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Plasma C-reactive protein, genetic risk score, and risk of common cancers in the Atherosclerosis Risk in Communities study

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Abstract

Purpose—Many studies, including the Atherosclerosis Risk in Communities (ARIC) cohort, reported a positive association between plasma C-reactive protein (CRP) – a biomarker of low-grade chronic inflammation – and colorectal cancer risk, although it is unclear if the association is causal. Our aims were to assess the associations of a CRP genetic risk score (CRP-GRS) created from single nucleotide polymorphisms (SNPs) with colorectal cancer risk, as well as examine plasma CRP and CRP-GRS in relation to common cancers in the ARIC cohort.

Methods—Cox proportional hazards models were used to prospectively estimate hazard ratios (HR) and (95% confidence interval, CI) of total, colorectal, lung, prostate, and breast cancers in relation to: 1) CRP-GRS among 8,657 Whites followed in 1987–2006 and 2) log-transformed plasma CRP among 7,603 Whites followed in 1996–2006. A weighted CRP-GRS was comprised of 20 CRP-related SNPs located in/near *CRP*, *APOC1*, *HNF1A*, *LEPR* and 16 other genes that were identified in genome-wide association studies.

Results—After multivariable adjustment, one standard deviation increment of the CRP-GRS was associated with colorectal cancer risk (HR=1.19; 95% CI, 1.03–1.37) but not with any other cancer. One unit of log-transformed plasma CRP was associated with the risk of total, colorectal, lung, and breast cancers: HRs (95% CIs) were 1.08 (1.01–1.15), 1.24 (1.01–1.51), 1.29 (1.08–1.54), and 1.27 (1.07–1.51), respectively. HRs remained elevated, although lost statistical significance for all but breast cancer, after excluding subjects with <2 years of follow-up.

Conclusions—The study corroborates a causative role of chronic low-grade inflammation in colorectal carcinogenesis.

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Keywords

inflammation; CRP; cancer risk; genetic risk score; genetic polymorphism; ARIC cohort

Introduction

Many cancers occur at sites of chronic irritation or injury. However, data about the link between low-grade systemic inflammation and cancer have been inconsistent. The most widely used biomarker of inflammation is circulating C-reactive protein (CRP) with concentrations above 10 mg/L indicating clinically significant inflammatory states, and values below that as low-grade chronic inflammation [1].

Circulating CRP has been associated with different types of incident cancer – total, colorectal, lung, breast, endometrial, prostate and ovarian, but consistent associations have been observed only for lung [2–8] and colorectal cancers [3, 4, 9–13]. Moreover, non-steroidal anti-inflammatory drugs (NSAIDs), which were reported to reduce CRP concentration [14], have been consistently shown to decrease colorectal cancer risk [15–17] with a 40% lower risk for those who regularly take NSAIDs versus those who do not [16]. Inverse associations have also been observed between aspirin use and the risk of lung, prostate, and breast cancers, but the protective effects of aspirin on these cancer endpoints are less well established [16].

Although these data implicate a role for inflammation in carcinogenesis, it has not been completely established whether low-grade systemic inflammation, reflected by increased CRP, contributes to carcinogenesis or merely indicates subclinical cancer. Recently, in the ARIC prospective cohort, we reported that for the highest versus lowest quartile of pre-diagnostic CRP the hazard ratio (HR) for colorectal cancer was 1.97 (95% CI, 1.13–3.43; $P=0.02$) [18]. This association remained (HR=1.77; 95% CI, 1.01–3.09; $P=0.05$) after excluding first two years of follow-up, which implies that the association is not explained solely by inflammation resulting from a subclinical tumor. However, the influence of subclinical colorectal cancer cannot be completely excluded due to its latency of 5–10 years or longer [19]. Further, as in all observational studies, the CRP–cancer association may be partially attributed to residual confounding by strong risk factors (e.g. obesity) that are associated with both plasma CRP and colorectal cancer risk [1, 20].

To investigate a potential causative role of inflammation in colorectal cancer development, we aimed to examine whether single-nucleotide polymorphisms (SNPs) that increase circulating CRP concentration are associated with incident colorectal cancer using the principles of Mendelian randomization [21, 22]. In this method, CRP-related SNPs are used as a proxy for plasma CRP. An advantage of using SNPs instead of plasma CRP is that given gene variants are randomly allocated at conception, an association between genetic polymorphisms and disease outcomes is not in theory affected by confounding factors, nor is it a consequence of the disease outcome [23]. This method is applicable because plasma CRP concentrations were shown to be 35–40% heritable in family studies [24] and, recently, genome-wide association studies (GWAS) have identified 20 genetic loci significantly associated with plasma CRP concentrations ($P<5\times 10^{-8}$) [25, 26]. The CRP-related SNPs were located in/near *CRP*, apolipoprotein C1 (*APOC1*), hepatocyte nuclear factor 1 homeobox (*HNF1A*), leptin receptor (*LEPR*), and 16 other genes. These loci together accounted for ~5% of the variation in plasma CRP concentrations in a meta-analysis of GWAS [25]. For comparison, BMI, the main non-genetic determinant of CRP, explained 5–15% of plasma CRP variation in the cohorts included in the meta-analysis. Of note, this meta-analysis included the ARIC cohort.

We hypothesized that there is a positive association between SNPs increasing CRP concentration and colorectal cancer risk among Whites in the ARIC cohort. We conducted two types of genetic analyses: one that examined the association of the CRP SNPs with colorectal cancer risk, and another that examined the causal association of inflammation reflected by plasma CRP with colorectal cancer using a Mendelian randomization instrumental-variables approach. To avoid multiple comparisons and create a variable that accounts for considerable variation in plasma CRP, we constructed a weighted CRP genetic risk score (CRP-GRS) based on 20 CRP-related SNPs established in the meta-analysis of GWAS [25, 26]. In addition, we prospectively examined other common incident cancers (prostate, lung, and breast), as well as total cancer in relation to plasma CRP and weighted CRP-GRS.

Methods

The ARIC study is a prospective cohort of atherosclerotic disease [27]. In brief, during 1987–1989 it enrolled and followed 15,792 White and African American men and women aged 45–64 years in four US communities: Forsyth County, NC; Jackson, MS; suburban Minneapolis, MN; and Washington County, Maryland [27]. Because of potential population stratification and insufficient power to conduct a separate genetic analysis among African-Americans (25% of the ARIC cohort), all genetic analyses were conducted among Whites only. For consistency, analyses for plasma CRP were similarly restricted. Local institutional review boards approved the ARIC protocol and all participants provided informed consent.

Baseline and three follow up visits in 1990–92, 1993–95, and 1996–98 (response rates were 93%, 86% and 81%, respectively) included interviews, laboratory measurements, and clinic examinations [27, 28]. Participants were asked to report their demographic characteristics, education, lifestyle behaviors, and medical history at each follow up. Trained personnel collected anthropometric measures and blood (serum and plasma) samples.

Genotyping

In the ARIC study, genotyping was conducted using the Affymetrix Genome-Wide Human SNP Array 6.0 and quality control was performed as described previously [29]. In brief, exclusions were: subjects not consenting to DNA use, samples with a mismatch between called and phenotypic sex, samples with genotype mismatch with 39 previously genotyped SNPs, suspected first-degree relatives of an included individual, and genetic outliers based on average IBS statistics and principal components analysis using EIGENSTRAT. After the filtering, 669,450 SNPs were used in an imputation to 2,543,887 autosomal SNPs from HapMap Phase II CEU samples using MACH v1.0.16. SNPs that had: MAF $\geq 1\%$, call rate $\geq 95\%$, and HWE- $p \geq 10^{-5}$ [29]. All 20 CRP-related SNPs identified in the GWAS meta-analysis [25, 26] were genotyped or imputed in ARIC. R-squared for MACH imputation (the squared correlation between imputed and true genotypes) in ARIC are shown in Supplementary Table 1.

CRP measurement

High-sensitivity CRP (mg/L) was measured in plasma frozen at -70°C at Visit 4 (1996–98) by an immunoturbidimetric assay using the Siemens (Dade Behring) BNII analyzer (Dade Behring, Deerfield, IL, USA). The reliability coefficient for CRP in blinded quality control replicates was 0.99 (421 blinded replicates) [30].

Cancer ascertainment

History of cancer at the baseline exam was ascertained by self-report. Incident cancers were ascertained for 1987–2006 by linkage to cancer registries and supplemented by hospital

records [18, 31]. Primary site, date of cancer diagnosis, and source of diagnostic information (e.g., a pathology report) were recorded. Information about stage, grade or benign tumors has not been collected.

For the analysis of CRP genes with incident cancers, the analytical cohort included 8,657 Whites who had clean genotyping data, consented to participate in studies of non-cardiovascular diseases and were free of cancer at Visit 1. They were followed from baseline until date of cancer diagnosis, death from other causes, loss to follow-up, or December 31, 2006, whichever occurred first. During this period, 1,929 total, 205 colorectal, 274 lung, 395 prostate, and 368 breast cancers occurred. All covariates for this analysis were ascertained at baseline.

Since plasma CRP was measured at Visit 4 (1996–98), the analytical cohort for the plasma analysis included 7,603 Whites followed from Visit 4 until date of cancer diagnosis, death from other causes, loss to follow-up, or December 31, 2006, whichever occurred first. These 7,603 subjects participated at Visit 4, were free of cancer at Visit 4, consented to participate in studies of non-cardiovascular diseases, and had plasma CRP measured. Overall, 1,159 incident cancers occurred including 120 colorectal, 156 lung, 253 prostate, and 176 breast cancers. Most covariates for this analysis were measured at Visit 4.

Statistical analysis

Proportional hazards regression models were used to estimate hazard ratios (HR) and 95% confidence interval (CI) of incident cancers in relation to weighted CRP-GRS and plasma CRP. Plasma CRP data were natural log-transformed to account for the non-linear associations with cancer. In a supplementary analysis, plasma CRP concentrations were analyzed as quartiles. Proportional hazards assumptions were tested using Schoenfeld residuals. There was no evidence that these assumptions were violated for any cancer.

To test the role of CRP-related polymorphisms identified in GWAS, we created a weighted genetic risk score (CRP-GRS) using the approach that is widely used in the studies of gene-disease associations [25, 32, 33]. We summed the number of risk (CRP-increasing) alleles from the 20 SNPs listed in Table 1. To account for the different associations of each SNP with log CRP concentration, we multiplied the number of risk alleles for the specific locus SNP_i (ranged from 0 to 2) by the appropriate β_i estimate for each SNP reported by the meta-analysis of GWAS of circulating CRP [25, 26]. In order to rescale CRP-GRS with a range of 0–40 (the maximum number of risk alleles for each individual), we divided the weighted CRP-GRS by the average effect size [32]:

$$\text{Rescaled weighted CRP-GRS} = (\beta_1 * SNP_1 + \beta_2 * SNP_2 + \dots + \beta_{20} * SNP_{20}) / ((\beta_1 + \dots + \beta_{20}) / 40).$$

CRP-GRS was analyzed as a continuous variable and as quintiles. To examine CRP-GRS score and individual SNPs (under the additive model assumption) in relation to log CRP concentration, a general linear model was applied.

Final multivariable-adjusted model for analysis of plasma CRP concentrations with cancers, included age (continuous), sex, ARIC center, education (<high school, high school, and >high school), as well as body-mass index (BMI) (<25, 25–29.9, and ≥30 kg/m²), waist circumference (continuous), smoking status (never, former, current), pack-years of smoking (continuous), current aspirin use (yes/no) and hormone therapy in women (former or current/never use) at Visit 4. For the analysis of total, colorectal and lung cancers, a variable combining sex and hormone therapy use was created: men, women never taking hormone therapy, and women who were former or current hormone therapy users. The model for

breast cancer was additionally adjusted for age at menarche (continuous), number of live births (continuous), and menopausal status at Visit 4 (yes/no). Due to strong correlation between BMI and waist ($r=0.89$), an individual waist circumference adjusted for BMI was computed by taking the residual values from a linear regression model with waist circumference as the dependent variable and BMI as the independent variable. By definition, the waist residuals provide a measure of waist circumference uncorrelated with BMI.

Because information on acute inflammatory diseases was not collected, in order to exclude an acute inflammatory response, we repeated analyses after excluding people with plasma CRP values >10 mg/L. Furthermore, to remove potential effects of undiagnosed cancer on plasma CRP, we conducted sensitivity analyses after excluding those with follow-up of less than 2 years.

Although confounding was not anticipated for the genetic analyses, we adjusted for major cancer risk factors measured at Visit 1 to account for potential confounding and increase the efficiency of the estimators. All the analyses described above were performed in SAS v9.1 (Cary, NC).

To examine the potential causal association between inflammation reflected by plasma CRP and the risk of colorectal and total cancer, we utilized a Mendelian randomization approach using the CRP-GRS as an instrumental variable. This approach is based on the assumptions that the CRP-GRS may serve as an objective proxy measure for plasma CRP if it is (1) associated with plasma CRP concentration; (2) not associated with potential confounders of the plasma CRP–cancer association, and (3) associated with cancer risk only through plasma CRP [34, 35].

To conduct this instrumental-variables statistical analysis, we used Stata, version 12 (Stata Corporation, College Station, Texas) and compared the estimates from two regression models: standard probit (command *probit*) and probit for instrumental-variables regression (command *ivprobit*) that were specifically designed for the analysis of binary outcomes [35–37]. This statistical method has been applied for studying causative effects of modifiable risk factors on cancer [22] and other outcomes [38]. The probit model is defined as $\Pr(y = 1 | x) = \Phi(x\beta)$, where Φ is the standard cumulative normal probability distribution and $x\beta$ is known as the probit coefficient. This instrumental-variables method uses the variation in plasma CRP that is explained by the CRP-GRS to estimate the causal association between inflammation reflected by plasma CRP and the cancer outcomes. We used F-statistics from the regression of plasma CRP on CRP-GRS to evaluate the strength of the instrument, with values greater than the conventional threshold of 10 indicating sufficient strength [35, 39]. The sample for this analysis ($n=6,429$) included all participants with data on plasma CRP at Visit 4 and the CRP-GRS and excluded the participants who had cancer diagnosed before Visit 4.

Results

Analysis of CRP-related polymorphisms with plasma CRP

Allele frequencies of the 20 CRP-related SNPs in the ARIC population were similar to those in the published GWAS for Whites (Table 1) [25, 26]. The location and potential function of SNPs, which were mostly unknown, may be found in Supplementary table 1. No SNPs composing the score were in linkage disequilibrium [40]. The CRP-GRS was normally distributed and accounted for 4% of the variance in log of plasma CRP level in unadjusted analysis (F -statistic=290); by comparison, BMI, the main non-genetic CRP determinant, explained 7% of the variance in log CRP concentration. In a multivariable adjusted model, the CRP-GRS was positively associated with log CRP concentration: $\beta_1=0.22$, $SE=0.01$ (P

<0.0001) per standard deviation (SD) increment of CRP-GRS. None of the participants' characteristics, other than plasma CRP concentration, was statistically significantly associated with CRP-GRS (Supplementary table 2).

Analysis of CRP-related polymorphisms with cancer

For colorectal cancer, the HR (95% CI) was 1.19 (1.03–1.37) per SD increment of the CRP-GRS in the multivariable analysis (Table 2). The HRs were similar for colon 1.20 (1.02–1.42) and rectal 1.18 (0.90–1.55) cancers. The association of the CRP-GRS with colorectal cancer changed only slightly after additional adjustment for log of plasma CRP concentration or after excluding participants with CRP>10mg/L. We did not find any associations of CRP-GRS with total, lung, prostate or breast cancers. The similar pattern was observed when we presented CRP-GRS as quintiles. For colorectal cancer, HRs (95% CI) were 1.00, 0.90 (0.56–1.45), 0.99 (0.62–1.58), 1.41 (0.92–2.16), and 1.39 (0.91–2.14) (P -trend=0.02) for quintiles 1 through 5 (data not shown). There was no indication of a trend across quintiles for any other cancer.

To further investigate the association between CRP-GRS and colorectal cancer risk, we conducted several sensitivity analyses. To test whether this association is driven by the SNPs most strongly associated with plasma CRP concentration, we reran the analysis after excluding the four SNPs located in the *APOC1*, *CRP*, *HNF1A*, *SALL1* genes (one at a time) from the CRP-GRS, but the association did not markedly change. Further, since many SNPs may be pleiotropic, i.e. may exert their effect on cancer through traits other than inflammation (i.e. obesity or diabetes), we repeated analysis after excluding SNPs related to metabolic syndrome (*APOC1*, *HNF1A*, *LEPR*, *GCKR*, *HNF4A*, and *PTPN2* genes) [25]. After each of these exclusions, the association between the CRP-GRS and colorectal cancer risk remained, which suggests that CRP-GRS influences colorectal cancer risk through inflammation.

Finally, to account for differences in genetic ancestry, we adjusted for principal components of genetic ancestry in Whites (derived from the GWAS) by entering them as continuous covariates in the Cox model. The estimates for colorectal cancer associated with the CRP-GRS did not materially change. We also tested for the interactions of the CRP-GRS with waist and BMI in relation to colorectal cancer risk in separate models, but none was observed, although the power was limited.

Analysis of plasma CRP with cancer

In this sample of Whites, plasma CRP was positively associated with BMI, waist, and smoking, and inversely associated with education (Table 3). The percentage of women and those who reported current use of aspirin or hormone therapy were higher among higher CRP levels. In a multivariable-adjusted model, plasma CRP concentration was associated with the risk of total, lung, and breast cancers; HRs (95% CIs) were 1.08 (1.01–1.15), 1.29 (1.08–1.54), and 1.27 (1.07–1.51) per 1 unit of log-transformed CRP concentration, respectively. Similarly, log-transformed CRP concentrations were associated with increased risk of colorectal cancer; HR (95% CI) per 1 unit of log-transformed CRP was 1.24 (1.01–1.51) (Table 4), while the HRs (95% CI) were 1.29 (1.03–1.62) for colon (n=98) and 1.01 (0.64–1.59) for rectal (n=24) cancers. The HRs for colorectal cancers were similar to those reported in an earlier analysis of the whole ARIC cohort, which included African Americans [18].

The HRs for all cancers remained increased after excluding people with follow-up of less than 2 years (4% of subjects, 18% of all cancers), although associations for total, colorectal, and lung cancers were attenuated and lost statistical significance, whereas the association for

breast cancer, strengthened (Table 4). The results were practically the same if we excluded only cancer cases that occurred within the first two years of follow-up. Similar associations were observed after excluding 5 years of follow-up (not shown) and for plasma CRP analyzed as quartiles (Supplementary table 3). After excluding participants with CRP > 10 mg/L (~8% of subjects), i.e. people who could have acute inflammation, all the associations remained virtually the same.

Multiplicative interactions of plasma CRP concentrations with BMI, waist circumference, smoking, and aspirin use were examined for all cancers. Interactions were also tested with sex for total, colorectal, and lung cancers and with ever use of hormone therapy for breast cancer. *P*-values for all interactions were >0.20, except for colorectal cancer the *P* value for an interaction of log CRP concentration with continuous waist was 0.07 and waist dichotomized at median, 0.09. Subgroup analyses were conducted after stratifying the waist at the median. The HR (95% CI) per unit increase of log CRP concentration for participants with waist below the median was 1.39 (1.08–1.78) and above the median, 1.07 (0.78–1.47) (Supplementary table 4). A similar pattern was observed for the analyses stratified by BMI categories: the HRs (95% CI) per 1 unit increase of log CRP concentration for colorectal cancer were 1.48 (1.01–2.17), 1.27 (0.89–1.81), and 0.95 (0.62–1.45) for BMI <25, 25–29.9, and ≥30 kg/m², respectively.

Analysis of genetic risk score, plasma CRP and colorectal cancer risk – Mendelian randomization approach

To examine the causal role of inflammation in the risk of colorectal cancer development, we conducted Mendelian randomization analysis using CRP-GRS as an instrument for inflammation reflected by circulating CRP (Figure 1). In addition, for comparison, we applied this approach to study the total cancer risk in relation to CRP. In multivariable-adjusted models, a standard probit regression analysis showed an almost statistically significant association of log-transformed plasma CRP with colorectal cancer risk but not with total cancer risk (Table 5). In the CRP-GRS-instrumented analysis, there was a moderate association with the risk of colorectal cancer but no association was observed for total cancer (Table 5).

Discussion

Main findings

In this prospective community-based study, we found that a genetic risk score created from CRP-related SNPs was associated with increased colorectal cancer risk, but not with risk for other cancers examined among Whites. To our knowledge, no other study has assessed a CRP-GRS in relation to cancer.

Further, we observed that plasma CRP was positively associated with incidence of total, lung, and breast cancers in addition to colorectal cancer (reported previously [18]). After excluding the first 2 years of follow-up, the magnitude of association was slightly changed for total cancer, lung cancer, and colorectal cancer (decreased) and breast cancer (increased). This variability could be explained by a decrease in power in the initially small analytical cohort (power calculations are presented in Supplementary table 5). The attenuation may be due, in part, to the effect of undiagnosed cancer on CRP concentrations. Of note, in our previous study in the whole ARIC cohort which had a larger sample size, for the highest versus lowest quartile of plasma CRP, the HRs for colorectal cancer only slightly changed (from 2.00 to 1.77) after excluding the first 2 years of follow-up and remained significant (95% CI, 1.01–3.09; *P*=0.05) [18]. We did not observe associations of plasma CRP with prostate cancer. Finally, using the CRP-GRS as a proxy for plasma CRP in a Mendelian

randomization instrumental-variables analysis, we found evidence that inflammation reflected by increased plasma CRP concentration may be a causal risk factor for the development of colorectal cancer.

This finding of a causal inference is in agreement with a potential biological mechanism suggesting that chronic systemic or local inflammation may lead to cancer due to the continuous production of pro-inflammatory cells and cytokines, as well as reactive oxygen and nitrogen species. Taken together, these processes could result in increased mutations and altered functions of important proteins [41, 42]. In addition, plasma CRP may have a pro-inflammatory effect by itself, via activating endothelial cells, monocytes and smooth muscle cells, inducing expression of adhesion molecules and chemoattractants, and activating the NF- κ B pathway [43]. Although the exact mechanisms underlying associations between systemic inflammation and CRP with specific cancers have not been established, the colon may be particularly prone to inflammation and carcinogenesis, due to several factors acting in concert: 1) the gastrointestinal tract is consistently exposed to environmental and dietary carcinogens; 2) the gut microbial flora causes permanent low-grade inflammation of colon mucosa; and 3) cells of the gastrointestinal tract are rapidly dividing leading to increased mutation rate [44–46]. This explanation agrees with the findings from our genetic analysis that showed associations with colorectal but not with other cancers. An alternative explanation for seeing the association for colorectal cancer only is that we lacked power to detect associations with other cancers, particularly if the effects were weaker. However, the analysis of CRP-GRS presented as quintiles did not show any indication of trend in associations with total or any other cancer. Nor did our instrumental variable analysis provide evidence for a causal association with total cancer.

Previous studies on plasma CRP and cancer risk

Most previous studies of plasma CRP and total cancer reported weak positive associations [2, 3, 6, 7], and a meta-analysis of 12 prospective studies reported HR=1.10 (95% CI, 1.02–1.18) [4] per unit increase in log CRP concentration, which is consistent with our findings. The observed positive association of CRP with lung cancer risk is also in agreement with many previous studies on lung cancer [2–8]. Furthermore, an absence of an association with prostate cancer is in accord with results from many other studies [4, 7, 47–50], whereas studies on plasma CRP and breast cancer are scarce and inconsistent. A meta-analysis of 5 studies reported a 1.10-fold (95% CI, 0.97–1.26) increase in breast cancer risk per a unit increase in log CRP concentration [4]. To our knowledge, only two prospective studies, the Rotterdam Study [6] and the Multiethnic Cohort Study [51] reported a statistically significant association of CRP concentration with breast cancer risk similar to ours. In all of these studies, the association did not change after excluding those with short follow up implying that inflammation preceded breast cancer occurrence. Although it is unclear why the findings are inconsistent, a positive association between inflammation and breast cancer parallels the finding from meta-analyses that consistently reported an inverse association between the use of NSAIDs and breast cancer risk [16, 52]. In addition, circulating CRP concentrations are often elevated in advanced stages of breast cancer and associated with shorter survival [53].

Most, but not all, previous studies found positive associations between plasma CRP and colorectal cancer risk similar to what we observed in ARIC. In brief, two meta-analyses reported slightly increased relative risks RR (95% CI) of colorectal cancer in relation to plasma CRP: 1.09 (0.98–1.21) [4] and 1.12 (1.01–1.25) [12] per 1 unit increase in log CRP concentration. The largest study to date – the European Prospective Investigation into Cancer and Nutrition reported RR (95% CI) of 1.09 (1.01–1.18) for colon and 1.02 (0.67–1.57, P -trend=0.65) for rectal cancer for a doubling in plasma CRP [9], which is in line with our findings.

In our study, both waist and BMI seem to weakly modify plasma CRP-colorectal cancer associations. Two studies [10, 11], but not others [3, 9], reported similarly that the association between plasma CRP and colorectal cancer was stronger among lean than in heavier individuals. We speculate that the association between plasma CRP concentration and colorectal cancer is weaker among obese participants because obesity is accompanied by inflammation [54] and there is less variability in the degree of inflammation between colorectal cancer cases and controls among obese than non-obese individuals.

Previous studies on CRP-associated genetic polymorphisms and cancer

Several studies investigated associations between individual CRP polymorphisms and risk of total cancer [6, 56], colorectal cancer [57–59], and prostate cancer [47] and reported inconsistent results. A study from Denmark examined the nine most common genotype combinations of the four non-coding SNPs in the CRP gene in relation to colorectal cancer risk, but did not observe any associations [56]. In contrast, the prospective CLUE II cohort study found that two CRP tag SNPs (rs2794521 and rs2808630) and CRP haplotypes were significantly associated with colorectal cancer risk [57]. The largest study to date by Slattery et al (2011) suggested that genetic variations in the CRP gene influence the risk of colon and rectal cancers [58]. Of note, all these studies examined SNPs located only in/near the CRP gene.

Recently, a Finnish study [22] examined the association of CRP-related SNPs, identified through two early GWAS [60, 61], with total cancer and four common cancers. They found rs1892534 in the *LEPR* gene to be associated with total cancer: HRs (95% CI) were 1.05 (0.90–1.23) and 1.20 (1.01–1.42) for subjects with one or two variant T alleles versus two CC alleles, respectively. Of note, rs1892534 was in linkage disequilibrium with another *LEPR* SNP – rs4420065 ($r^2=1$) that was included in our CRP-GRS. Although our goal was not to examine individual SNPs because of multiple comparisons and pleiotropic effects of individual SNPs, we tried to reproduce the findings from the Finnish study. We observed a similar association of rs1892534 with total cancer HR=1.09 (95% CI, 1.02–1.16) per each risk allele increase.

Strengths and limitations

An important strength of our study is that it was conducted within a large prospective ARIC cohort that has complete genotyping information about the CRP-related SNPs for all participants, accurate measurements of high-sensitivity CRP, cancer ascertainment through cancer registries and detailed data about anthropometric and lifestyle characteristics. This ample information allowed us to create the weighted CRP-GRS based on 20 CRP-related SNPs and conduct the Mendelian randomization analysis after multivariable adjustment. The CRP-GRS has advantages over a single measurement of plasma CRP because it is more accurately measured, in theory not confounded with other known colorectal cancer risk factors and it may reflect lifetime CRP concentrations, i.e. may provide some additional information beyond a single CRP measurement [55]. Further, the CRP-GRS offers advantages over individual SNPs because it avoids multiple comparisons and explains more variation in plasma CRP. Finally, others have shown that the analyses of weighted genetic risk scores based on multiple SNPs with weights obtained from other studies (in our case, from GWAS) are less biased than the traditional analyses of individual SNPs [62]. Thus, in our study, the CRP-GRS, which explained 4% of plasma log transformed CRP (F -statistic=290), was not related to the confounders of plasma CRP-cancer association, and appeared to be a rather strong instrument for plasma CRP. Hence, we have evidence that two of the assumptions for using the Mendelian randomization approach were satisfied. Further, our sensitivity analyses, in which individual SNPs most strongly associated with plasma CRP were excluded, showed that CRP-GRS-colorectal cancer association was robust and

provided some reassurance that CRP-GRS exerts its effect on colorectal cancer risk mainly through inflammation (i.e. the third assumption of no pleiotropy). However, the fact that the CRP-GRS–colorectal cancer association was only slightly attenuated after adjustment for plasma CRP suggests that CRP-GRS has some pleiotropic effects and may influence colorectal cancer development not only through plasma CRP but through some other pathways. It is not possible to account for all of these pathways because the functions of most SNPs constituting CRP-GRS are unknown. Alternatively, the association between CRP-GRS and colorectal cancer risk may be due to chance.

Another limitation of our study is low power for subgroup analyses; this precluded our examining associations in African Americans. An additional limitation is that plasma CRP was measured only once, although studies reported good tracking of CRP over multiple years with a reliability coefficient for serum CRP concentration 0.59 [3, 63, 64]. If misclassification occurred, it would most likely result in attenuation of circulating CRP–cancer associations [64, 65].

Although our findings do not have immediate clinical applications, this study is a step towards understanding the role of inflammation in cancer etiology. It provides support to the hypothesis of a causative role of low-grade systemic inflammation in colorectal cancer carcinogenesis. In addition, the study underlines that using a genetic risk score has advantages over a single plasma CRP measurement and over individual polymorphisms, because it provides more information about inflammation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Associations between the CRP-GRS, inflammation and incident colorectal cancer: Mendelian randomization analysis. CRP-GRS is used as an instrumental variable for studying the role of inflammation (reflected by plasma CRP) in colorectal cancer development.

Table 1

Association between 20 SNPs affecting plasma CRP concentrations in GWAS [25, 26] and plasma CRP concentrations among Whites, ARIC, 1996–98.

Gene	SNP	Chromosome	CRP-raising allele in ARIC	Other allele for CRP	GWAS Beta coefficient ^a (SE)	ARIC Beta coefficient ^{a,b} (SE)	P-value
1. <i>APOC1</i>	rs4420638	19	A:0.83	G	0.24 (0.009)	0.25 (0.02)	<.0001
2. <i>CRP</i>	rs2794520	1	C:0.67	T	0.16 (0.006)	0.15 (0.02)	<.0001
3. <i>HNF1A</i>	rs1183910	12	G:0.68	A	0.15 (0.006)	0.12 (0.02)	<.0001
4. <i>SALL1</i>	rs10521222	16	C:0.97	T	0.10 (0.015)	0.24 (0.07)	0.0009
5. <i>LEPR</i>	rs4420065	1	C:0.62	T	0.09 (0.005)	0.06 (0.02)	0.002
6. <i>HNF4A</i>	rs1800961	20	C:0.60	T	0.09 (0.015)	0.13 (0.05)	0.009
7. <i>IL6R</i>	rs4129267	1	C:0.60	T	0.08 (0.005)	0.10 (0.02)	<.0001
8. <i>GCKR</i>	rs1260326	2	T:0.41	C	0.07 (0.005)	0.13 (0.02)	<.0001
9. <i>PPPIR3B</i>	rs9987289	8	A:0.08	G	0.07 (0.011)	-0.01 (0.03)	0.81
10. <i>BCL7B</i>	rs13233571	7	C:0.88	T	0.05 (0.009)	0.06 (0.03)	0.02
11. <i>IL1F10</i>	rs6734238	2	G:0.59	A	0.05 (0.006)	0.05 (0.02)	0.008
12. <i>NLRP3</i>	rs12239046	1	C:0.63	T	0.05 (0.006)	0.02 (0.02)	0.39
13. <i>PABPC4</i>	rs12037222	1	A:0.23	G	0.05 (0.007)	0.04 (0.02)	0.06
14. <i>IRF1</i>	rs4705952	5	G:0.75	A	0.04 (0.007)	0.03 (0.02)	0.12
15. <i>ASCL1</i>	rs10745954	12	A:0.51	G	0.04 (0.006)	0.04 (0.02)	0.03
16. <i>GPRC6A</i>	rs6901250	6	A:0.32	G	0.04 (0.006)	-0.003 (0.02)	0.88
17. <i>PSMGI</i>	rs2836878	21	G:0.70	A	0.03 (0.006)	0.04 (0.02)	0.05
18. <i>RGS6</i>	rs4903031	14	G:0.21	A	0.03 (0.007)	0.08 (0.02)	0.0002
19. <i>RORA</i>	rs340029	15	T:0.61	C	0.03 (0.006)	0.03 (0.02)	0.12
20. <i>PTPN2</i>	rs2847281	18	A:0.60	G	0.03 (0.006)	-0.01 (0.02)	0.45

^a Beta coefficient represents difference in log transformed concentration of CRP (mg/l) per each additional risk allele.

^b Adjusted for age, sex, and center

Table 2

Hazard ratios (HR) of incident total, colorectal, lung, prostate, and breast cancers per standard deviation of CRP genetic risk score among Whites, ARIC, 1987–2006.

Cancer	No. of cancers	Person-years	HR (95% CI)	
			Model 1 ^a	Model 2
Total cancer	1929	137,320	0.99 (0.94–1.03)	0.99 (0.95–1.04) ^{b,c}
Colorectal cancer	205	146,737	1.18 (1.02–1.35)	1.19 (1.03–1.37) ^{b,c}
Lung cancer	274	147,365	0.97 (0.87–1.08)	0.99 (0.87–1.11) ^{b,c}
Prostate cancer	395	67,083	0.95 (0.86–1.05)	0.94 (0.86–1.04) ^b
Breast cancer	368	75,536	1.03 (0.93–1.14)	0.99 (0.85–1.15) ^{b,d}

^aModel 1: Adjusted for age, sex (but prostate and breast cancers), and ARIC center

^bModel 2: Adjusted for age, ARIC center, education, BMI, waist, aspirin use, smoking status, and pack-years of smoking

^cAdditionally adjusted for sex-hormone therapy

^dAdditionally adjusted for hormone therapy use, menopausal status, age at menarche and number of live births

Table 3

Mean value or prevalence of characteristics across quartiles of plasma C-reactive protein (CRP) in 7,603 Whites free of cancer at Visit 4 (1996–98), ARIC

Characteristics Mean (SD) or frequency (%)	CRP (mg/L) quartiles			
	<1.1 n=1900	1.1–2.4 n=1901	2.5–5.6 n=1901	>5.6 n=1901
Age (SD) (y)	62.7 (5.8)	62.9 (5.6)	62.8 (5.6)	62.9 (5.5)
Sex (% Female)	41.5	45.4	56.7	67.3
BMI (SD) (kg/m ²)	25.9 (3.9)	27.9 (4.5)	29.0 (5.1)	30.7 (6.2)
Waist (SD) (cm)	95.1 (11.7)	100.3 (12.4)	103.3 (13.7)	107.1 (15.6)
>High school education ^a , (%)	55.4	50.5	46.4	42.9
Current smokers, (%)	10.5	12.2	15.1	17.3
40 pack-years of smoking, (%)	16.7	21.0	23.6	25.8
Aspirin, current use (%)	54.7	56.8	59.3	64.7
Current hormone therapy, (% of women)	29.2	34.7	45.9	59.2
Menopausal status (% of women)	91.8	91.7	91.9	92.4
Age at menarche ^a (SD) (for women)	13 (1.7)	12.9 (1.6)	12.9 (1.5)	12.8 (1.6)
Number of live births ^a (SD) (for women)	2.8 (1.5)	3.0 (1.6)	3.0 (1.6)	3.0 (1.5)

^aReported at Visit 1, 1987–89. All other variables were reported at Visit 4

Hazard ratios (HR) of incident total, colorectal, lung, prostate, and breast cancers in relation to log transformed C-reactive protein (CRP) among Whites, ARIC, 1996–2006

Table 4

Cancer	No. of cases	Person-years	HR (95% CI) per 1 unit of log CRP	
			Full analytical cohort	Excluding follow-up 2 y
Total cancer ^{a,b}	1159	64,281	1.08 (1.01–1.15)	1.05 (0.98–1.13)
Colorectal cancer ^{a,b}	120	67,880	1.24 (1.01–1.51)	1.13 (0.91–1.41)
Lung cancer ^{a,b}	156	68,036	1.29 (1.08–1.54)	1.13 (0.92–1.39)
Prostate cancer ^a	253	30,496	0.94 (0.81–1.09)	0.93 (0.79–1.11)
Breast cancer ^{a,c}	176	35,888	1.27 (1.07–1.51)	1.40 (1.16–1.70)

^a Adjusted for age, ARIC center, education, BMI, waist, aspirin use, smoking status, and pack-years of smoking

^b Additionally adjusted for sex-hormone therapy use.

^c Additionally adjusted for hormone therapy use, menopausal status, age at menarche, and number of live births

Mendelian randomization approach: standard and instrumental probit model analyses of plasma CRP and cancer using CRP-GRS as an instrumental variable

Table 5

	No. of cases ^a	Standard probit regression ^b		Instrumental-variable probit regression ^b	
		Beta coefficient (95% CI) per 1 unit of log CRP	P-value	Beta coefficient (95% CI) per 1 unit of log CRP ^c	P-value
Colorectal cancer	105	0.08 (−0.003, 0.17)	0.06	0.44 (0.12, 0.75)	0.006
Total cancer	997	0.03 (−0.01, 0.07)	0.16	0.003 (−0.18, 0.19)	0.98

^aThe number of cases is lower than in Tables 2 and 4 because the sample for this analysis included participants with data on plasma CRP at Visit 4 and on CRP-GRS.

^bAdjusted for age, ARIC center, sex-hormone therapy use, education, BMI, waist, aspirin use, smoking status, and pack-years of smoking

^cChange per unit increase in log CRP using the CRP-GRS as an instrument for plasma CRP