56
Hypermethylation						
<i>CDKN2A (p16)^j</i>	28 (41)	17 (45)	0.8 (0.4 – 1.9)	0.68	0.8 (0.3 – 1.9)	0.54
<i>CYP27B1^l</i>	58 (84)	31 (82)	1.2 (0.4 – 3.4)	0.74	0.8 (0.2 – 3.0)	0.72
<i>ER alpha^j</i>	31 (45)	20 (53)	0.7 (0.3 – 1.6)	0.45	0.6 (0.3 – 1.5)	0.27
<i>IGF2^e</i>	52 (75)	28 (74)	1.1 (0.4 – 2.7)	0.85	0.8 (0.3 – 2.0)	0.57
<i>MGMT^h</i>	28 (41)	16 (42)	0.9 (0.4 – 2.1)	0.88	0.9 (0.4 – 2.1)	0.80
<i>MLH1^l</i>	63 (91)	34 (89)	1.2 (0.3 – 4.7)	0.76	0.7 (0.2 – 3.1)	0.67
<i>NGFR^a</i>	20 (29)	12 (32)	0.9 (0.4 – 2.1)	0.78	0.9 (0.3 – 2.2)	0.75
<i>PER2^g</i>	62 (90)	35 (92)	0.8 (0.2 – 3.1)	0.70	0.6 (0.1 – 3.0)	0.56
<i>SEPT9^a</i>	32 (46)	18 (47)	1.0 (0.4 – 2.1)	0.92	0.9 (0.4 – 2.1)	0.81

* - Odds of adenoma status given no methylation detected in the candidate gene. Note: *CYP24A* was not included in the table due to all subjects being unmethylated in the promoter.

Adjusted for: a – vitamin D and multivitamin use;

b – vitamin C and vitamin D use and physical activity;

c – multivitamin, vitamin C, and vitamin D use, physical activity, ever smoking, currently drinking alcohol, being married, and age group;

d – multivitamin use, vitamin C and D use, and ever smoking;

e – OR adjusted for vitamin C and D use and physical activity;

f – multivitamin use, vitamin C use, vitamin D use, physical activity, ever smoking, age, and being married;

g – multivitamin and vitamin D use, physical activity, and age group;

h – vitamin D use;

i – vitamin D use and age group;

j – vitamin D use, physical activity and age;

k – vitamin C and D use, physical activity and age;

l – vitamin D use, current drinking alcohol, and age group. OR: odds ratio; CI: confidence interval