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ORIGINAL PAPER

Serum CRP and IL-6, genetic variants and risk of colorectal adenoma in a multiethnic population

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Abstract Chronic inflammation, which is suspected to play a role in the development of colorectal cancer (CRC), has rarely been studied in colorectal adenoma. We investigated the inter-relationships of serum levels of the inflammatory proteins CRP and in IL-6, single nucleotide polymorphisms (SNPs) in the *CRP* (rs1205, rs1130864, rs1800947) and *IL6* (rs1800795) genes, and lifestyle factors with colorectal adenoma in a sigmoidoscopy-based case–control study of 271 adenoma cases and 539 age-, sex-, and race/ethnicity-matched controls in Hawaii. We found no association of serum CRP or IL-6 levels with the risk of adenoma. A multiple regression with stepwise selection identified elevated BMI, Caucasian and Native Hawaiian versus Japanese race/ethnicity, and current smoking as being associated with significantly higher serum CRP and IL-6 levels. Female versus male gender was also associated with higher CRP levels and older age with higher IL-6 levels. The C allele of rs1205 and the A allele of rs1130864 were significantly associated with higher serum CRP levels (p_{trend}: 0.0002 and 0.01, respectively), as well as with a decreased adenoma risk [rs1205: OR for CT and CC vs. TT = 0.69 (95% CI: 0.48–0.98) and 0.53 (0.34–0.83), respectively, $p_{trend} = 0.008$; rs1130864: OR for GA and AA versus GG = 0.65 (0.45–0.93) and 0.74 (0.31–1.76), respectively, $p_{trend} = 0.04$]. The findings of lower serum CRP and IL-6 levels in Japanese (a group with a high CRC risk) and of a decreased adenoma risk observed for alleles associated with higher circulating CRP levels suggest a protective effect for CRP in early colorectal neoplasia that warrants further study.

Keywords CRP · IL-6 · SNPs · Colorectal adenoma

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Introduction

The inflammatory and malignant processes share a number of characteristics, including the release of reactive oxygen species, tissue degradation, angiogenesis, and anti-apoptotic effects. Consequently, there has been a long-standing interest in the role of chronic inflammation in carcinogenesis. Colorectal neoplasia is a particularly suitable disease in which to study this relationship because of its strong associations with inflammatory bowel disease and non-steroidal anti-inflammatory drug (NSAID) use, and because of the importance of the host innate immunity in maintaining homeostasis in the normal gut [1].

Cytokines are the mediators of inflammation. These inflammatory proteins, secreted by activated lymphocytes, exert their action locally on the cells expressing their receptors, including the immune cells and endothelial cells

of the gut. In addition, cytokines reach the blood stream and can, therefore, be used as biomarkers of inflammation. Circulating levels of cytokines are known to increase many fold in response to infection and tissue damage, including the cytokine interleukin-6 (IL-6) and the inflammatory protein, C-reactive protein (CRP), which is produced by the liver in response to IL-6. However, cytokines can be upregulated even in the absence of an obvious acute inflammatory stimulus, and their variation within the reference range has been shown to reflect chronic inflammation and predict the onset of a number of chronic diseases, such as cardiovascular diseases [2–4].

Several studies have examined the relationship between circulating levels of CRP and CRC risk with inconsistent findings; four studies found no association [5–8], one reported a possible protective effect [9], and three studies showed a positive association [10–12]. To our knowledge, only two studies examined systemic markers of inflammation and the risk of colorectal adenoma (a precursor lesion for most CRC) by measuring circulatory CRP alone, or both CRP and IL-6 levels, respectively [13, 14]. Both studies reported no association with CRP, while a positive association was reported for IL-6 [13].

In addition to exogenous (e.g., infection) and endogenous (e.g., obesity) factors that can affect circulatory levels of cytokines [15], individuals may also have constitutively higher expression of these proteins due to their genetic make up. Indeed, common single nucleotide polymorphisms (SNPs) in the *IL6* and *CRP* genes have been associated with increased circulatory concentrations of their respective protein. The allele frequencies for these SNPs vary among ethnic/racial groups and may contribute to intra and inter-population differences in adenoma risk. Only a few studies have examined the association of these SNPs with CRC [7, 16, 17], and even fewer studies have focused on adenoma [18]. We are not aware of any colorectal adenoma study that examined both circulatory levels and genetic variants for inflammatory proteins.

Given the growth promoting effects of inflammation, we hypothesized that increased circulating levels of CRP and IL-6, two pro-inflammatory proteins, may increase the risk of colorectal adenoma. We further hypothesized that SNPs associated with higher levels of these cytokines would confer an increased risk for the development of colorectal adenoma. We tested these hypotheses in a sigmoidoscopy-based case–control study conducted in the multiethnic population of Hawaii.

Methods

Subjects were identified from two flexible sigmoidoscopy screening clinics in Honolulu (the Hawaii site of the Prostate, Lung, Colorectal, and Ovarian Cancer (PLCO) screening trial and the Kaiser Permanente Hawaii Gastroenterology Screening Clinic) between January 1996 and June 2002. Cases were individuals diagnosed with a first colorectal adenoma who were at least 75% Caucasian or Japanese, or any amount of Native Hawaiian ancestry. Controls were randomly selected among individuals with no lesion and were matched to cases on age, sex, ethnicity (Japanese, Caucasian or Hawaiian), screening date (±3 months), and recruitment clinic. The participation rate was 66.1 and 68.0% for cases and controls, respectively [12]. This study was IRB-approved, and all participants signed an informed consent.

In-person interviews were conducted with all study participants at their homes by trained interviewers to obtain various demographic and lifestyle information. The survey instruments included a detailed food frequency questionnaire focusing on dietary intake and supplement use during the 12 months preceding the sigmoidoscopy. An additional questionnaire administered at the time of the blood draw focused on smoking status, number of cigarettes smoked per day, and use of NSAIDs during the preceding 2 weeks. Since these factors may affect circulatory CRP and IL-6 levels, they were considered in the analyses.

Blood samples were obtained after a 10 h fast from 810 study participants (271 cases and 539 controls). Forty cc of blood was collected and processed within 2 h and stored at −80°C. Serum CRP was measured by a high-sensitivity turbidity assay from Pointe Scientific, Inc (Canton, MI), whereas serum IL-6 was measured by a high-sensitivity ELISA assay from R&D Systems (Minneapolis, MN). The intra-batch assay variability was 3.6 and 7.2% for CRP and IL-6, respectively, and the inter-batch assay variability was 6.1% for CRP and 9.3% for IL-6 measurements, based on blind duplicate samples analyzed with the study samples.

DNA was isolated from blood lymphocytes using QIAmp 96 DNA Blood Kit (Qiagen, Valencia, CA) and genotyped for SNPs in *CRP* (rs1205, rs1130864, and rs1800947) and *IL6* (rs1800795) with the 5′ nuclease Taqman allelic discrimination assay (Applied Biosystems, Foster city, CA). Laboratory personnel were blinded to the case–control status of the samples, and quality control (QC) duplicate samples were included on each plate. The average genotyping call rate was 99%, and the genotype concordance among 65 blind duplicate samples was greater than 99%. Among controls, all ethnic-specific genotype frequencies were in Hardy–Weinberg equilibrium ($p > 0.05$).

Multiple analysis of covariance was used to determine the differences in crude and adjusted serum CRP and IL-6 levels by sex-racial/ethnic groups, covariate level, and genotype. Appropriate transformations of the CRP and IL-6 protein measurements were identified using the Box-Cox method. Values were transformed back to their natural scale for the purpose of presentation in the tables.

Case–control differences in mean values and percentages were tested using two-sample *t*-tests and chi-square tests, respectively. Multiple linear regression models were used to test for differences in mean CRP and IL-6 levels by variable categories, and for stepwise selection of variables contributing to the variation in their serum levels. The potential inclusion of variables in a model was limited to those factors that improved the fit of the model based on a significance level of 0.10. The criterion for a variable to remain in a model was set at $p = 0.15$. All explanatory variables competed to enter the models, except case–control status, which was forced into the models. Unconditional logistic regression was used to estimate the adenoma odds ratios (ORs) and 95% confidence intervals (CIs) for increasing quartiles of serum CRP and IL6 and for adjusting for the effects of other covariates associated with adenoma risk. These covariates included the study matching criteria: age, sex, and race/ethnicity, as well as BMI and smoking status. Separate analyses were conducted after exclusion of NSAID users. Likewise, adjustment for NSAID use did not affect the ORs; therefore, we present the models without adjustment for NSAID use. Statistical analyses were carried out using SAS version 9.1 (SAS Institute, Cary, NC).

Results

Table 1 shows the baseline characteristics for the adenoma cases and controls. Cases and controls were of similar age, sex, and race/ethnicity. The majority of study participants were men and more cases than controls ever smoked. Body size characteristics, such as current BMI, waist-to-hip ratio, and height did not differ significantly between cases and controls. NSAID use within 2 weeks prior to blood draw was slightly more common among controls (46.8% vs. 42.3%) ($p = 0.22$), whereas the mean serum concentration of IL-6 and CRP did not differ between cases and controls. The only dietary variable that differed between cases and controls was processed meat consumption, with a significantly higher intake among cases ($p = 0.0004$).

In the stepwise multiple linear regression, the variables competing to enter the models were the same for the IL-6 and CRP models and included age, sex, race, BMI, waist circumference, hip circumference, waist-to-hip ratio, height, smoking status, NSAID use, glucocorticoid use, physical activity (METs), and education (years of schooling). The stepwise procedure first identified BMI, followed by age, race/ethnicity (Native Hawaiian vs. Japanese and Caucasian vs. Japanese), and smoking status (current vs. former or

Table 1 Baseline characteristics of the adenoma cases and controls

	Cases ($n = 271$)	Controls ($n = 539$)	p value
Age (years)	62.5 (±6.8)	62.0 (±6.6)	0.35
Sex (% males) (*n*)	67.9 (184)	68.8 (371)	0.79
Race/ethnicity (%) (*n*)			0.72
Japanese	39.9 (109)	37.4 (202)	
Caucasian	42.0 (114)	45.3 (244)	
Hawaiian	18.0 (49)	17.3 (93)	
Education (years)	14.8 (±3.1)	15.0 (±3.2)	0.25
Smoking status (%) (*n*)			0.14
Current	13.7 (37)	10.2 (55)	
Past	48.2 (131)	45.3 (244)	
Never	38.2 (103)	44.5 (240)	
No. of cigarettes per day[a]	14.8 (±9.4)	15.6 (±11.3)	0.74
Drinking (%) (*n*)			0.46
Never	34.3 (93)	37.3 (201)	
Past	20.3 (55)	21.9 (118)	
Current	45.4 (123)	40.8 (220)	
Red meat (g/day)	63.7 (±51.9)	58.1 (±49.6)	0.14
Processed meat (g/day)	35.3 (±36.4)	27.5 (±25.8)	0.0004
BMI (kg/m^2)	26.9 (±4.6)	26.7 (±4.7)	0.54
Waist-to-hip ratio	0.91 (±0.09)	0.90 (±0.08)	0.67
Physical activity (METs)	60.5 (±13.4)	60.6 (±14.2)	0.91
NSAID use (%) (*n*)[b]	42.3 (115)	46.8 (127)	0.22
Serum IL-6 (pg/mL)	2.4 (±2.3)	2.4 (±2.5)	0.93
Serum CRP (mg/L)	1.9 (±2.9)	2.1 (±3.9)	0.59

Mean (and standard deviation) unless otherwise indicated

[a] For current smokers, during the 2 weeks preceding the blood draw

[b] NSAID use during the 2 weeks preceding the blood draw

Table 2 Mean (95% confidence limits) serum IL-6 and CRP by case–control status and covariates ($n = 810$)

	IL-6 (pg/mL) Mean (95% CL)	CRP (mg/L) Mean (95% CL)
Case–control status		
Cases	1.89 (1.73–2.07)	1.01 (0.89–1.14)
Controls	1.93 (1.80–2.08)	1.02 (0.93–1.13)
*p**	0.69	0.82
Race/ethnicity		
Japanese	1.61 (1.48–1.75)	0.80 (0.71–0.90)
Caucasian	1.99 (1.84–2.15)	1.37 (1.23–1.53)
Hawaiian	2.20 (1.96–2.48)	0.96 (0.81–1.12)
p	**<0.0001**	**<0.0001**
Age (years)		
<55	1.56 (1.37–1.79)	0.95 (0.79–1.14)
55–59	1.68 (1.52–1.86)	0.98 (0.85–1.12)
60–64	1.95 (1.75–2.17)	1.09 (0.94–1.26)
65–69	2.19 (1.98–2.42)	1.09 (0.95–1.25)
>70	2.23 (1.95–2.54)	0.91 (0.76–1.10)
p	**<0.0001**	0.79
Sex		
Male	1.91 (1.78–2.04)	0.77 (0.71–0.85)
Female	1.93 (1.76–2.12)	1.33 (1.17–1.51)
p	0.80	**<0.0001**
BMI (kg/m^2)		
<20	1.38 (1.08–1.74)	0.45 (0.29–0.65)
20–25	1.70 (1.57–1.84)	0.79 (0.70–0.88)
26–30	2.01 (1.83–2.20)	1.20 (1.06–1.37)
>30	2.78 (2.44–3.17)	1.94 (1.61–2.36)
p	**<0.0001**	**<0.0001**
Physical activity (METs)		
<51.8	2.02 (1.81–2.24)	1.17 (1.01–1.35)
51.8–58.2	1.99 (1.79–2.21)	1.16 (1.01–1.34)
58.3–67.2	1.84 (1.65–2.04)	1.01 (0.88–1.17)
>67.2	1.83 (1.65–2.03)	0.84 (0.73–0.97)
p	0.17	**0.0005**
Smoking status at blood draw		
Never	1.64 (1.53–1.77)	0.85 (0.76–0.93)
Former	1.83 (1.70–1.97)	0.97 (0.88–1.07)
Current	2.35 (2.04–2.70)	1.29 (1.06–1.56)
p	**<0.0001**	**0.0001**

Mutually adjusted for the variables in the Table

Bold values indicate those *p* values that were statistically significant

* *p* for difference in mean levels across categories of the variable

never smoking), as statistically significant predictors of serum IL-6 levels. For CRP, female gender, BMI, race/ethnicity, current smoking, and physical activity were selected and were significantly associated with serum levels.

Table 2 displays these relationships. Case–control status was not associated with serum CRP or IL-6 levels. Among the three ethnic/racial groups studied, the lowest mean serum level for both IL-6 (1.61 pg/ml) and CRP (0.80 mg/L) was observed in Japanese, while Native Hawaiians had the highest mean IL-6 level (2.20 pg/ml), and Caucasians had the highest mean CRP level (1.37 mg/L). Women had significantly higher mean CRP level (1.33 vs. 0.77 mg/L, $p < 0.0001$) than men, while IL-6 levels did not differ significantly by sex ($p = 0.80$). IL-6 levels significantly increased with age ($p < 0.0001$), while CRP did not. Both serum IL-6 and CRP levels significantly increased with BMI ($p < 0.0001$ for each). Increased physical activity was associated with decreased serum CRP and IL-6 levels, but not significantly so for IL-6. Current smokers had a significantly higher mean IL-6 ($p < 0.0001$) and CRP levels ($p = 0.0001$) compared to former and never smokers. The coefficient for the correlation between serum IL-6 and CRP levels was 0.5.

Table 3 Adenoma odds ratios (95% confidence intervals) by quartile of serum IL-6 and CRP

IL6 (pg/mL)	Cases/controls	OR (95% CI)*	OR (95% CI)**
<1.09	68/135	1.00	1.00
1.09–1.69	65/135	1.00 (0.66–1.53)	0.99 (0.65–1.52)
1.70–2.79	72/132	1.10 (0.72–1.67)	1.02 (0.66–1.57)
>2.79	66/137	0.96 (0.63–1.48)	0.87 (0.55–1.37)
p trend		0.96	0.91
CRP (mg/L)			
<0.30	47/102	1.00	1.00
0.30–0.69	78/164	1.00 (0.64–1.55)	0.97 (0.62–1.52)
0.70–2.00	71/130	1.21 (0.76–1.92)	1.16 (0.72–1.86)
>2.00	71/139	1.14 (0.71–1.82)	1.00 (0.60–1.66)
p trend		0.76	0.82

* Adjusted for sex, age, and race

** Further adjusted for smoking status and BMI

The adenoma ORs ratios for increasing quartile of IL-6 and CRP levels are presented in Table 3, adjusting for the matching criteria (age, sex, race/ethnicity), and further for smoking and BMI. Neither inflammatory protein was associated with adenoma risk.

To compare allele frequencies across ethnic/racial groups and with other populations, we genotyped the same SNPs among the population controls enrolled in our previous CRC case–control study [19, 20]. The frequency for the C allele of *CRP* rs1205 was 31.7% in Japanese, 64.7% in Caucasians, and 33.1% in Native Hawaiians. The corresponding frequencies for the A allele of *CRP* rs1130864 were 5.9, 28.5, and 12.1% and those for the C allele of *CRP* rs1800947, 5.0, 4.9, and 4.8%. None of the *CRP* SNPs were in high linkage disequilibrium with each other. For *IL6* rs1800795, the frequency for the C allele was 0% in Japanese, 36.5% in Caucasians, and 16.9% in Native Hawaiians.

Table 4 Mean serum CRP and IL-6 levels (and 95% confidence limits) by genotype in cases and controls ($n = 810$)

	SNP	Genotype	*N*	Adjusted mean* (95% CL)
CRP (mg/L)	rs1205	TT	236	0.92 (0.79–1.06)
		CT	370	1.12 (0.98–1.28)
		CC	201	1.30 (1.11–1.53)
		p trend		0.0002
	rs1130864	GG	551	1.04 (0.93–1.17)
		GA	230	1.12 (0.95–1.31)
		AA	27	1.49 (1.04–2.19)
		p trend		0.012
	rs1800947	GG	730	0.93 (0.68–1.26)
		CG+CC	75	1.09 (0.95–1.27)
IL-6 (pg/mL)	rs1800795	GG	531	1.90 (1.73–2.07)
		GC	210	2.23 (1.98–2.51)
		CC	65	2.35 (1.96–2.82)
		p trend		0.07

* Adjusted for age, sex, race, smoking status, and BMI

** For each SNP, the test for trend was based on a count of the allele associated with increased protein level in past studies

Table 5 Adenoma odds ratio (95% confidence intervals) by genotype

Gene	SNP	Cases/ controls	OR* (95% CI)	OR** (95% CI)
CRP	rs1205			
	TT	96/140	1.00	1.00
	CT	119/250	0.69 (0.48–0.98)	0.67 (0.47–0.96)
	CC	55/146	0.53 (0.34–0.83)	0.53 (0.34–0.83)
	p trend		0.008	0.008
	rs1130864			
	GG	200/351	1.00	1.00
	GA	62/167	0.65 (0.45–0.93)	0.66 (0.46–0.95)
	AA	8/19	0.74 (0.31–1.76)	0.75 (0.32–1.80)
	p trend		0.04	0.05
	rs1800947			
	GG	241/488	1.00	1.00
	CG+CC	27/48	1.17 (0.71–1.93)	1.18 (0.71–1.96)
IL6	rs1800795			
	GG	173/357	1.00	1.00
	GC	74/136	1.34 (0.88–2.03)	1.32 (0.87–2.00)
	CC	22/43	1.30 (0.71–2.39)	1.24 (0.67–2.27)
	p trend		0.24	0.32

* Adjusted for age, sex, and ethnicity

** Further adjusted for smoking status (ever vs. never) and BMI

Table 4 shows the adjusted means for serum levels of CRP or IL-6 by genotype for all study participants combined. For rs1205, the C allele was strongly associated with increased levels of CRP ($p = 0.0002$). The A allele for *CRP* rs1130864 and the C allele for *IL6* rs1800795 were associated with higher circulating levels of their respective protein, but the test for gene–dosage effect was statistically significant only for rs1130864 ($p = 0.01$).

Table 5 shows the associations of the *CRP* and *IL6 SNPs* with adenoma risk, adjusting for age, sex, and race/ethnicity. For *CRP* rs1205, the C allele related to higher serum protein levels was associated with a decreased risk of adenoma (OR = 0.53, 95% CI = 0.34–0.83) (p for trend: 0.008). A similar inverse association was observed for the A allele of *CRP* rs1130864 ($p = 0.04$). The other two SNPs were not associated with adenoma. Further adjusting for BMI and ever smoking did not materially change the risk estimates (Table 5). Finally, no interaction with race was detected for any of the SNPs.

Discussion

In this study, we found no association between serum concentrations of the inflammatory proteins CRP and IL-6 and colorectal adenoma risk. However, we found that Japanese, a group at high risk for CRC, had lower circulating levels of CRP and IL-6, compared to whites and Native Hawaiians. We also studied genetic polymorphisms in the *CRP* and *IL6* genes that had been reported to be associated with serum levels. We confirmed that the C allele for *CRP* rs1205 and the A allele for rs1130864 were associated with higher serum CRP concentrations. We also found that these alleles were associated with a decreased adenoma risk with a gene–dosage effect.

For this study, we selected specific genetic variants in *CRP* and *IL6* based on their published associations with serum CRP or IL-6 concentrations. Two of the CRP polymorphisms studied are located in the 3′ untranslated region (rs1130864 is a C/T substitution at position 3014 and rs1205 a C/T substitution at position 3872). The third *CRP* SNP (rs1800947) is a synonymous C/G polymorphism at codon 188, whereas the *IL6* polymorphism (rs1800795) is located at position −174 (C/G). Except for rs1800947, we confirmed in our study that the ancestral alleles for the other two *CRP* SNPs were associated with higher serum levels. A similar trend was suggested for the C allele of *IL6* rs1800795 and serum IL-6 levels. These relationships have been observed in the past [21–23]. In the present study, rs1800795 was monomorphic in Japanese and, thus, this group appears to lack the allele associated with higher IL-6 expression.

We are aware of only one prior study, which examined inflammation-related gene polymorphisms and their association with adenoma risk [18]. This recent study of 244 cases and 231 controls found no association between the

IL6 rs1800795 polymorphism and adenoma risk, but found that heterozygosity at the *IL1B*-31C/T variant and possession of the minor allele for *IL8* -251A polymorphism was associated with an increased adenoma risk. No *CRP* SNP was investigated. Overall, however, the results of this past study suggested that a pro-inflammatory genetic profile may be a risk factor for early colorectal neoplasia, concordant with our a priori hypothesis. This hypothesis was based on the proposed growth promoting effects of inflammatory cytokines in the setting of chronic sub-clinical inflammation, as well as on some early reports of a positive association of circulating CRP levels with CRC. Indeed, three prospective cohort studies reported that increased pre-diagnostic CRP levels may be associated with an increased incidence of CRC [10–12]. However, since the latency period for CRC is over a decade long and since inflammation is part of the host response to tumor development [24], increased CRP levels may have been the consequence rather than a cause of the tumor in these studies. In fact, this association has not been reproduced in a number of other cohort studies [5–8].

To our knowledge, only one past study investigated the circulatory CRP levels and adenoma risk. Consistent with our findings, this case–control study nested in the CLUE II cohort study reported no association [14]. Interestingly, this same cohort study was the first to report an association between circulatory CRP levels and CRC risk [10].

Although we found no association between serum IL-6 or CRP concentrations and adenoma risk, the two *CRP* SNPs that we and others found associated with higher CRP serum levels conferred a reduced risk of adenoma in our study. This conflicts with our a priori hypothesis but is consistent with the lower CRP and IL-6 levels observed for Japanese in this study, since this group is at high risk for CRC [25, 26]. This is also consistent with our finding that the genotypes yielding lower serum CRP concentrations were more common among Japanese. Similar to our results for colorectal adenoma, it has recently been shown in a Dutch prospective study that individuals homozygous for the T allele in *CRP* rs1205, related to lower serum CRP levels, had an increased risk of lung cancer, while their risk of colorectal cancer did not change [7].

If our results were to be replicated in other studies, they might be explained by CRP properties other than their well-described pro-inflammatory activity. Namely, CRP is also known to play an important role in the maintenance of tissue homeostasis, by which it recognizes damaged cells and mediates their removal by binding to lysed or permeabilized cells, as well as to intact apoptotic cells [27]. Gershov and colleagues showed that increased CRP concentrations are associated with enhanced phagocytosis of apoptotic cells and may, thus, contribute to their clearance [27]. Therefore, constitutively higher CRP levels may yield a more effective tissue repair and elimination of initiated cells and, as the result, exert a protective effect against adenoma development.

The use of NSAIDs has been shown to protect against both adenoma and CRC development. However, whether such effect is a consequence of their beneficial effects on inflammation or of other properties is still unclear [28]. In our study, approximately 40% of cases and controls reported NSAID use. Neither the adjustment for NSAID use, nor separate analysis after exclusion of NSAID users had any significant effect on the adenoma risk estimates. However, this sub-group analysis yielded less precise OR estimates due to the reduced sample size.

As in past reports, BMI was the single major determinant of both IL-6 and CRP circulating levels in our study. Similarly, the IL-6 and CRP levels that we observed for Japanese were similar to previous reports [29]; however, the CRP levels for Caucasians in our study were much lower compared to those reported for the ATBC study (1.17 mg/L vs. 2.60 mg/L) [11]. Although the mean age and BMI were comparable for the two studies, the ATBC study included only men who were current smokers, whereas only 10% of controls and 13% of adenoma cases in our study reported current smoking. Since smoking affects CRP levels, this is a likely contributor to the observed differences.

In contrast, we detected much higher IL-6 levels compared to a recent adenoma study conducted in whites [13]. In this study, half of the participants had undetectable levels of IL-6 (less than 0.104 pg/mL), while only a quarter had IL-6 levels higher than 0.36 pg/mL [13]. By comparison, only a quarter of our study participants had IL-6 levels lower than 1.09 pg/mL (Table 3) and only a few had undetectable IL-6 levels. These differences may explain why we did not reproduce the positive association between IL-6 and adenoma risk reported by Kim et al. [13].

Several potential study limitations should be considered when interpreting our findings. A relatively large number of statistical comparisons were conducted, which may have led to false positive findings. The statistical power to detect interactions was limited due to the small size of the study. Finally, serum levels of cytokines were only measured once and after the diagnosis of adenoma. Although it has been shown that circulating CRP levels are relatively stable over time [30], it is well established that these levels are influenced by lifestyle (e.g., smoking) and underlying infections and, thus, may not represent the inflammatory state of the target tissue. To address this limitation, we genotyped our subjects for "functional" SNPs, since they may better represent long-term (lifetime) exposure to differential CRP or IL-6 levels, or result in a less vigorous constitutional inflammatory response. Since such a constitutional response would occur in every tissue in the body

where these genes are expressed, including the colonic mucosa, it is plausible that genotype may better reflect the capacity of local tissue to mount an inflammatory response. Therefore, using SNPs in our study may have partially addressed the issue of measuring systemic versus local inflammation. Thus, one may expect genetic variants to better reflect risk of adenoma than serum measurements. This may explain why, in this study, the association between CRP and adenoma was only seen with the SNPs and not with serum levels.

In summary, while we observed no association between serum levels of the inflammatory markers CRP and IL-6 and colorectal adenoma in this study, our analyses revealed inverse associations between SNPs linked to constitutively higher CRP concentrations and adenoma risk. Significant ethnic/racial differences were observed in circulating CRP and IL-6 levels that were consistent with both the ethnic/racial distributions of the genotypes under study and CRC risk. These findings suggest a protective effect of CRP against early colorectal neoplasia. Further investigations are needed to improve our understanding of the role of these inflammatory proteins in the etiology of colorectal neoplasia.

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