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Evaluation of Pro-inflammatory Markers Plasma C-reactive Protein and Urinary Prostaglandin-E2 Metabolite in Colorectal Adenoma Risk

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Abstract

C-reactive protein (CRP) is a pro-inflammatory protein with potential as a biomarker in predicting colon cancer risk. However, little is known regarding its association with risk of colorectal adenomas, particularly by subtypes. We conducted a colonoscopy-based matched case-control

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study to assess whether elevated plasma CRP levels may be associated with colorectal adenoma risk and further whether this association may be modified by urinary prostaglandin E2 metabolite (PGE-M), a biomarker of systemic prostaglandin E2 production. Included in the study were 226 cases with a single small tubular adenoma, 198 cases with multiple small tubular adenomas, 283 cases with at least one advanced adenoma, and 395 polyp-free controls. No apparent association between CRP level and risk of single small tubular adenomas was found ($p_{\text{trend}}=0.59$). A dose-response relationship with CRP level was observed for risk of either multiple small tubular adenomas (OR = 2.01, 95% CI = 1.10–3.68 for the highest vs lowest tertile comparison; $p_{\text{trend}} = 0.03$) or advanced adenomas (OR = 1.81, 95% CI = 1.10–2.96 for the highest vs lowest tertile comparison; $p_{\text{trend}}= 0.02$). In a joint analysis of CRP level and PGE-M, risk of multiple or advanced adenoma was greatest among those with highest levels of both CRP and PGE-M in comparison to those with low CRP and low PGE-M (OR = 3.72, 95% CI = 1.49–9.72). Our results suggest that elevated CRP, particularly in the context of concurrent elevated PGE-M, may be a biomarker of multiple or advanced adenoma risk in a screening age population.

Keywords

Colorectal Adenoma Risk; C-reactive Protein; Prostaglandin-E2 Metabolite; Inflammation; Biomarkers

Introduction

Discovery of reliable biomarkers for assessment of both colorectal adenoma and adenocarcinoma risk in a screening-aged population has presented challenges to date. Markers of inflammation may have utility in assessing individual risk for colorectal cancer screening. Chronic inflammation involving the mucosal surface and submucosa of the colon and rectum previously has been postulated as a mechanism for both pre-cancerous colorectal adenoma development and potential colorectal cancer progression [1, 2]. C-reactive protein (CRP) is a stable, non-specific inflammatory protein produced primarily by hepatocytes in an acute phase reactive setting in response to increased circulating interleukin-6 (IL-6), interleukin-1-beta (IL-1 β), and tumor necrosis-alpha levels (TNF- α) [3]. As such, CRP may serve as a biomarker for systemic inflammation, and has potential value in monitoring chronic disease status [4]. Recently, a role for locally-produced CRP within the colorectal mucosa has been proposed in the development of colorectal cancer. For example, somatic mutations in the CRP gene promoter may be involved in colorectal tumor progression [5, 6]. Previous case-control studies of CRP and risk of incidental colorectal cancer have generally indicated a positive association strongest in males and in lesions located within the colon but not the rectum [7–14]. Given the association between chronic inflammation and colorectal cancer, the utility of CRP in risk stratification of colorectal adenomas has been hypothesized, particularly in advanced adenomas with higher likelihood for evolution to malignancy [15]. Some previous studies have suggested a positive association with advanced adenomas, adenomas with predominant villous character, adenomas stratified based on larger size (>0.5 mm), and multiple adenomas on endoscopic examination [15–17]. Although this may provide some evidence for the use of CRP as a biomarker for clinically significant adenomas, other studies have detected either a null or even inverse relationship

between CRP and colorectal adenoma risk [18–24]. However, many of these previous studies were limited in their sample sizes, and often did not evaluate whether risk differed by small tubular or advanced lesions. Single small tubular lesions are the most prevalent lesion type in most populations and these lesions are considered to have a very low probability of progression to cancer [25]. Thus, a lack of association with CRP in previous studies may be due to attenuation from a mixture of small tubular and advanced lesions in analyses if the association varies by adenoma subtype. Further studies are needed in order to address these potential limitations.

Strong evidence relating inflammation to colorectal neoplasia involves the prostaglandin pathway and the cyclooxygenase-2 (COX-2) enzyme, where COX-2 activation leads to prostaglandin-E2 production and an eventual pro-inflammatory state. Inhibition of COX-2 by nonsteroidal anti-inflammatory drugs (NSAIDs) reduces systemic inflammation, and previous studies have associated the regular use of NSAIDs with a reduced risk of colorectal adenomas [26–29]. We and others have found a stable metabolite of prostaglandin E2 isolated from urine, prostaglandin E2 metabolite (PGE-M), is associated with risks of advanced or multiple colorectal adenomas and colorectal cancer [30–34]. However, no study has jointly evaluated plasma CRP levels and urinary PGE-M levels with colorectal neoplasia risk. In this study, we evaluated the relationships between CRP levels and adenoma risk among individuals with single small tubular (non-advanced) adenomas, multiple small tubular (non-advanced) adenomas, and advanced adenomas in a case-control, colonoscopy-based study using a matched comparison to no-polyp controls. Additionally, we have assessed whether the associations of plasma CRP with colorectal adenoma risk are independent of or modified by urinary PGE-M levels.

Materials and methods

Tennessee Colorectal Polyp Study

The Tennessee Colorectal Polyp Study, or TCPS, is a colonoscopy-based, case-control study designed and implemented at two academic institutions (Vanderbilt University Medical Center and the Veterans' Affairs (VA) Tennessee Valley Health Care Nashville Campus) between February 1, 2003 and October 29, 2010. Approval for these studies was granted by the Vanderbilt University Investigational Review Board, the Veterans' Affairs Institutional Review Board, and the Veterans' Affairs Research and Development Committee. Eligible participants between the ages of 40 and 75 were identified from colonoscopy schedules at the above institutions. Further details of this study have been published previously [30, 35]. All study participants within this analysis provided written informed consent prior to their scheduled colonoscopy. Potential participants were excluded if they had a family history of hereditary colorectal cancer syndromes or any previous history of colorectal adenomas, inflammatory bowel disease, or any cancer (excluding non-melanoma skin cancers). In total, there were 12,585 eligible participants; 7,621 (61%) signed a written consent to participate and participated in at least one component of the study.

In the days following the colonoscopy, participants were asked to complete a standard telephone interview and a mailed, 108-item food frequency questionnaire involving their dietary choices [36]. The telephone interview was conducted by trained interviewers with

standard questions regarding participant medication use, lifestyle choices, demographics, anthropometry, medical history, family history, and reproductive history.

All colonoscopies were completed by a trained gastroenterologist. Cases and controls were assigned based on the histological analysis of the pathology samples obtained from the colonoscopy. Any participants found to have colorectal adenocarcinoma on biopsy were eliminated from the analysis. Participants assigned to the no-polyp control group underwent a complete colonoscopy to the cecum without evidence of hyperplastic polyps, adenomas, or other rare polyp types. Those assigned to the single small tubular adenoma group expressed only an individual tubular adenoma less than 1 cm in diameter without pathologic evidence of high-grade dysplasia. Individuals assigned to the multiple small tubular adenoma group had two or more individual tubular adenomas that were less than 1 cm and without high-grade dysplasia. Individuals in the advanced adenoma group expressed at least one adenoma which had at least one of the following components: (a) equal to or greater than 1 cm in size; (b) expressed at least 25% villous character; and/or (c) contained high-grade dysplasia. Individuals within the advanced adenoma group may have expressed additional small tubular adenomas on biopsy. Additionally, participants in all three case groups may have expressed concurrent hyperplastic polyps at the time of colonoscopy and participants with advanced adenoma may have serrated adenomas (n=34).

Identification of Cases and Controls for Matched Analysis

Individual matching for cases and controls was conducted in a similar fashion to previously published studies [30]. Briefly, we randomly selected a single control participant to match the index case from one or more case groups (single small tubular adenoma, multiple small tubular adenoma, or advanced adenoma). Controls and cases were matched individually based on age (± 5 years), sex (male or female), and race (Caucasian or non-Caucasian). In addition, participants were matched based on at least one of the following factors: study site (Vanderbilt University or VA), collection date of plasma (± 90 days or same season), and NSAID use for a minimum of 3 times per week for at least one year (grouped into never users, former users, or current users). Additionally, eligibility for this analysis required that participants refrain from using any aspirin or NSAID product for a minimum of 48 hours prior to their colonoscopy, as their acute use has been previously shown to lower CRP and PGE-M levels [37, 38]. This was instructed to individuals prior to colonoscopy at these institutions, but did end up eliminating 274 (6.2%) of all participants initially eligible for this matched analysis. Eligible participants were selected among those who completed their telephone interview, food frequency questionnaire, and provided plasma for CRP analysis while abstaining from NSAID use for 48 hours prior to colonoscopy. The sample size was further limited due to funding constraints; thus, this analysis included 395 control participants without evidence of polyps, 226 expressing only a single small tubular adenoma, 198 with multiple small tubular adenomas, and 283 with advanced adenomas.

Plasma Analysis of C-Reactive Protein and Prostaglandin-E2 Metabolite

In the TCPS, we asked participants to donate a 15 mL blood sample immediately prior to their colonoscopy which was drawn into separate serum, EDTA, and heparin tubes. The blood was separated into fractions including red blood cells, buffy coat (white blood cells),

and plasma shortly after collection. Each individual component was isolated and within 6 hours processed and stored in a -80°C freezer for future use. Beginning in 2004, we asked participants to donate a sample of urine, which was collected and frozen within 6 hours at -80°C for future use. In total, 5,824 individuals donated blood and 4,404 individuals donated urine for future analyses.

Plasma C-reactive protein levels were measured after taking 100 μL of isolated plasma from the collected sample. Briefly, all samples were processed using a C-reactive Protein High Sensitivity Wide Range Kit (Pointe Scientific, Canton, MI, USA) with according to the manufacturer's protocol. The diluted plasma was placed in a buffered solution and then incubated with rabbit anti-human antibodies against CRP which were conjugated to latex particles. After washing steps, the absorbance of the antibody-antigen complex at 570 nm was measured using an ACE Clinical Chemistry System (Alfa Wassermann, Inc., West Caldwell, NJ, USA). A single value for CRP was recorded which was accompanied by a standard curve and high and low controls on each plate with samples from the matched cases and controls measured on the same plate. The lower limit of detection for CRP was 0.1 mg/L; however, if a value was below the detection level, the CRP level was estimated by using the limit of detection divided by the square root of 2, as described previously [39]. The intra-individual coefficient of variation was 18.0%.

Detection of PGE-M (11- α -hydroxy-9,15-dioxo-2,3,4,5-tetranor-prostane-1,20-dioic acid) was completed using 0.75 ml of urine thawed from stored samples, as described previously (30, 40). The urine was titrated to a pH of 3 and treated with methyloxime HCL to convert to an *O*-methyloxime derivative. Purification of the sample took place using a C18 Sep Pak with an added internal deuterated PGE-M control standard. The purified samples underwent liquid chromatography using a Zorbax Eclipse XDB-C18 column which was attached to a ThermoFinnigan Mass Spectrometry Pump (Thermo Fisher Scientific, Waltham, MA, USA). In order to quantitate the level of PGE-M, measurements were taken of the m/z peak areas of the predominant peak ion products at a m/z ratio of 336 (representing the PGE-M product) and 339 (representing the deuterated internal standard) using a selected reaction monitoring mode on the MS. The ratio of these values was determined to be the level of urinary PGE-M in the cases and controls. The lower limit of detection for PGE-M was 40 pg. The coefficient of variation was calculated as 6.1% for between batches and 7.8% for within batches. Urinary creatinine was measured using a Sigma kit (Sigma Co., Inc., St. Louis, MO, USA) and levels of urinary PGE-M were reported as ng PGE-M/mg creatinine.

Statistical Analysis

To describe demographic and lifestyle factors and potential confounders, frequencies and means with standard deviations were calculated for individual case groups and their matched controls. Tests for statistical significance between groups were conducted using Wilcoxon Signed Rank Test for continuous variables, McNemars Test for categorical variables, or conditional logistic regression models adjusted for age, sex, and/or study site, as appropriate. Given that CRP is right-skewed, the geometric mean was calculated for cases and controls.

The differences in the geometric means were evaluated in linear regression models. CRP levels were categorized into tertiles based on the distribution among controls. Odds ratios

and 95% confidence intervals (95% CI) were calculated based on conditional logistic regression modeling. Tests for linear trend were conducted by including the categorical variables as continuous parameters in the models. Multiple small tubular and advanced adenoma cases were combined in some statistical analyses. Given that CRP is associated with inflammation and a variety of chronic conditions, we evaluated several potential confounders which may influence the relationship between plasma CRP levels and adenoma risk. Potential confounders included age, race (Caucasian or non-Caucasian), study site (Vanderbilt or Veterans' Affairs), educational status (high school or less, some college, college graduate, or professional/graduate school), household income (less than \$15,000, \$15,000–\$30,000, \$30,000–\$50,000, or \$50,000 or more), reason for colonoscopy (asymptomatic screening, family history of colorectal cancer, diagnostic, or other), regular exercise (yes/no), cigarette use (never, former, and current), regular alcohol use (never, former, and current), NSAID use (never, former, and current), daily red meat intake (grams/day), total energy intake consumed daily (kcal/day), statin use (never, former, or current), urinary PGE-M levels, post-menopausal status in females (yes/no), and hormone replacement therapy use in females (never/ever use). Inflammation may be an intermediate marker between body mass index (BMI, kilograms/meters²) and colorectal neoplasia risk; thus, BMI was only assessed as a potential effect modifier. A factor was considered a confounder if it was statistically significant in its association with case status and with CRP level within controls. Age, educational attainment, and study site were adjusted in all models as potential confounders.

Effect modification was evaluated using either stratified (for sex) or joint analysis (for BMI, red meat intake, cigarette smoking, NSAID use, and PGE-M). Continuous potential effect modifiers were categorized into low (below median) and high (above median) based on the distribution among controls. Likelihood ratio tests for multiplicative interaction were used to compare the models with and without interaction terms. Categorical variables were used in all models to test interaction. For all analyses, p values of ≤ 0.05 (two-sided) were considered statistically significant. SAS software version 9.4 (SAS Institute, Inc., Cary, NC, USA) was used to carry out all statistical analysis.

Results

Matching factors and other demographic and lifestyle characteristics for each adenoma case group and their matched controls are displayed in Table 1. Cases with either multiple small tubular adenomas or advanced adenomas tended to be older and more likely to be active or former smokers, have lower educational attainment, and consume more red meat than controls. Cases with multiple small tubular adenomas contained a significantly higher percentage of males and consumed more total calories, exercised less, and had a higher BMI than all other groups. Participants who were found to have advanced adenomas were also less likely to regularly use NSAID products compared to all other groups. Controls were more likely to have a family history of colorectal cancer as their indication for the procedure, while advanced adenoma cases were more likely to have received colonoscopy for diagnostic reasons. Urinary PGE-M levels were significantly elevated when compared to matched controls only in the multiple small tubular and advanced adenoma groups.

CRP levels were statistically significantly elevated in participant groups with multiple small tubular adenomas and advanced adenomas compared to no-polyp controls (Table 2). The difference was most striking with multiple small tubular adenomas, which showed a 29.4% difference in the geometric mean compared to no-polyp controls.

Higher levels of CRP were associated with a statistically significant increased adenoma risk in a dose-response manner for multiple small tubular and advanced adenoma case groups (OR 2.01 (95% CI = 1.10–3.68) for multiple small tubular adenomas, OR 1.81 (95% CI = 1.10–2.96) for advanced adenomas for highest vs. lowest tertile) (Table 3). Further analysis of advanced adenoma cases did not detect any differences between cases with multiple advanced adenomas and cases with a single advanced adenoma (data not shown).

In order to assess whether sex was a potential effect modifier in this study, we conducted stratified analysis by sex. Sex-specific findings were very similar to the overall analysis (data not shown). Additional potential effect modifiers based on dietary or lifestyle factors were evaluated using joint analysis. No significant modifying effect of cigarette smoking was observed (Table 3). Comparison of ‘never’ to ‘ever’ smokers in a joint analysis yielded similar results to a ‘current’ to ‘former/never’ comparison (data not shown). No significant modifying effect was found related to NSAID use (Table 3), BMI, and red meat intake (data not shown).

No elevated risk of single small tubular adenomas was found with increased levels of either plasma CRP or urinary PGE-M (Table 4). However, analysis of both multiple small tubular adenomas and advanced adenomas suggested a role of CRP in risk based on PGE-M levels. There was no apparent association between CRP and either multiple small tubular adenoma or advanced adenoma risk among those with PGE-M levels below the median. In contrast, the risk for these two adenoma case groups was elevated with increasing CRP levels among participants with PGE-M levels above the median, although the tests for interaction were not statistically significant. There was a statistically significant association between advanced adenoma risk and elevated CRP levels (2nd and 3rd tertile) in participants with higher PGE-M levels compared to the lowest CRP tertile and low PGE-M. A similar pattern was observed in the risk of multiple small tubular adenomas, although no OR was statistically significant presumably due to a limited sample size. In sensitivity analysis, similar patterns were also observed when limited to non-users of NSAIDs (data not shown).

Discussion

In our study, we found that high circulating plasma CRP was associated with an elevated risk of both multiple small tubular adenomas and advanced adenomas in a dose-response manner. Further, it appears that the increased risk of advanced adenoma associated with CRP levels is likely dependent upon urinary PGE-M levels, where high CRP may only be associated with increased risk if PGE-M level is also elevated. This relationship likely holds true for risk associated with multiple small tubular adenomas, although statistical power to adequately assess this relationship was not likely sufficient in this study. Our study is among the first to show a strong association between both multiple and advanced adenoma risk and increases in CRP levels, with a novel finding that these increases in CRP are largely

dependent upon activation of the prostaglandin pathway, as shown with elevations in urinary PGE-M. This implies the importance of prostaglandin-specific inflammation as a potential mechanism for the risk of clinically important precursor colorectal lesions.

C-reactive protein is a known pro-inflammatory protein produced and released into the circulation primarily by hepatocytes in response to elevations in IL-6, IL-1 β , and TNF- α [3]. Highly elevated levels of CRP are typically associated with acute inflammatory conditions, such as infections, trauma, or acute flares of autoimmune diseases. It has been shown that CRP levels are elevated among individuals with chronic conditions of inflammation, as seen in colorectal cancer [12–15]. Known cytokines associated with inflammation are dysregulated within adenoma tissues and considered to initiate an inflammatory state [2]. Additionally, research in mouse models have shown that colonic epithelial dysplasia provokes a cytokine-driven inflammatory response which upregulates circulatory inflammatory markers such as TNF- α and IL-6, which are known to alter CRP production [41, 42]. A recent report also hypothesized that local production of CRP in the colorectal cancers may be altered as somatic mutations in the CRP gene were observed in tumor but not normal tissues [5, 6]. Therefore, it is conceivable that CRP levels may also be elevated among patients with adenomas, particularly among those with multiple or advanced adenomas. Previous studies have not provided consistent results regarding the association between CRP levels and colorectal adenoma risk in a screening age population [16–24]. However, many of these studies combined all adenomas into a single category, which likely included large proportions of single small tubular adenomas which may not provoke an inflammatory response sufficient to raise CRP levels [16, 20, 21]. In our study, we did not find any evidence for an apparent association between circulating CRP level and risk of single small tubular adenomas. On the other hand, we showed the risk of multiple small tubular adenomas or advanced adenomas was elevated with an increasing circulating CRP level in a dose-response manner. Our findings were supported by several previous studies that reported a potential association of circulating CRP levels with adenomas containing advanced features including villous components, high-grade dysplasia, or adenomas which are larger in size, although at times sample sizes for these previous studies were not large enough to reach statistical significance [15–17].

We found that the association of circulating CRP levels with risk of multiple or advanced adenomas are likely to be modified by urinary PGE-M levels. To our knowledge, there are no previous studies jointly evaluating urinary PGE-M levels with plasma CRP levels in association to adenoma risk. PGE-M is a stable metabolite produced from the breakdown product of prostaglandin E2 (PGE-2), which itself is produced often in settings of inflammation and in parallel with COX-2 enzyme activation [43]. Both PGE-2 overproduction and COX-2 activation have been well detailed as a carcinogenic mechanism within the colonic epithelium [44]. We witnessed no clear association between circulating CRP levels and adenoma risk was found among participants with a low urinary PGE-M. Interestingly, among participants with high PGE-M, a potential association exists between circulating CRP levels and risk of multiple or advanced adenomas, although not all point estimates of risk were statistically significant; this is perhaps due to a small sample size. This implicates the role of a COX-2 dependent mechanism for the inflammatory changes seen in the setting of multiple small tubular and advanced adenomas and reflected in

elevation in their CRP levels. This possible mechanism is further suggested by our observation that CRP is associated with advanced adenoma risk among individuals not currently using NSAIDs but demonstrates no association among those currently using NSAIDs. The specific roles that localized CRP produced in the colorectal mucosa in relation to the COX-2 pathway in both advanced and multiple small tubular adenoma development will require further investigation. The pattern for multiple small tubular adenomas is less clear but may suggest a combination of factors leads to the risk of multiple small lesions including inflammation related to the COX-2 pathway and will also need to be further investigated.

There are multiple strengths to this study. Although we cannot exclude the possibility of residual confounding, we used a careful individual matching strategy in an attempt to limit confounding. We also conducted extensive evaluation of other potential confounders which we determined in our analysis to be contributing to confounding or has been detailed in past studies to affect CRP [45]. All control participants underwent a full colonoscopy to the cecum which reduced misclassification of the outcome. We were able to separately evaluate risks according to clinically significant (either multiple small tubular or advanced) and insignificant (single small tubular) lesions. We were also the first to compare both plasma CRP levels and urinary PGE-M levels from the same participants within a study assessing colorectal adenoma risk. It should be noted, however, that two of the previous studies involving colorectal adenoma or adenocarcinoma risk and urinary PGE-M levels have involved participants within our Tennessee Colorectal Polyp Study so additional studies in other populations are needed to confirm this finding [30, 32]. The analysis comparing PGE-M and CRP may also be limited by potential confounding by acute NSAID use, which we attempted to control through matching and with removal of participants who took NSAIDs 48 hours prior to sample collection. Furthermore, it is possible that multiple small tubular or advanced adenomas themselves lead to activation of an inflammatory response and thus increase circulating CRP levels. If this is correct, the association between circulating CRP and adenoma risk would not be causal. Nevertheless, the positive association observed in our study suggested that circulating CRP could serve as a potential biomarker to stratify patients into high and low risk groups for colorectal cancer screening.

In summary, this study assesses the potential viability of C-reactive protein as a biomarker for risk of colorectal adenomas in a screening population and its possible role in tumorigenesis. It appears that elevated CRP may be associated with increased risk of multiple small tubular adenomas or advanced adenomas, particularly in the setting of elevated urinary PGE-M and COX-2- mediated inflammation. As advanced or multiple pre-cancerous lesions are most at risk for progressing to adenocarcinoma, non-invasive testing for CRP elevation may be able to risk stratify individuals for a more urgent colonoscopy screening and detection of at-risk lesions. Further studies, including prospective evaluations and evaluation of the serrated pathway to colorectal carcinogenesis, are warranted to confirm and expand on our findings.

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Abbreviations

BMI	Body Mass Index
CRP	C-reactive Protein
CI	Confidence intervals
COX-2	Cyclooxygenase-2
IL-1β	Interleukin-1 Beta
IL-6	Interleukin-6
NSAID	Nonsteroidal Anti-inflammatory Drug
PGE-M	Prostaglandin E2-Metabolite
TCPS	Tennessee Colorectal Polyp Study
TNF-α	Tumor Necrosis Factor-alpha
VA	Veteran's Affairs

References

1. Terzi J, Grivennikov S, Karin E, Karin M. Inflammation and colon cancer. *Gastroenterology*. 2010; 138(6):2101–2114. [PubMed: 20420949]
2. McLean MH, Murray GI, Stewart KN, et al. The inflammatory microenvironment in colorectal neoplasia. *PLOS One*. 2011; 6(1):e15366. [PubMed: 21249124]
3. Pepys MB, Hirshfield GM. C-reactive protein: a critical update. *J Clin Invest*. 2003; 111(12):1805–1812. [PubMed: 12813013]
4. Ansar W, Ghosh S. C-reactive protein and the biology of disease. *Immunol Res*. 2013; 56:131–142. [PubMed: 23371836]
5. Wang MY, Zhou HH, Zhang SC, et al. Recurrent mutations at C-reactive protein gene promoter SNP position -286 in human cancers. *Cell Res*. 2014; 24:505–508. [PubMed: 24418758]
6. Su HX, Zhou HH, Wang MY, et al. Mutations of C-reactive protein (CRP)-286 SNP, APC and p53 in colorectal cancer: Implication for CRP-Wnt crosstalk. *PLOS One*. 2014; 9(7):e102418. [PubMed: 25025473]
7. Erlinger TP, Platz EA, Rifai N, Helzlsouer KJ. C-reactive protein and the risk of incident colorectal cancer. *J Amer Med Assoc*. 2004; 291(5):585–590.
8. Zhang SM, Buring JM, Lee IM, Cook NR, Ridker PM. C-reactive protein levels are not associated with increased risk for colorectal cancer in women. *Ann Intern Med*. 2005; 142(6):425–432. [PubMed: 15767620]

9. Il'yasova D, Colbert LH, Harris TB, et al. Circulating levels of inflammatory markers and cancer risk in the healthy aging and body composition cohort. *Cancer Epidem Biomar*. 2005; 14(10):2413–2418.
10. Gunter MJ, Stolzenberg-Solomon R, Cross AJ, et al. A prospective study of serum C-reactive protein and colorectal cancer risk in men. *Cancer Res*. 2006; 66(4):2483–2487. [PubMed: 16489056]
11. Aleksandrova K, Jenab M, Boeing H, et al. Circulating C-reactive protein concentrations and risks of colon and rectal cancer: A nested case-control study within the European prospective investigation into cancer and nutrition. *Am J Epidemiol*. 2010; 172(4):407–418. [PubMed: 20634278]
12. Chan AT, Ogino S, Giovannucci EL, Fuchs CS. Inflammatory markers are associated with risk of colorectal cancer and chemopreventative response to anti-inflammatory drugs. *Gastroenterology*. 2011; 140(3):799–808. [PubMed: 21115010]
13. Toriola AT, Cheng TY, Neuhauser ML, et al. Biomarkers of inflammation are associated with colorectal cancer risk in women but are not suitable as early detection markers. *Int J Cancer*. 2013; 132(11):2648–2658. [PubMed: 23161620]
14. Wu J, Cai Q, Li H, et al. Circulating C-reactive protein and colorectal cancer risk: a report from the Shanghai men's health study. *Carcinogenesis*. 2013; 34(12):2799–2803. [PubMed: 23985781]
15. Chiu HM, Lin JT, Chen TH, et al. Elevation of C-reactive protein level is associated with synchronous and advanced colorectal neoplasm in men. *Am J Gastroenterol*. 2008; 103(9):2317–2325. [PubMed: 18844617]
16. Tsilidis KK, Erlinger TP, Rifai N, et al. C-reactive protein and colorectal adenoma in the CLUE-II cohort. *Cancer Cause Control*. 2008; 19:559–567.
17. Otake T, Uezono K, Takahashi R, et al. C-reactive protein and colorectal adenomas: Self defense forces health study. *Cancer Sci*. 2009; 100(4):709–714. [PubMed: 19469014]
18. Kim S, Keku TO, Martin C, et al. Circulating levels of inflammatory cytokines and risk of colorectal adenomas. *Cancer Res*. 2008; 68(1):323–328. [PubMed: 18172326]
19. Ognjanovic S, Yamamoto J, Saltzman B, et al. Serum CRP and IL-6, genetic variants and risk of colorectal adenoma in a multi-ethnic population. *Cancer Cause Control*. 2010; 21:1131–1138.
20. Chan AT, Sima CS, Zuber AG, Ridker PM, Hawk ET, Bertagnolli MM. C-reactive protein and risk of colorectal adenoma according to Celecoxib treatment. *Cancer Prev Res*. 2011; 4(8):1172–1180.
21. Gunter MJ, Cross AJ, Huang WY, et al. A prospective evaluation of C-reactive protein levels and colorectal adenoma development. *Cancer Epidem Biomar*. 2011; 20(3):537–544.
22. Kong SY, Bostick RM, Flanders WD, et al. Oxidative balance score, colorectal adenoma, and markers of oxidative stress and inflammation. *Cancer Epidem Biomar*. 2012; 23(3):545–554.
23. Tao S, Haug U, Kuhn K, Brenner H. Comparison and combination of blood-based inflammatory markers with faecal occult blood tests for non-invasive colorectal cancer screening. *Brit J Cancer*. 2012; 106(8):1424–1430. [PubMed: 22454079]
24. Crockett SD, Mott LA, Barry EL, et al. C-reactive protein and risk of colorectal adenomas or serrated polyps: A prospective study. *Cancer Prev Res*. 2014; 7(11):1122–1127.
25. O'Brien MJ, Winawer SJ, Zuber AG, et al. The national polyp study: patient and polyp characteristics associated with high-grade dysplasia in colorectal adenomas. *Gastroenterology*. 1990; 98(2):371–379. [PubMed: 2403953]
26. Cole BF, Logan RF, Halabi S, et al. Aspirin for the chemoprevention of colorectal adenomas: meta-analysis of the randomized trials. *J Natl Cancer I*. 2009; 101:256–266.
27. Bertagnolli MM, Eagle CJ, Zuber AG, et al. Celecoxib for the prevention of sporadic colorectal adenomas. *New Engl J Med*. 2006; 355(9):873–884. [PubMed: 16943400]
28. Arber N, Eagle CJ, Spicak J, et al. Celecoxib for the prevention of colorectal adenomatous polyps. *New Engl J Med*. 2006; 355(9):885–895. [PubMed: 16943401]
29. Baron JA, Sandler RS, Bresalier RS, et al. A randomized trial of Rofecoxib for the chemoprevention of colorectal adenomas. *Gastroenterology*. 2006; 131(6):1674–1682. [PubMed: 17087947]

30. Shrubsole MJ, Cai Q, Wen W, et al. Urinary prostaglandin E2 metabolite and risk for colorectal adenoma. *Cancer Prev Res.* 2012; 5(2):336–342.
31. Cai Q, Gao YT, Chow WH, et al. Prospective study of urinary prostaglandin E2 metabolite and colorectal cancer risk. *J Clin Oncology.* 2006; 24(31):5010–5016.
32. Johnson JC, Schmidt CR, Shrubsole MJ, et al. Urine PGE-M: A metabolite of prostaglandin E2 as a potential biomarker of advanced colorectal neoplasia. *Clin Gastro Hepatol.* 2006; 4(11):1358–1365.
33. Bezawada N, Song M, Wu K, et al. Urinary PGE-M levels are associated with risk of colorectal adenomas and chemopreventative response to anti-inflammatory drugs. *Cancer Prev Res.* 2014; 7(7):758–765.
34. Maresso KC, Vilar E, Hawk ET. Urinary PGE-M in colorectal cancer: Predicting more than risk? *Cancer Prev Res.* 2014; 7(10):969–972.
35. Shrubsole MJ, Wu H, Ness RM, Shyr Y, Smalley WE, Zheng W. Alcohol drinking, cigarette smoking, and risk of colorectal adenomatous and hyperplastic polyps. *Am J Epidemiol.* 2008; 167(9):1050–1058. [PubMed: 18304959]
36. Buchowski MS, Schlundt DG, Hargreaves MK, Hankin JH, Signorello LB, Blot WJ. Development of a culturally sensitive food frequency questionnaire for use in the Southern Community Cohort Study. *Cell Mol Bio.* 2003; 49:1295–1304. [PubMed: 14984001]
37. Ikonomidis I, Andreotti F, Economou E, Stefanadis C, Toutouzas P, Nihoyannopoulos P. Increased proinflammatory cytokines in patients with chronic stable angina and their reduction by aspirin. *Circulation.* 1999; 100:793–798. [PubMed: 10458713]
38. Kim SB, Kim SH, Chang JW, et al. Effects of celecoxib on high-sensitivity C-reactive protein levels in chronic peritoneal dialysis patients. *Renal Failure.* 2004; 26:381–384. [PubMed: 15462105]
39. Hornung RW, Reed LD. Estimation of average concentration in the presence of non-detectable values. *Appl Occup Environ Hygiene.* 1990; 5(1):46–51.
40. Murphey LC, Williams MK, Sanchez SC, et al. Quantification of the major urinary metabolite of PGE-2 by a liquid chromatographic/mass spectrometric assay: determination of cyclooxygenase specific PGE2 synthesis in healthy humans and those with lung cancer. *Anal Biochem.* 2004; 334:266–275. [PubMed: 15494133]
41. Grivennikov S, Karin E, Terzic J, et al. IL-6 and STAT-3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. *Cancer Cell.* 2009; 15(2):103–113. [PubMed: 19185845]
42. Popivanova BK, Kitamura K, Wu Y, et al. Blocking TNF-alpha in mice reduces colorectal carcinogenesis associated with chronic colitis. *J Clin Invest.* 2008; 118(2):560–570. [PubMed: 18219394]
43. Greenhough A, Smartt HJ, Moore AE, et al. The COX2/PGE2 pathway: key roles in the hallmark of cancer and adaptation to the tumour microenvironment. *Carcinogenesis.* 2009; 30(3):377–386. [PubMed: 19136477]
44. Backlund MG, Mann JR, Dubois RN. Mechanisms for the prevention of gastrointestinal cancer: The role of prostaglandin E2. *Oncology.* 2005; 69(Suppl 1):28–32. [PubMed: 16210874]
45. Sung KC. Seasonal variation of C-reactive protein in apparently healthy Koreans. *Int J Cardiol.* 2006; 107(3):338–342. [PubMed: 16503255]

Table 1

Demographic and lifestyle factors of study participants: the Tennessee Colorectal Polyp Study.

Characteristics	Single Small Tubular Adenoma (226 Pairs)			Multiple Small Tubular Adenoma (198 Pairs)			Advanced Adenoma (283 Pairs)		
	Matched Controls	Cases	P value	Matched Controls	Cases	P value	Matched Controls	Cases	P value
Matching Factors									
Age (mean $\pm$ SD) *	56.6 $\pm$ 7.2	57.5 $\pm$ 7.1	<0.001	58.9 $\pm$ 6.9	60.0 $\pm$ 6.5	<0.001	57.4 $\pm$ 7.4	58.9 $\pm$ 7.3	<0.001
Sex †									
Male	164 (72.6)	164 (72.6)		157 (79.3)	157 (79.3)		203 (71.7)	203 (71.7)	
Female	62 (27.4)	62 (27.4)		41 (20.7)	41 (20.7)		80 (28.3)	80 (28.3)	
Race †									
Non-Caucasian	12 (5.3)	12 (5.3)		18 (9.1)	18 (9.1)		38 (13.4)	38 (13.4)	
Caucasian	214 (94.7)	214 (94.7)		180 (90.9)	180 (90.9)		245 (86.6)	245 (86.6)	
Study Site (%) †			0.28			0.41			0.13
Vanderbilt University	154 (68.1)	159 (70.4)		121 (61.1)	115 (58.1)		188 (66.4)	178 (62.9)	
VA Nashville	72 (31.9)	67 (29.6)		77 (38.9)	83 (41.9)		95 (33.6)	105 (37.1)	
NSAID Medication Use (%) †,‡			0.47			0.54			<0.001
Never	69 (31.7)	83 (37.1)		65 (34.2)	65 (37.4)		87 (32.2)	124 (49.8)	
Former	23 (10.6)	19 (8.5)		15 (7.9)	14 (8.0)		23 (8.5)	16 (6.4)	
Current	126 (57.8)	122 (54.5)		110 (57.9)	95 (54.6)		160 (59.3)	109 (43.8)	
Non-Matching Factors									
Reason for Colonoscopy (%) †,‡			0.90			0.52			0.003
Asymptomatic Screening	135 (59.7)	137 (60.6)		123 (62.1)	112 (56.6)		166 (58.7)	164 (58.0)	
Family History of CRC	27 (11.9)	31 (13.7)		21 (10.6)	22 (11.1)		35 (12.4)	20 (7.1)	
Diagnostic	45 (19.9)	41 (18.1)		32 (16.2)	43 (21.7)		55 (19.4)	84 (29.7)	
Other	19 (8.4)	17 (7.5)		22 (11.1)	21 (10.6)		27 (9.5)	15 (5.3)	
Educational Attainment (%) †,‡			0.52			0.02			0.01
High School or Less	46 (21.2)	61 (27.4)		43 (22.8)	67 (38.7)		59 (22.1)	86 (34.3)	
Partial College	59 (27.2)	59 (26.5)		52 (27.5)	44 (25.4)		79 (29.6)	70 (27.9)	
College Graduate	49 (22.6)	48 (21.5)		45 (23.8)	30 (17.3)		54 (20.2)	48 (19.1)	
Grad./Professional School	63 (29.0)	55 (24.7)		49 (25.9)	32 (18.5)		75 (28.1)	47 (18.7)	

Characteristics	Single Small Tubular Adenoma (226 Pairs)			Multiple Small Tubular Adenoma (198 Pairs)			Advanced Adenoma (283 Pairs)		
	Matched Controls	Cases	P value	Matched Controls	Cases	P value	Matched Controls	Cases	P value
BMI (mean $\pm$ SD) ^{*,‡}	27.6 $\pm$ 5.3	28.8 $\pm$ 5.8	0.02	28.0 $\pm$ 5.3	29.5 $\pm$ 5.9	0.03	27.9 $\pm$ 5.6	28.3 $\pm$ 5.2	0.42
Regular Exercise (%) ^{†,‡}			0.71			0.01			0.57
No	96 (43.8)	101 (45.1)		84 (44.0)	102 (58.6)		125 (46.1)	121 (47.6)	
Yes	123 (56.2)	123 (54.9)		107 (56.0)	72 (41.4)		146 (53.9)	133 (52.4)	
Total Energy Intake (kcal/day) ^{*,‡}	2216.5 $\pm$ 972.8	2243.6 $\pm$ 1017.8	0.98	2282.9 $\pm$ 1057.0	2485.4 $\pm$ 1203.9	0.06	2238.2 $\pm$ 1018.4	2253.0 $\pm$ 1181.7	0.97
Red Meat Intake (g/day; mean $\pm$ SD) ^{*,‡}	66.1 $\pm$ 71.4	67.2 $\pm$ 66.9	0.92	65.8 $\pm$ 69.5	79.3 $\pm$ 65.1	0.04	64.2 $\pm$ 72.0	71.6 $\pm$ 62.0	0.04
Cigarette Use (%) ^{†,‡}			0.43			0.001			<0.001
Never	100 (45.7)	100 (44.6)		79 (41.4)	50 (28.7)		130 (48.0)	81 (31.9)	
Former	78 (35.6)	91 (40.6)		81 (42.4)	67 (38.5)		95 (35.1)	101 (39.8)	
Current	41 (18.7)	33 (14.7)		31 (16.2)	57 (32.8)		46 (17.0)	72 (28.3)	
Alcohol Use (%) ^{†,‡}			0.27			0.06			0.01
Never	122 (55.7)	116 (51.8)		108 (56.5)	82 (47.1)		152 (56.1)	115 (45.5)	
Former	50 (22.8)	46 (20.5)		44 (23.0)	58 (33.3)		61 (22.5)	78 (30.8)	
Current	47 (21.5)	62 (27.7)		39 (20.4)	34 (19.5)		58 (21.4)	60 (23.7)	
Statin Medication Use (%) ^{†,‡}			0.26			0.17			0.66
Never	134 (65.0)	129 (60.6)		103 (63.6)	71 (50.7)		145 (65.3)	124 (61.1)	
Former	4 (1.9)	5 (2.3)		2 (1.2)	2 (1.4)		4 (1.8)	3 (1.5)	
Current	68 (33.0)	79 (37.1)		57 (35.2)	67 (47.9)		73 (32.9)	76 (37.4)	
HRT Use (%) ^{†,‡,§}			0.18			1.00			0.30
Never	28 (49.1)	23 (37.7)		17 (42.5)	15 (42.9)		34 (45.3)	29 (39.2)	
Ever	29 (50.9)	38 (62.3)		23 (57.5)	20 (57.1)		41 (54.7)	45 (60.8)	
Post-Menopausal (%) ^{†,‡,§}			0.25			0.08			0.10
Premenopausal	19 (33.3)	16 (26.2)		11 (27.5)	4 (11.4)		25 (33.3)	17 (23.0)	
Postmenopausal	38 (66.7)	45 (73.8)		29 (72.5)	31 (88.6)		50 (66.7)	57 (77.0)	
Urine PGE-M Level ^{*,‡} (ng/mg Creatinine; mean $\pm$ SD)	2.4 $\pm$ 0.8	2.4 $\pm$ 0.8	0.87	2.4 $\pm$ 0.8	2.5 $\pm$ 0.7	0.09	2.3 $\pm$ 0.8	2.6 $\pm$ 0.7	0.04

^{*} P values were derived from Wilcoxon Signed Rank test.

[†] P values were derived from McNemar's test.

†P values were derived from conditional logistic models adjusted for age, sex, and location of colonoscopy (VA or VUMC).

§Only female participants from the analysis were included in this category.

Table 2

CRP levels by case and control status, the Tennessee Colorectal Polyp Study.

Study Group	n	Geometric Mean (Q1–Q3)	Difference (%) [*]	P [*]
No-polyp Controls	395	2.77(1.31–5.65)	ref	
Single Small Tubular Adenoma	226	3.00(1.59–5.97)	11.8	0.21
Multiple Small Tubular Adenoma	198	3.43(1.82–6.96)	26.71	0.011
Advanced Adenoma	283	3.32(1.69–7.16)	20.78	0.023

^{*}The geometric mean difference and associated p value were calculated using a linear regression model based on log transformed values of CRP between specific study groups. The differences were calculated by dividing the estimated geometric mean from the study groups from the geometric mean of no-polyp controls.

Table 3

Overall and subgroup analyses of colorectal adenoma risk associated with CRP level, The Tennessee Colorectal Polyp Study.

CRP Subgroup	Controls	Adenoma Case Group					
		Multiple Small Tubular			Multiple Small Tubular or Advanced		
		n	OR (95% CI) *	n	OR (95% CI) *	n	OR (95% CI) *
Overall							
	CRP T1 (low)	132	63 1.00 (ref)	41	1.00 (ref)	69	1.00 (ref)
	CRP T2	132	87 1.06 (0.63–1.76)	74	1.85 (1.02–3.34)	99	1.38 (0.85–2.23)
	CRP T3	131	76 0.89 (0.53–1.48)	83	2.01 (1.10–3.68)	117	1.81 (1.10–2.96)
	P _{trend}		0.59		0.03		0.007
Cigarette Use							
Never/Former							
	CRP T1 (low)	111	55 1.00 (ref)	24	1.00 (ref)	49	1.00 (ref)
	CRP T2	106	78 1.19 (0.68–2.08)	48	2.06 (1.04–4.09)	70	1.41 (0.82–2.42)
	CRP T3	98	58 0.79 (0.46–1.38)	45	2.01 (0.99–4.09)	63	1.57 (0.88–2.78)
	Current						
	CRP T1 (low)	15	7 0.60 (0.20–1.80)	11	3.39 (0.93–12.35)	12	1.52 (0.54–4.22)
	CRP T2	20	8 0.44 (0.17–1.16)	19	4.37 (1.50–12.70)	19	1.70 (0.77–3.76)
	CRP T3	24	18 0.91 (0.38–2.19)	27	6.98 (2.32–21.02)	41	2.91 (1.41–6.01)
	P _{interaction} †		0.20		0.62		0.83
NSAID Use							
Never/Former							
	CRP T1 (low)	56	24 1.00 (ref)	17	1.00 (ref)	31	1.00 (ref)
	CRP T2	56	40 1.36 (0.61–3.03)	31	1.88 (0.74–4.80)	47	1.90 (0.82–4.41)
	CRP T3	51	38 1.29 (0.60–2.79)	31	1.81 (0.74–4.43)	62	2.27 (1.02–5.08)
	Current						
	CRP T1 (low)	69	38 1.19 (0.40–3.54)	18	0.55 (0.20–1.50)	30	0.42 (0.18–1.00)
	CRP T2	70	46 1.07 (0.38–3.04)	36	1.07 (0.44–2.62)	42	0.53 (0.24–1.19)
	CRP T3	70	38 0.82 (0.31–2.21)	41	1.31 (0.54–3.19)	38	0.60 (0.26–1.39)
	P _{interaction} †		0.47		0.39		0.66
							0.85

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* Adjusted for age, educational attainment, and study site.
† P values were derived from log likelihood ratio testing.

Table 4
Association of colorectal adenoma risk according to plasma CRP and urinary PGE-M levels, The Tennessee Colorectal Polyp Study

PGE-M* and CRP Levels	Controls		Adenoma Case Group									
			Single Small Tubular			Multiple Small Tubular						
			n	OR (95% CI) [†]	n	OR (95% CI) [†]	n	OR (95% CI) [†]	n	OR (95% CI) [†]		
Low PGE-M												
			34	30	1.00 (ref)	8	1.00 (ref)	13	1.00 (ref)	21	1.00 (ref)	
			43	43	0.78 (0.34–1.76)	13	1.48 (0.35–6.37)	21	1.96 (0.61–6.32)	34	1.67 (0.65–4.29)	
			41	35	0.46 (0.18–1.13)	17	1.39 (0.41–4.73)	21	1.29 (0.43–3.86)	38	1.23 (0.51–2.96)	
High PGE-M												
			38	29	0.66 (0.25–1.69)	14	1.10 (0.27–4.53)	21	1.35 (0.40–4.51)	35	1.22 (0.47–3.17)	
			38	52	0.99 (0.42–2.34)	24	1.98 (0.52–7.56)	39	4.16 (1.32–13.11)	63	3.04 (1.21–7.64)	
			40	54	0.99 (0.44–2.20)	29	3.00 (0.70–12.86)	51	5.31 (1.55–18.18)	80	3.72 (1.42–9.72)	
P _{interaction} [‡]					0.08			0.90		0.08		0.25

* Low PGE-M was defined as <9.99 ng/mg creatinine and high PGE-M was defined as ≥9.99 ng/mg creatinine.

[†] Adjusted for age, educational attainment, and study site.

[‡] P values were derived from log likelihood ratio testing.