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Research Article

Association of *CRP* genetic variants with blood concentrations of C-reactive protein and colorectal cancer risk

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Abbreviations: CRP, C-reactive protein; EPIC, European Prospective Investigation into Cancer and Nutrition; SNP, single nucleotide polymorphism; MR, Mendelian Randomization

Novelty and Impact: Positive associations between blood concentrations of the inflammatory marker C-reactive protein (CRP) and risk of colorectal cancer have been observed in several large prospective studies, but these observations may be influenced by bias and it is unknown whether they reflect a causal relationship. We observed that genetically determined higher CRP concentrations were associated with higher risk of colorectal cancer, supporting the hypothesis that elevated CRP is directly involved in colorectal carcinogenesis.

ABSTRACT

High blood concentrations of C-reactive protein (CRP) have been associated with elevated risk of colorectal cancer in several prospective studies including the European Prospective Investigation into Cancer and Nutrition (EPIC), but it is unknown whether these observations reflect a causal relationship. We aimed to investigate whether *CRP* genetic variants associated with lifelong higher CRP concentrations translate into higher colorectal cancer risk. We conducted a prospective nested case-control study within EPIC including 727 cases diagnosed between 1992 and 2003 and 727 matched controls selected according to an incidence-density sampling protocol. Baseline CRP concentrations were measured in plasma samples by a high sensitivity assay. Tagging single nucleotide polymorphisms (SNPs) in the *CRP* gene (rs1205, rs1800947, rs1130864, rs2808630, rs3093077) were identified via HapMap. The causal effect of CRP on colorectal cancer risk was examined in a Mendelian Randomization approach utilizing multiple *CRP* genetic variants as instrumental variables. The SNPs rs1205, rs1800947, rs1130864 and rs3093077 were significantly associated with CRP concentrations and were incorporated in a CRP allele score which was associated with 13% higher CRP concentrations per allele count (95% confidence interval 8%, 19%). Using the CRP-score as instrumental variable, genetically 2-fold higher CRP concentrations were associated with higher risk of colorectal cancer (odds ratio 1.74, 95% confidence interval 1.06, 2.85). Similar observations were made using alternative definitions of instrumental variables. Our findings give support to the hypothesis that elevated circulating CRP may play a direct role in the etiology of colorectal cancer.

INTRODUCTION

High blood concentrations of the inflammatory marker C-reactive protein (CRP) have been associated with moderately elevated risk of colorectal cancer in several prospective studies[1]. Recent data from the European Prospective Investigation into Cancer and Nutrition (EPIC) study showed a positive association between circulating CRP and risk of colon but not rectal cancer, and that the association with colon cancer was independent of a variety of metabolic factors including body mass index (BMI), waist circumference, elevated glycated hemoglobin (HbA1c), elevated C-peptide and reduced high density lipoprotein cholesterol (HDL-C) [2]. However, findings from observational studies relating circulating CRP to risk of colorectal cancer are prone to bias by reverse causation due to inflammatory processes that can originate from occult cancer. Furthermore, CRP concentrations are influenced by a variety of life-style factors such as obesity [3], physical activity [4] and diet [5], that could also influence colorectal cancer risk. Thus, even after adjustment for various potentially confounding factors, as was done in the study in EPIC [2], residual confounding factors may still distort findings. Hence, from standard observational studies it cannot necessarily be assumed that elevated CRP is directly involved in colorectal carcinogenesis. Mendelian Randomization (MR) is an approach to elucidate whether intermediate modifiable traits such as biomarker concentrations are causally related to disease risk. Under the assumption of the random assortment of alleles at gamete formation, genetic variants associated with biomarker levels can be used as relatively unbiased proxies for biomarker concentrations because they are generally unrelated to confounding factors that typically distort findings from conventional epidemiological studies [6] and they cannot be altered by disease occurrence, thereby circumventing reverse causation. The aim of our study was to investigate whether *CRP* genetic variants associated with lifelong differences in CRP concentrations translate into differences in colorectal cancer risk. We first estimated the association of those genetic variants with colorectal

cancer risk and then performed a formal MR approach by instrumental variable analysis to examine the causal association between elevated CRP concentrations and colorectal cancer risk.

METHODS

Study population

This study was conducted using a nested case-control design within the European Prospective Investigation into Cancer and Nutrition (EPIC), a large prospective cohort with more than 520 000 participants from 10 countries, aged 25-70 years at recruitment between 1992 and 2000[7]. All participants gave written informed consent. At recruitment, anthropometric measurements and blood samples were taken and the participants completed questionnaires on medical history, medication, sociodemographic and lifestyle characteristics[7-9]. The EPIC study was approved by the ethics review board of the International Agency for Research on Cancer (Lyon, France) and the local review boards of the participating institutions.

Follow-up procedures

Incident cancer cases were identified through record linkage with regional cancer registries at all study centers except those in Germany, France, Greece, and Naples (Italy), where active follow-up using a combination of methods, including health insurance records, cancer and pathology registries, and direct contact of participants or next-of-kin, was used. For the present study, the closure dates, defined as the latest date of complete follow-up for both cancer incidence and vital status, ranged from December 1999 to June 2003 for study centers using registry data, and from June 2000 to December 2002 for study centers using active follow-up methods.

Nested case-control study

Colorectal cancer was defined based on the International Statistical Classification of Diseases, Injury and Causes of Death (10th Revision) as a combination of tumors of the colon (C18.0-C18.7), tumors that were overlapping or unspecified (C18.8-C18.9), and tumors of the rectum (C19- C20). For location-specific analyses, overlapping and unspecified tumors were grouped among all colon cancers (C18:0–C18:9). A total of 727 incident cases of colorectal cancer (483 colon, 244 rectal) with available blood samples and DNA were included in the present study. This number differs from the study on CRP levels and risk of colorectal cancer (1096 cases) [2] in EPIC, because DNA samples from Denmark were unavailable due to local technical and organizational issues that delayed sample retrieval. Using risk set sampling, for each case one control was randomly selected among participants free of cancer at the time of diagnosis of the index case, matched on sex, age at blood collection (2-months to 4-year intervals), study center, and fasting status (<3, 3-6, or >6 hours). Women were additionally matched on menopausal status (premenopausal, perimenopausal, postmenopausal, or surgically menopausal). Premenopausal women were matched on phase of the menstrual cycle at blood collection (early follicular, late follicular, ovulatory, early luteal, mid-luteal, or late luteal), and postmenopausal women were matched on current use of hormone replacement therapy (yes/no). The latter matching criteria were used because the nested case-control study was designed to be used for several biomarker studies, including studies on hormonal factors in relation to colorectal cancer risk.

Tagging SNP selection and genotyping procedures

A set of tagging single nucleotide polymorphisms (SNPs) was selected to cover variations in the *CRP* gene common to populations of European descent. The tagging SNPs were selected via

HapMap 22/phaseII CEPH (Utah residents with ancestry from northern and western Europe) population data applying stringent criteria (minor allele frequency >5% and pairwise $r^2 \geq 0.80$), according to Tagger software[10] implemented in the Haploview program [11] and from a genome-wide association study [12]. The final list of selected tagging SNPs was: rs1205 (C>T, 3' untranslated region), rs2794520 (C>T, 3' flanking region), rs1800947 (C>G, exon 2), rs1130864 (G>A, 3' untranslated region), rs2808630 (T>C, 3' untranslated region), rs2794521 (T>C, 5' flanking region), rs3093077 (A>C, 3' untranslated region). Some of the selected SNPs have also been identified in more recently published genome-wide association studies on CRP-levels [12-14]. All SNPs have been previously associated with circulating CRP concentrations in epidemiological studies[13, 15-17]. The selected SNPs were genotyped using TaqMan methodology. Genotype call rates were >99.2% for all assays. Of the genotyped SNPs, rs2794520 was in high agreement (kappa=0.99) and linkage disequilibrium ($r^2 \geq 0.80$) with rs1205 and rs2794521 was in high agreement with rs2808630 (kappa=0.99). We omitted rs2794520 and rs2794521 from analysis. We assumed an additive genetic model for all *CRP*-SNP genotypes. Due to the very low number of homozygote variant genotypes in rs1800947 (n=8 with GG genotype) and rs2808630 (n=4 with CC genotype), for these two SNPs the homozygote variants were grouped together with heterozygotes for categorical analyses.

Laboratory analyses

Plasma CRP was measured using a high sensitivity assay (Beckman-Coulter, Woerden, the Netherlands) as described previously[2].

Circulating levels of C-peptide, HbA1c, insulin-like growth factor 1 (IGF-1), total cholesterol, triglycerides, HDL-C, low density lipoprotein cholesterol (LDL-C), total and high-molecular weight adiponectin, leptin, soluble leptin receptor (sOB-R), and 25-hydroxy vitamin D have been determined within separate research projects in the same nested case-control study as described previously [18-21].

Statistical analysis

Hardy-Weinberg Equilibrium was tested in control participants using the Chi-Squared test. Baseline characteristics between cases and controls and between SNP genotypes (among controls) were compared using paired t-test, Wilcoxon signed rank test, or Mc Nemar's test. CRP-values were naturally log-transformed.

To verify whether selected SNPs can be utilized as instrumental variables for MR analysis, the association between selected SNPs and CRP concentrations was quantified using linear regression models with robust variance in controls. Geometric mean CRP concentrations and 95% confidence intervals (95% CI) by genotype and the estimated percent difference in CRP levels per minor allele (with genotypes coded 0, 1 or 2 according to the number of minor alleles) are presented. We created an unweighted CRP-score by counting the alleles of the *CRP* SNPs that were individually associated with higher CRP concentrations. In addition, a weighted allele score was created by summing each genotype multiplied by its estimated coefficient from the linear regression, divided by the sum of weights[22]. The weighted CRP-score was divided in tertiles for categorical analyses.

Using the expectation-maximation algorithm, haplotype frequencies were estimated from the SNPs rs1800947, rs1130864, rs1205, rs2808630 and rs3093077 (JMP Genomics/SAS 9.3). Haplotypes with a frequency lower than 5% were pooled [23]. Haplotype-specific differences in CRP concentrations were estimated among controls using an additive model with the haplotype associated with the highest CRP concentration as reference.

To explore whether individual SNPs, CRP-scores and *CRP* haplotypes are associated with risk of colorectal, colon or rectal cancer, we used conditional logistic regression models controlling for matching variables to estimate odds ratios (OR) that can be interpreted as incidence rate ratios. Sex-specific associations are shown for colorectal cancer, but due to limited sample size, sex-specific associations for colon and rectal cancer are not shown. We also investigated whether adjustment for covariates including smoking, education, alcohol consumption, dietary intake and physical activity influenced the risk estimates. In addition, we examined whether ORs were different after additional adjustment for measured CRP.

Finally, in a formal MR approach, the causal effect of low-grade inflammation reflected by CRP on colorectal cancer risk was quantified by instrumental variable analysis using two-stage least squares regression adjusted for matching factors. The first stage comprised the linear regression of log-transformed CRP concentrations on the genetic instrument, resulting in predicted values of CRP concentrations. The second stage comprised a logistic regression of colorectal cancer on the predicted CRP concentrations. Instrument strength for MR analysis was evaluated using the F-statistic[24] from the first stage regression. An F-value >10 is considered the minimally required instrument strength [24] for unbiased instrumental variable estimation [25]. We used different definitions of instrumental variables to explore the robustness of associations. To compare the association between genetically raised CRP (instrumental variable analysis) and measured CRP

in relation to colorectal cancer, we also present the multivariable adjusted ORs from the conditional logistic regression analysis for the association between measured CRP concentration and risk of colorectal cancer in the previously used dataset with 1096 case-control pairs (previously published with slightly different adjustment) [2], as well as in our data set of 727 case-control pairs. As a sensitivity analysis and to produce results comparable with two recently published MR studies on CRP and cancer[26, 27], we also conducted a MR analysis using probit models. Interpretation of coefficients from probit models is less intuitive than odds ratios from logistic regression approaches. In brief, coefficients from probit regression models indicate the estimated change in the probability of the outcome for a one unit change of the exposure. We computed probit coefficients using standard (measured CRP, multivariable adjusted for matching factors and covariables) and instrumental variable (adjusted for matching factors) probit regression. All reported p-values are two-sided. Instrumental variable analyses were performed using the STATA, Version 12.1 (StataCorp, College Station, Texas, USA). All other analyses were performed using SAS Enterprise Guide, Version 4.3 (SAS Institute Inc., Cary, North Carolina, USA).

RESULTS

Genotype frequencies of all *CRP*-SNPs were consistent with Hardy-Weinberg equilibrium (data not shown). Colorectal cancer cases had a higher BMI and waist circumference than controls, were more often physically inactive and had a higher intake of alcohol, total energy and red and processed meat (Table 1). Compared to controls, cases had lower concentrations of total cholesterol, LDL-C, HDL-C, adiponectin, sOB-R and 25-hydroxy vitamin D. None of the

potentially confounding lifestyle, dietary or metabolic factors differed substantially across *CRP*-SNP genotypes or the CRP-score values (supplemental table 1).

Association between genotype and circulating CRP

The SNPs rs1205 and rs1800947 were associated with lower CRP concentrations per minor allele, while the minor alleles of rs1130864 and rs3093077 were associated with higher CRP concentrations (Table 2). rs2808630 was not associated with CRP. The CRP-score was created by counting the alleles associated with higher CRP concentrations, i.e. the C-alleles of rs1205, rs1800947, and rs3093077, and the A-allele of rs1130864. Each allele count of the unweighted CRP-score was associated with 13% higher CRP, explaining 2% of inter-individual variation in CRP concentrations. The weighted CRP-score was associated with 29% higher CRP per score unit and explained 3% of inter-individual variation.

Five *CRP* haplotypes with a frequency >5% were identified. The haplotype C-G-C-T-C was associated with 128% higher CRP concentrations than haplotype G-G-T-T-A (Supplemental Table 2).

Association between genetic variation in the *CRP* gene and risk of colorectal cancer

The T-allele of rs1205, which was associated with 19% lower CRP, was also associated with lower risk of colorectal cancer (p-trend 0.01) (Table 3). The A-allele of rs11308864, which was associated with 15% higher CRP, was associated with higher risk of colorectal cancer overall (p-trend 0.04) and in women (p-trend 0.04), but not in men. SNPs rs1800947, rs3093077 and rs2808630 were not associated with colorectal cancer risk. Both the unweighted and weighted CRP-scores were positively associated with risk of colorectal and colon cancer and less pronounced with risk of rectal cancer. None of the observed associations was considerably

influenced by adjustment for potentially confounding factors such as education, physical activity, smoking status, alcohol or red and processed meat intake (data not shown). Associations were only weakly attenuated by adjustment for measured CRP concentrations. For example, the highest versus lowest category in the unweighted CRP-score was associated with an OR of colorectal cancer of 1.50 (95% CI 1.01-2.22, p-trend 0.02) before and an OR of 1.45 (95% CI 0.97-2.15, p-trend 0.07) after adjustment for log-transformed CRP concentration. With the haplotype C-G-C-T-C as reference, none of the identified common haplotypes was clearly associated with risk of colorectal, colon or rectal cancer (supplemental Table 3).

Association between circulating CRP and risk of colorectal cancer

In multivariable adjusted conditional logistic regression models, two-fold higher circulating CRP concentration was associated with a moderately higher risk of colorectal cancer (OR 1.06, 95% CI 1.00-1.13) in the full dataset (1096 case-control pairs) (Figure 1). The association was similar in the present dataset of 727 case-control pairs (OR 1.04, 95% CI 0.97, 1.12).

Associations between CRP and risk of colorectal cancer by using instrumental variables

Using the unweighted CRP-score as instrumental variable, genetically 2-fold higher CRP was associated with 74% higher (95% CI 1.06, 2.85) risk of colorectal cancer (Figure 1). With the weighted CRP-score as instrumental variable, 2-fold higher CRP was associated with 57% higher risk of colorectal cancer (95% CI 0.99, 2.48). A 48% higher risk of colorectal cancer (95% CI 0.94, 2.32) was observed when using *CRP* haplotypes as instrumental variables. Using rs1205 (F-value 21.6) as instrumental variable, genetically higher CRP was associated with significantly higher risk of colorectal cancer. SNPs rs1800947, rs1130864, and rs3093077 were of weaker instrument strength (F-values <15), resulting in wider confidence intervals for MR estimates.

Genetically raised CRP through rs1130864 was significantly associated with higher risk of colorectal cancer, while no association was observed with rs1800947 as instrumental variable and -contrary to the expected- a lower risk of colorectal cancer was observed using rs3093077 as instrumental variable. Using the unweighted or weighted CRP-score as instrumental variables, genetically raised CRP was positively associated with colorectal cancer risk in both men and women, as well as with colon and rectal cancer, although confidence intervals were wide for these subgroup analyses (supplemental figure 1).

Associations between CRP and risk of colorectal cancer by using standard and instrumental variable probit regression

In standard probit analysis multivariable adjusted for matching factors and covariables, a one-unit increase in log-transformed CRP was associated with a statistically significantly higher probability of colorectal cancer (beta 0.07, 95% CI 0.01, 0.13). In the instrumental variable probit analysis, significant associations between genetically raised CRP and risk of colorectal cancer were observed with the unweighted CRP-score (beta 0.40, 95% CI 0.09, 0.72), rs1205 (beta 0.56, 95% CI 0.25, 0.88) and rs1130864 (beta 0.57, 95% CI 0.18, 0.96) as instrumental variables.

DISCUSSION

In this study, we observed that the *CRP* SNPs rs1205 and rs1130864 were significantly associated with risk of colorectal cancer in the direction expected from their association with CRP concentrations. In a formal MR approach using multiple genetic variants of the *CRP* gene and a set of alternative instrumental variable definitions, we observed that genetically raised CRP concentrations were associated with higher risk of colorectal cancer, with significant MR estimates for the unweighted CRP-score, rs1205 and rs1130864 as instrumental variables. These

findings give support to the hypothesis that elevated CRP is directly involved in colorectal carcinogenesis.

There is strong evidence for inflammatory processes playing a role in the development of colorectal cancer [28], which is convincingly supported by the observed association between local inflammation of the colorectal mucosa due to inflammatory bowel disease and colorectal cancer risk [29, 30]. Furthermore, there is evidence that reduction of systemic inflammation induced by weight loss also has tissue-specific consequences, i.e. reduced inflammation in the colorectal mucosa [31]. However, as to date no specific mechanism has been suggested to explain how elevated circulating CRP could directly influence colorectal carcinogenesis [32] and experimental studies on the direct effect of CRP on colorectal cancer cells are scarce. Furthermore, studies investigating whether genetic variation in the CRP gene is associated with inflammatory and/or carcinogenic processes beyond CRP concentrations are scarce. There is some suggestion that CRP itself exerts proinflammatory effects[33], although in a recent study the inflammatory status of random colonic biopsies was largely unrelated to circulating CRP[34]. On the other hand, it has been shown that CRP induces the expression of adhesion cells, which is an important step in tumorigenesis [35] and could serve as an alternative causal process.

Standard epidemiological studies relating blood concentrations of CRP to risk of colorectal cancer have not always been consistent. As to date, 7 out of 15 prospective studies published between 2003 and 2012 observed significant positive associations between circulating CRP and risk of colorectal cancer [36, 37]. In a meta-analysis from 2008, CRP concentrations were positively associated with risk of colorectal cancer, with stronger associations for colon than for rectal cancer and stronger associations in men than in women [1]. In the most recent and so far largest study in women, the Women's Health Initiative Observational Study, a modest positive association between baseline CRP concentrations and colorectal cancer risk was observed [36].

We are aware of three MR studies investigating the potentially causal association between CRP concentrations and risk of colorectal cancer[26, 27, 38]. In the first study, common genotype combinations of 4 *CRP*-SNPs (rs1205, rs1130864, rs3093077, and rs3091244) were associated with up to 72% higher CRP concentrations, but not with higher risk of colorectal cancer (197 cases) [38]. In the second study using a CRP-score from two CRP-associated SNPs (rs2794520 of the *CRP* gene and rs1169300 of the *HNF1A* gene) in an instrumental variable probit model, a non-significantly higher probability of colorectal cancer (116 cases, beta per one unit in log-transformed CRP 0.07, 95% CI -0.44, 0.58) was observed [26]. The third and most recently published MR study on the association between CRP and risk of cancer is in line with our findings[27]: In this study a weighted score of 20 CRP-related SNPs located in or near 20 genes including *CRP*, *APOC1*, *HNF1A*, and *LEPR* was significantly associated with risk of colorectal cancer in instrumented probit analysis (105 cases, beta per one unit in log-transformed CRP 0.44, 95% CI 0.12, 0.75). Similar to our observations, the coefficient from standard probit analysis was substantially weaker (beta 0.08, 95% CI -0.003, 0.17) than the instrumental variable probit coefficient. Studies that related *CRP* genetic variation to risk of colorectal cancer without a formal MR approach have not always been consistent[39-41]. While in the so far largest study SNPs in the *CRP* gene including rs1205 were significantly associated with risk of colon cancer (1,574 cases) [40], the SNPs rs1205 and rs1130864 (which were significantly associated with colorectal cancer risk in our study) were not individually associated with risk of colorectal cancer in the Rotterdam Study (189 cases)[39] and the CLUE II cohort (208 cases)[41]. In CLUE II, however, two CRP haplotypes were significantly associated with colorectal cancer risk[41].

Given that our MR study gives support to elevated CRP playing a causal role in the etiology of colorectal cancer, the critical evaluation of the MR assumptions for the instrumental variables approach [42] is particularly important. In our setting, the three assumptions for instrumental

variables were: (1) instrumental variable is associated with CRP concentrations, (2) it is independent of factors that may confound the association between CRP and colorectal cancer, and (3) it is associated with colorectal cancer only through CRP (no pleiotropy, i.e. genetic variants having multiple functions). Regarding the first assumption, four *CRP* SNPs included in our study were significantly associated with CRP concentrations, and all but two (*CRP* haplotypes and rs3903077) instrumental variables had F-values >12, thereby not falling below the "weak instruments" threshold (F-value <10) that is typically applied in MR studies [24]. Weak instrumental variables may produce biased effect estimates when there is confounding in the exposure-disease relationship[25], but the size of bias is inversely related to the F-value [43]. Therefore, the MR estimates based on the CRP-score (F-value >35) can be considered more reliable than the MR estimates based on rs3903077 (F-value 9.1) or the *CRP* haplotypes (F-value 8.1). In terms of the second MR assumption, as expected given the random assortment of alleles at conception, none of the potentially confounding factors varied substantially across CRP SNP genotypes or the CRP scores, suggesting that the second assumption is fulfilled. Regarding the third MR assumption, various potentially mediating metabolic factors including C-peptide, HbA1c, and adipokines did not vary across genotypes, arguing against pleiotropic effects invalidating the MR approach [42]. Furthermore, the risk of unknown pleiotropy is reduced by using multiple genetic instruments [42]. The risk of pleiotropy would have been even lower, if CRP-associated SNPs from different genes would have been taken into account[27]. Although CRP-associated SNPs in other genes were not available in the present study, our approach of including only genetic variants located in the CRP gene yielded similar results as in the study by Prizment et al [27], in which CRP-associated SNPs located in or near 20 different genes were utilized. Nevertheless, we found little attenuation of the association between *CRP* genetic variation and colorectal cancer risk after additional adjustment for measured CRP concentrations,

indicating that the third assumption, also referred to as exclusion restriction assumption [44], may be violated. While the weak attenuation may simply reflect measurement error in the one-time measured CRP concentrations, a violation of the exclusion restriction assumption may result in biased MR estimates and thus we cannot exclude that the instrumental-variable odds ratios overestimated the true association[43, 44].

Our study has some limitations such as the modest sample size leading to wide confidence intervals. Therefore, replication in a larger study is desirable. Especially the systematic examination of potentially differential associations by site and sex will only be feasible in larger settings. MR analysis using genetic instruments with two-stage least squares regression can be biased when both the variance explained by the instruments and the sample size are small [45]. Even though our sample size was limited, the CRP-scores had F-values >35 and explained 2-3% of the inter-individual variance in CRP concentrations. However, we cannot exclude that the association between *CRP* genetic variation and risk of colorectal cancer is due to chance. It is also a limitation of our study is that we were not able to verify whether CRP or *CRP* genetic variation was associated with other biomarkers of inflammation and/or colonic inflammation. Furthermore, it should be noted that we cannot exclude that the here employed *CRP* SNPs are in linkage disequilibrium with other genetic markers that are associated with colorectal cancer risk via a CRP-independent pathway. Population stratification may have confounded our MR estimates, but given the relatively homogenous European study population and the use of multiple genetic variants as instrumental variables this source of bias is rather unlikely[42]. In general, potential bias by geographical differences and/or differential follow-up procedures (e.g. active follow-up versus registry data) is likely small in this nested case-control study, because both cases and controls originate from the same source population with the same geographical background and center-specific follow-up procedures and were matched by study center (in

addition to other matching factors). Nevertheless, the selected control group may not be fully representative to the source population, since per each case one control participant was selected based on matching criteria. However, this mainly results in a loss of precision of risk estimates, and potential bias is likely to be small and expected to be random.[46]

CONCLUSION

This MR study utilizing multiple genetic variants of the *CRP*-gene as instrumental variables gives support to the hypothesis that elevated CRP is directly involved in colorectal carcinogenesis. Given the modest sample size, the results require cautious interpretation, particularly when referring to sex-specific analyses and subtypes of colorectal cancer. Our findings warrant confirmation by larger MR studies and experimental studies investigating a potential mechanism of action for CRP as a direct contributor to colorectal carcinogenesis.

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Figure 1. Estimates of the association of circulating (observational) and genetically raised (instrumental variable analysis) CRP levels with risk of colorectal cancer

* OR per 2-fold higher CRP concentration on the original scale corresponds to a difference in log-transformed CRP concentrations of log 2.

† Observational OR (95% CI) from conditional logistic regression adjusted for smoking status (never, former, current or missing data), education (no school degree/primary school, technical/professional school, secondary school, university degree, or missing data), alcohol consumption (nondrinker or g/day), and physical activity (inactive, moderately inactive, moderately active, active, or missing data), body mass index and waist circumference

‡ Instrumental variable analysis by 2-stage least-squares regression, adjusted for matching factors

F-values (indicator of instrument strength) derived from first-stage regression.

rs2808630 not displayed because of insufficient instrument strength (F-value=0.46)

	Ca/Co	F-value	OR (95% CI)*
Observed (full dataset)†	1096/1096	-	1.06 (1.00, 1.13)
Observed (present dataset)†	727/727	-	1.04 (0.97, 1.12)
Instrumental variable analysis ‡			
unweighted CRP-score	721/726	35.1	1.74 (1.06, 2.85)
weighted CRP-score	721/726	38.4	1.57 (0.99, 2.48)
CRP Haplotypes	727/727	8.1	1.48 (0.94, 2.32)
rs1205	721/725	21.6	2.37 (1.22, 4.60)
rs1800947	720/726	12.9	1.13 (0.54, 2.34)
rs1130864	720/725	13.2	2.40 (1.03,5.57)
rs3093077	721/722	9.1	0.59 (0.22, 1.59)

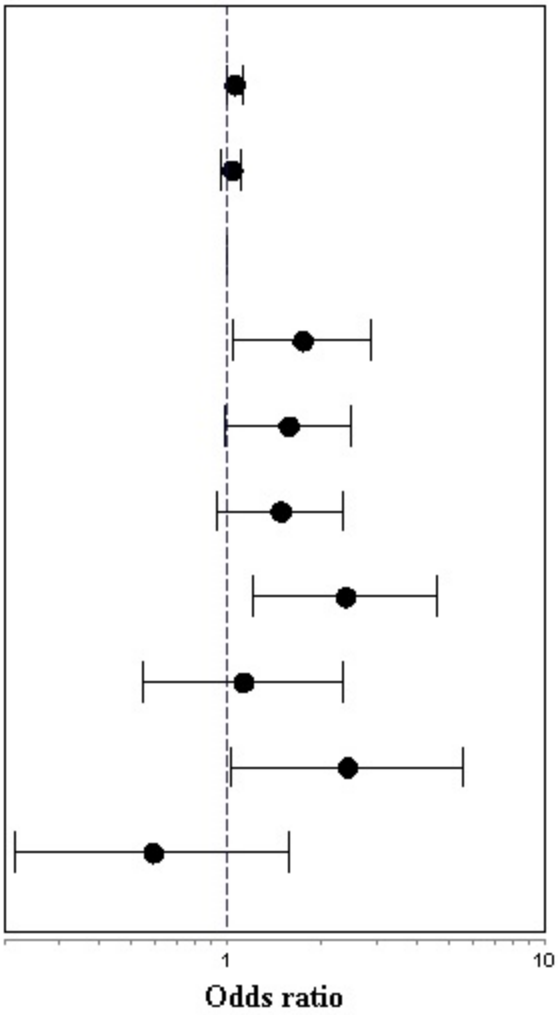

Table 1. Baseline characteristics of incident colorectal cancer cases and matched controls (n=1,454)

	Controls (n=727)	Cases (n=727)	p-value
Female sex, n (%)	396 (54.5)	396 (54.5)	*
Age, years, mean (SD)	58.9 (7.9)	59.0 (7.9)	*
Current smoking, n (%)	150 (20.6)	138 (19.0)	0.41
University degree, n (%)	119 (16.4)	115 (15.8)	0.76
Physically inactive, n (%)	75 (10.3)	101 (13.9)	0.02
Height, cm, mean (SD)	166 (8.9)	167 (9.2)	0.02
Body mass index, kg/m2, mean (SD)	26.4 (3.8)	27.1 (4.3)	0.002
Waist circumference, cm, mean (SD)	88.6 (11.9)	90.6 (13.1)	0.0002
Alcohol intake, g/day, median (IQR)	5.84 (0.89-16.10)	6.15 (0.76-19.89)	0.11
Fiber, g/day, median (IQR)	21.89 (17.74-26.82)	21.76 (17.16-27.38)	0.73
Energy, kcal/day, median (IQR)	1952 (1608- 2401)	2051 (1641- 2490)	0.05
Fruits and vegetables, g/day, median (IQR)	397.1 (259.7-566.2)	383.2 (258.9-553.6)	0.58
Red and processed meat, g/day, median (IQR)	69.81 (45.22-102.4)	71.99 (46.55-106.5)	0.28
Fish, g/day, median (IQR)	21.91 (10.19-39.64)	20.29 (9.86-35.74)	0.21
CRP, mg/L, median (IQR)	2.25 (1.07- 4.36)	2.86 (1.12- 5.20)	0.01
C-peptide (ng/mL), median (IQR)	3.50 (2.68- 5.09)	3.75 (2.80- 5.31)	0.29
HbA1c (%), median (IQR)	5.70 (5.50- 6.00)	5.80 (5.50- 6.10)	0.18
IGF1 (ng/mL), median (IQR)	211.9 (170.5-262.7)	214.4 (170.5-262.2)	0.80
Total cholesterol (mmol/L), median (IQR)	6.39 (5.60- 7.19)	6.21 (5.49- 6.94)	0.02
LDL cholesterol (mmol/L), median (IQR)	4.35 (3.70- 5.16)	4.23 (3.58- 4.87)	0.02
HDL cholesterol (mmol/L), median (IQR)	1.34 (1.14- 1.61)	1.31 (1.08- 1.58)	0.004
Triglycerides (mmol/L), median (IQR)	1.40 (0.96- 2.00)	1.41 (0.97- 2.10)	0.22
Total adiponectin (µg/mL), median (IQR)	6.59 (4.90- 9.01)	6.16 (4.48- 8.51)	0.002
HMW adiponectin (µg/mL), median (IQR)	3.30 (2.14- 4.99)	3.27 (2.01- 4.83)	0.31
Leptin (ng/mL), median (IQR)	9.25 (4.29-19.80)	9.59 (4.76-18.20)	0.41

Soluble Leptin Receptor (ng/mL), median (IQR)	21.40 (17.60-26.40)	20.20 (16.30-24.50)	<0.0001
25-hydroxy vitamin D (nmol/L), median (IQR)	59.20 (44.30-78.00)	55.65 (39.90-71.90)	0.001

SD, standard deviation, IQR, inter-quartile range, HMW, high-molecular weight

P-values for the difference between cases and controls were determined by Mc Nemar's test for variables expressed as %, by student's paired t-test for variables expressed as means, and by Wilcoxon’s signed rank test for variables expressed as medians

* matching variable

Table 2. Association between CRP polymorphisms, CRP-genetic score and CRP concentration among controls (n=727)

SNP	MAF	n	Mean (95% CI), mg/L
rs1205[a]	0.34		
CC		302	2.47 (2.20; 2.77)
CT		342	2.04 (1.81; 2.29)
TT		81	1.71 (1.39; 2.10)
p-trend			0.001
Percent difference in CRP per T-allele			-19% (-30%; -8%)
rs1800947[a]	0.07		
CC		633	2.24 (2.07; 2.44)
CG/GG		93	1.68 (1.37; 2.06)
p-trend			0.004
Percent difference in CRP per G-allele			-30% (-51%; -10%)
rs1130864[a]	0.31		
GG		346	2.01 (1.80; 2.25)
GA		317	2.20 (1.96; 2.48)
AA		62	2.91 (2.30; 3.67)
p-trend			0.01
Percent difference in CRP per A-allele			15% (3%; 26%)
rs2808630	0.27		
TT		386	2.28 (2.05; 2.53)
CT		279	1.94 (1.71; 2.19)
CC		58	2.60 (1.96; 3.44)
p-trend			0.61
Percent difference in CRP per C-allele			-3% (-15%; 9%)
rs3093077[a]	0.07		
AA		621	2.06 (1.89; 2.24)

AC/CC	101	2.91 (2.40; 3.53)
p-trend		0.002
Percent difference in CRP per C-allele		32% (12%; 53%)
Unweighted CRP-score		
0 or 1	24	1.47 (0.98; 2.20)
2	80	1.69 (1.39; 2.06)
3	157	1.79 (1.51; 2.12)
4	222	2.41 (2.09; 2.79)
5	153	2.19 (1.86; 2.58)
6 or 7	90	3.05 (2.54; 3.67)
p-trend		<0.0001
Percent difference in CRP per score unit		13% (8%; 19%)
Weighted CRP-score		
Tertile 1	261	1.73 (1.53; 1.96)
Tertile 2	190	2.25 (1.92; 2.64)
Tertile 3	275	2.59 (2.30; 2.92)
p-trend		<0.0001
Percent difference in CRP per score unit		29% (18%; 40%)

MAF=Minor allele frequency in controls, CRP, C-reactive protein

[a] incorporated in CRP-score

Models were unadjusted.

Table 3. Association between CRP genetic variation and risk of colorectal cancer (n=1,454)

CRP genotype	% difference in CRP[a]	Colorectal cancer			Men			Women			Colon cancer			Rectal cancer		
		Ca/Co	OR	(95% CI)	Ca/Co	OR	(95% CI)	Ca/Co	OR	(95% CI)	Ca/Co	OR	(95% CI)	Ca/Co	OR	(95% CI)
rs1205																
CC	0	358/302	1.00	(Ref.)	161/129	1.00	(Ref.)	197/173	1.00	(Ref.)	241/201	1.00	(Ref.)	117/101	1.00	(Ref.)
CT	-21	292/342	0.74	(0.60-0.92)	131/165	0.64	(0.46-0.89)	161/177	0.83	(0.62-1.10)	201/227	0.75	(0.58-0.98)	91/115	0.71	(0.49-1.04)
TT	-44	71/81	0.76	(0.53-1.08)	37/35	0.84	(0.50-1.41)	34/46	0.67	(0.41-1.10)	38/54	0.59	(0.37-0.93)	33/27	1.11	(0.63-1.97)
p-trend				0.01			0.09			0.07			0.01			0.60
per T allele	-19		0.82	(0.70-0.96)		0.82	(0.65-1.03)		0.82	(0.67-1.01)		0.76	(0.63-0.93)		0.93	(0.72-1.21)
rs1800947																
CC	0	636/633	1.00	(Ref.)	289/286	1.00	(Ref.)	347/347	1.00	(Ref.)	426/429	1.00	(Ref.)	210/204	1.00	(Ref.)
CG/GG	-35	84/93	0.91	(0.67-1.23)	39/44	0.88	(0.56-1.37)	45/49	0.93	(0.61-1.42)	54/54	1.02	(0.69-1.51)	30/39	0.75	(0.46-1.24)
p-trend				0.77			0.75			0.92			0.78			0.41
per G allele	-30		0.96	(0.72-1.28)		0.93	(0.61-1.42)		0.98	(0.66-1.45)		1.05	(0.73-1.53)		0.82	(0.52-1.31)
rs1130864																
GG	0	315/346	1.00	(Ref.)	154/154	1.00	(Ref.)	161/192	1.00	(Ref.)	209/226	1.00	(Ref.)	106/120	1.00	(Ref.)
GA/AA	13	405/379	1.17	(0.94-1.44)	174/175	0.97	(0.71-1.34)	231/204	1.35	(1.01-1.79)	271/257	1.14	(0.88-1.49)	134/122	1.21	(0.84-1.74)
p-trend				0.04			0.42			0.04			0.14			0.14
per A allele	15		1.19	(1.01-1.40)		1.10	(0.87-1.41)		1.26	(1.01-1.57)		1.16	(0.95-1.41)		1.25	(0.93-1.67)
rs2808630																
TT	0	370/386	1.00	(Ref.)	171/175	1.00	(Ref.)	199/211	1.00	(Ref.)	245/265	1.00	(Ref.)	125/121	1.00	(Ref.)
CT	-18	279/279	1.04	(0.84-1.29)	125/131	0.99	(0.72-1.35)	154/148	1.09	(0.81-1.47)	186/181	1.10	(0.85-1.44)	93/98	0.93	(0.64-1.36)
CC	12	70/58	1.26	(0.86-1.85)	32/23	1.45	(0.83-2.52)	38/35	1.11	(0.65-1.89)	48/35	1.53	(0.95-2.46)	22/23	0.88	(0.46-1.68)

p-trend				0.30			0.38			0.54			0.10			0.63
per C allele	-30		1.09	(0.93-1.27)		1.11	(0.88-1.39)		1.07	(0.86-1.33)		1.18	(0.97-1.43)		0.94	(0.71-1.23)
rs3093077																
AA	0	634/621	1.00	(Ref.)	288/285	1.00	(Ref.)	346/336	1.00	(Ref.)	418/412	1.00	(Ref.)	216/209	1.00	(Ref.)
AC/CC	29	87/101	0.84	(0.62-1.15)	41/41	0.97	(0.60-1.57)	46/60	0.76	(0.51-1.14)	62/70	0.86	(0.59-1.26)	25/31	0.79	(0.46-1.37)
p-trend				0.29			0.90			0.21			0.47			0.41
per C allele	32		0.85	(0.63-1.15)		0.97	(0.60-1.57)		0.78	(0.53-1.15)		0.88	(0.61-1.25)		0.79	(0.46-1.37)
unweighted CRP-score																
0 or 1 or 2	0	93/104	1.00	(Ref.)	48/44	1.00	(Ref.)	45/60	1.00	(Ref.)	54/67	1.00	(Ref.)	39/37	1.00	(Ref.)
3,4	23	342/379	1.02	(0.75-1.39)	154/181	0.84	(0.54-1.31)	188/198	1.26	(0.81-1.96)	233/251	1.17	(0.79-1.73)	109/128	0.83	(0.50-1.37)
5	25	165/153	1.20	(0.84-1.70)	71/68	1.01	(0.60-1.70)	94/85	1.43	(0.88-2.33)	108/100	1.34	(0.86-2.11)	57/53	1.01	(0.57-1.77)
6 or 7	46	121/90	1.50	(1.01-2.22)	56/37	1.39	(0.77-2.50)	65/53	1.62	(0.94-2.76)	85/65	1.63	(1.01-2.65)	36/25	1.32	(0.66-2.66)
p-trend				0.02			0.12			0.06			0.03			0.28
per score unit	13		1.08	(1.00-1.17)		1.08	(0.96-1.22)		1.09	(0.98-1.21)		1.10	(1.00-1.21)		1.06	(0.93-1.21)
weighted CRP-score																
Tertile 1	0	226/261	1.00	(Ref.)	111/124	1.00	(Ref.)	115/137	1.00	(Ref.)	144/166	1.00	(Ref.)	82/95	1.00	(Ref.)
Tertile 2	23	187/190	1.14	(0.86-1.49)	79/86	1.05	(0.70-1.58)	108/104	1.21	(0.84-1.75)	127/128	1.16	(0.83-1.62)	60/62	1.09	(0.68-1.75)
Tertile 3	33	308/275	1.27	(1.00-1.62)	139/120	1.28	(0.89-1.84)	169/155	1.26	(0.91-1.75)	209/189	1.27	(0.94-1.72)	99/86	1.27	(0.84-1.92)
p-trend				0.05			0.17			0.17			0.12			0.25
per score unit	65		1.15	(0.99-1.35)		1.20	(0.95-1.52)		1.11	(0.90-1.37)		1.17	(0.96-1.42)		1.12	(0.86-1.46)

[a] based on unadjusted mixed model

OR, odds ratio; models were conditional logistic regression models, controlled for matching factors, without further adjustment

Supplemental Table 1a. Baseline characteristics by CRP-SNPs rs1205 and rs1800947 in control participants (n=727)

	rs1205				rs1800947		
	CC (n=302)	CT (n=342)	TT (n=81)	p-value	CC (n=633)	CG/GG (n=93)	p-value
Female sex, n (%)	173 (57.3)	177 (51.8)	46 (56.8)	0.34	347 (54.8)	49 (52.7)	0.70
Age, years, mean (SD)	58.9 (7.9)	59.1 (7.8)	58.6 (8.5)	0.85	58.9 (8.0)	59.3 (7.0)	0.63
Current smoking, n (%)	58 (19.2)	71 (20.8)	21 (25.9)	0.41	124 (19.6)	26 (28.0)	0.06
University degree, n (%)	48 (15.9)	58 (17.0)	12 (14.8)	0.87	105 (16.6)	13 (14.0)	0.52
Physically inactive, n (%)	35 (11.6)	36 (10.5)	4 (4.9)	0.22	69 (10.9)	6 (6.5)	0.19
Height, cm, mean (SD)	166 (8.9)	166 (9.0)	166 (9.0)	0.67	166 (9.0)	166 (8.8)	0.80
Body mass index, kg/m2, mean (SD)	26.3 (3.8)	26.4 (3.7)	27.0 (3.9)	0.17	26.4 (3.8)	26.6 (3.8)	0.61
Waist circumference, cm, mean (SD)	88.1 (12.4)	88.6 (11.7)	89.8 (11.0)	0.30	88.5 (11.9)	89.1 (12.0)	0.64
Alcohol intake, g/day, mean (SD)	12.8 (17.8)	12.9 (18.9)	10.7 (16.6)	0.53	12.8 (18.6)	11.7 (15.7)	0.58
Fiber, g/day, mean (SD)	23.0 (7.7)	23.0 (8.0)	21.5 (6.7)	0.22	22.8 (7.8)	23.4 (7.3)	0.51
Energy, kcal/day, mean (SD)	2059 (601)	2074 (677)	1963 (508)	0.45	2056 (609)	2064 (764)	0.91
Fruits and vegetables, g/day, mean (SD)	448 (266)	446 (267)	432 (244)	0.68	443 (261)	460 (286)	0.56
Red and processed meat, g/day, mean (SD)	76.2 (46.8)	77.8 (44.4)	76.9 (47.0)	0.76	77.2 (45.0)	76.4 (50.3)	0.87
Fish, g/day, mean (SD)	30.2 (30.9)	31.0 (30.5)	30.3 (27.9)	0.87	29.6 (29.4)	37.0 (35.6)	0.03
CRP, mg/L, median (IQR)	2.62 (1.35- 5.03)	2.12 (0.96- 4.17)	1.76 (0.83- 3.73)	0.003	2.42 (1.11- 4.63)	1.65 (0.80- 3.38)	0.01
C-peptide (ng/mL), median (IQR)	3.54 (2.60- 5.15)	3.41 (2.69- 5.10)	3.58 (2.73- 4.62)	0.96	3.51 (2.65- 5.05)	3.44 (2.76- 5.10)	0.98
HbA1c (%), median (IQR)	5.70 (5.50- 6.10)	5.70 (5.50- 6.00)	5.70 (5.50- 6.00)	0.81	5.70 (5.50- 6.00)	5.70 (5.60- 6.10)	0.66
IGF1 (ng/mL), median (IQR)	214.8 (165.5-274.2)	214.5 (175.8-261.9)	193.1 (173.2-241.9)	0.68	213.8 (170.7-261.6)	205.9 (169.8-273.2)	0.70
Total cholesterol (mmol/L), median (IQR)	6.45 (5.66- 7.25)	6.31 (5.57- 7.15)	6.49 (5.74- 7.05)	0.41	6.39 (5.60- 7.22)	6.37 (5.63- 7.02)	0.82
LDL cholesterol (mmol/L), median (IQR)	4.45 (3.82- 5.28)	4.31 (3.67- 5.02)	4.28 (3.66- 5.00)	0.14	4.36 (3.70- 5.18)	4.33 (3.70- 5.00)	0.77
HDL cholesterol (mmol/L), median (IQR)	1.36 (1.15- 1.61)	1.34 (1.13- 1.62)	1.27 (1.09- 1.54)	0.31	1.35 (1.14- 1.61)	1.31 (1.07- 1.65)	0.71
Triglycerides (mmol/L), median (IQR)	1.40 (0.98- 1.92)	1.40 (0.94- 1.99)	1.42 (0.94- 2.42)	0.50	1.41 (0.97- 2.00)	1.30 (0.88- 1.97)	0.50
Total adiponectin (µg/mL), median (IQR)	6.68 (4.98- 9.17)	6.51 (4.90- 8.96)	6.05 (4.65- 9.15)	0.66	6.63 (4.93- 8.99)	6.21 (4.76- 9.09)	0.52
HMW adiponectin (µg/mL), median (IQR)	3.45 (2.31- 5.11)	3.16 (2.11- 4.91)	3.29 (2.12- 4.87)	0.69	3.34 (2.21- 4.98)	3.29 (2.00- 5.07)	0.59
Leptin (ng/mL), median (IQR)	9.25 (4.31-20.10)	8.97 (4.01-18.50)	11.30 (4.86-22.50)	0.57	9.44 (4.44-19.80)	8.03 (3.78-17.25)	0.28
Soluble Leptin Receptor (ng/mL), median (IQR)	21.20 (17.60-26.50)	21.90 (17.80-26.55)	19.65 (16.70-24.30)	0.05	21.20 (17.60-26.40)	21.60 (18.00-26.50)	0.67
25-hydroxy vitamin D (nmol/L), median (IQR)	61.05 (45.90-79.00)	58.50 (41.90-77.60)	55.00 (44.30-72.30)	0.25	59.50 (44.00-78.10)	57.20 (45.90-76.90)	0.80

SD, standard deviation; IQR, inter-quartile range ; CRP, C-reactive protein; , HbA1c, glycated hemoglobin; LDL, low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol; HMW, high-molecular weight

P-values were determined by Chi-Square test for categorical variables, by analysis of variance for variables expressed as means, and by Kruskal-Wallis test for variables expressed as medians

Supplemental Table 1b. Baseline characteristics by CRP-SNPs rs1130864 and rs2808630 in control participants (n=727)

	rs1130864				rs2808630			
	GG (n=346)	GA (n=317)	AA (n=62)	p-value*	TT (n=386)	CT (n=279)	CC (n=58)	p-value
Female sex, n (%)	192 (55.5)	168 (53.0)	36 (58.1)	0.69	211 (54.7)	148 (53.0)	35 (60.3)	0.59
Age, years, mean (SD)	58.9 (8.0)	58.7 (7.8)	59.9 (8.2)	0.65	58.9 (7.8)	58.6 (8.0)	60.4 (8.0)	0.58
Current smoking, n (%)	70 (20.2)	69 (21.8)	11 (17.7)	0.74	80 (20.7)	61 (21.9)	9 (15.5)	0.56
University degree, n (%)	60 (17.3)	49 (15.5)	9 (14.5)	0.75	63 (16.3)	44 (15.8)	11 (19.0)	0.84
Physically inactive, n (%)	31 (9.0)	39 (12.3)	5 (8.1)	0.31	37 (9.6)	31 (11.1)	7 (12.1)	0.74
Height, cm, mean (SD)	166 (9.1)	166 (8.8)	166 (9.0)	0.58	166 (8.7)	166 (9.4)	165 (8.4)	0.64
Body mass index, kg/m2, mean (SD)	26.5 (3.7)	26.4 (3.9)	26.3 (3.5)	0.78	26.5 (3.7)	26.3 (4.0)	26.1 (3.0)	0.31
Waist circumference, cm, mean (SD)	88.5 (11.4)	88.6 (12.5)	88.5 (11.8)	0.92	88.7 (11.5)	88.3 (12.7)	88.1 (10.5)	0.64
Alcohol intake, g/day, mean (SD)	11.7 (17.2)	13.3 (18.4)	14.6 (22.9)	0.16	13.1 (19.2)	12.6 (18.0)	11.3 (12.8)	0.50
Fiber, g/day, mean (SD)	22.7 (7.6)	23.2 (8.1)	21.9 (6.7)	0.89	22.6 (7.6)	23.0 (8.0)	24.2 (7.6)	0.14
Energy, kcal/day, mean (SD)	2015 (606)	2107 (670)	2045 (555)	0.20	2055 (614)	2068 (679)	2036 (501)	0.99
Fruits and vegetables, g/day, mean (SD)	435 (262)	453 (257)	461 (312)	0.33	457 (267)	427 (262)	460 (247)	0.43
Red and processed meat, g/day, mean (SD)	75.4 (42.4)	78.3 (48.7)	81.0 (47.8)	0.29	79.0 (46.1)	76.1 (46.4)	70.6 (38.9)	0.17
Fish, g/day, mean (SD)	29.7 (30.2)	31.1 (30.2)	33.0 (32.7)	0.40	31.1 (29.4)	31.1 (32.8)	24.3 (23.5)	0.28
CRP, mg/L, median (IQR)	2.05 (1.01- 3.91)	2.33 (1.05- 4.74)	2.65 (1.60- 4.75)	0.05	2.39 (1.11- 4.62)	2.01 (0.93- 3.82)	2.87 (1.36- 5.15)	0.06
C-peptide (ng/mL), median (IQR)	3.38 (2.65- 5.09)	3.52 (2.64- 5.00)	3.70 (2.79- 4.38)	0.91	3.60 (2.72- 5.09)	3.25 (2.57- 4.80)	3.94 (2.51- 5.79)	0.24
HbA1c (%), median (IQR)	5.70 (5.50- 5.90)	5.80 (5.60- 6.10)	5.70 (5.40- 6.00)	0.06	5.80 (5.50- 6.00)	5.70 (5.50- 6.00)	5.70 (5.50- 6.05)	0.35
IGF1 (ng/mL), median (IQR)	209.3 (171.6-259.2)	214.5 (167.2-269.0)	210.3 (178.5-284.7)	0.91	211.0 (173.2-262.1)	214.3 (169.8-264.7)	212.9 (156.7-253.5)	0.82
Total cholesterol (mmol/L), median (IQR)	6.39 (5.60- 7.04)	6.39 (5.67- 7.33)	6.34 (5.47- 7.15)	0.56	6.34 (5.58- 7.13)	6.41 (5.59- 7.34)	6.66 (5.76- 7.22)	0.36
LDL cholesterol (mmol/L), median (IQR)	4.34 (3.64- 5.08)	4.39 (3.80- 5.22)	4.28 (3.89- 5.14)	0.49	4.26 (3.70- 5.03)	4.44 (3.69- 5.22)	4.67 (4.05- 5.37)	0.11
HDL cholesterol (mmol/L), median (IQR)	1.33 (1.17- 1.61)	1.36 (1.13- 1.63)	1.31 (1.08- 1.56)	0.42	1.33 (1.11- 1.59)	1.38 (1.17- 1.62)	1.34 (1.18- 1.61)	0.26
Triglycerides (mmol/L), median (IQR)	1.40 (0.95- 2.05)	1.42 (0.96- 2.00)	1.40 (0.99- 1.77)	0.86	1.40 (0.96- 1.99)	1.40 (0.95- 2.02)	1.48 (1.01- 2.02)	0.83
Total adiponectin (µg/mL), median (IQR)	6.65 (4.96- 9.15)	6.46 (4.80- 8.84)	6.97 (5.47- 8.82)	0.41	6.43 (4.87- 8.91)	6.70 (4.96- 9.09)	6.19 (4.83- 8.95)	0.56
HMW adiponectin (µg/mL), median (IQR)	3.29 (2.15- 5.03)	3.23 (2.06- 4.87)	3.59 (2.57- 5.15)	0.26	3.29 (2.13- 4.88)	3.36 (2.26- 5.07)	3.18 (2.04- 4.56)	0.59
Leptin (ng/mL), median (IQR)	9.74 (4.47-21.70)	9.00 (3.94-18.50)	9.71 (5.23-18.20)	0.67	9.44 (4.50-20.10)	9.10 (4.12-18.10)	9.06 (3.71-24.10)	0.71
Soluble Leptin Receptor (ng/mL), median (IQR)	21.00 (17.80-26.20)	21.80 (17.60-26.70)	21.10 (15.80-27.00)	0.70	21.00 (17.20-25.80)	22.10 (18.00-26.50)	20.75 (17.60-26.50)	0.22
25-hydroxy vitamin D (nmol/L), median (IQR)	57.60 (43.80-75.10)	60.85 (43.50-79.85)	61.40 (47.30-81.60)	0.49	58.00 (42.10-78.50)	58.90 (46.40-75.50)	63.65 (46.30-80.80)	0.28

SD, standard deviation; IQR, inter-quartile range; CRP, C-reactive protein; , HbA1c, glycated hemoglobin; LDL, low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol; HMW, high-molecular weight

P-values were determined by Chi-Square test for categorical variables, by analysis of variance for variables expressed as means, and by Kruskal-Wallis test for variables expressed as medians

Supplemental Table 1c. Baseline characteristics by CRP-SNPs rs3093077 (n=727)

	rs3093077		
	AA (n=621)	AC/CC (n=101)	p-value
Female sex, n (%)	336 (54.1)	60 (59.4)	0.32
Age, years, mean (SD)	59.1 (8.1)	58.1 (6.7)	0.23
Current smoking, n (%)	134 (21.6)	16 (15.8)	0.19
University degree, n (%)	95 (15.3)	21 (20.8)	0.16
Physically inactive, n (%)	62 (10.0)	12 (11.9)	0.56
Height, cm, mean (SD)	166 (9.0)	165 (8.5)	0.53
Body mass index, kg/m2, mean (SD)	26.5 (3.7)	26.1 (4.1)	0.35
Waist circumference, cm, mean (SD)	88.8 (11.9)	86.6 (11.9)	0.10
Alcohol intake, g/day, mean (SD)	13.0 (18.7)	11.5 (15.7)	0.45
Fiber, g/day, mean (SD)	22.9 (7.8)	22.5 (7.6)	0.59
Energy, kcal/day, mean (SD)	2067 (638)	1987 (583)	0.24
Fruits and vegetables, g/day, mean (SD)	445 (264)	450 (269)	0.87
Red and processed meat, g/day, mean (SD)	77.3 (46.1)	75.2 (42.0)	0.68
Fish, g/day, mean (SD)	30.6 (30.2)	30.2 (31.0)	0.90
CRP, mg/L, median (IQR)	2.15 (1.03- 4.17)	2.90 (1.52- 5.65)	0.003
C-peptide (ng/mL), median (IQR)	3.52 (2.68- 5.01)	3.28 (2.67- 5.28)	0.92
HbA1c (%), median (IQR)	5.70 (5.50- 6.00)	5.70 (5.50- 6.00)	0.67
IGF1 (ng/mL), median (IQR)	209.0 (169.0- 264.7)	231.9 (187.2- 260.9)	0.22
Total cholesterol (mmol/L), median (IQR)	6.39 (5.65- 7.25)	6.32 (5.55- 6.98)	0.11
LDL cholesterol (mmol/L), median (IQR)	4.37 (3.71- 5.22)	4.17 (3.55- 4.86)	0.08
HDL cholesterol (mmol/L), median (IQR)	1.34 (1.13- 1.62)	1.40 (1.19- 1.61)	0.23
Triglycerides (mmol/L), median (IQR)	1.41 (0.96- 2.02)	1.14 (0.92- 1.78)	0.03
Total adiponectin (µg/mL), median (IQR)	6.57 (4.90- 9.02)	6.87 (5.25- 9.01)	0.42
HMW adiponectin (µg/mL), median (IQR)	3.29 (2.12- 4.99)	3.59 (2.40- 5.09)	0.47
Leptin (ng/mL), median (IQR)	9.25 (4.24-19.05)	9.44 (4.71-21.70)	0.51
Soluble Leptin Receptor (ng/mL), median (IQR)	21.50 (17.60- 26.50)	21.10 (17.80- 26.20)	0.97
25-hydroxy vitamin D (nmol/L), median (IQR)	59.70 (44.50- 78.10)	56.10 (39.70- 75.80)	0.08

SD, standard deviation; IQR, inter-quartile range ; CRP, C-reactive protein; , HbA1c, glycated hemoglobin; LDL, low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol; HMW, high-molecular weight

P-values were determined by Chi-Square test for categorical variables, by analysis of variance for variables expressed as means, and by Kruskal-Wallis test for variables expressed as medians

Supplemental Table 1d. Baseline characteristics by weighted and unweighted CRP-score in control participants (n=727)

	Unweighted CRP-score					Weighted CRP-score			
	0,1, 2 (n=104)	3,4 (n=379)	5 (n=153)	6, 7 (n=90)	p-value	Tertile 1	Tertile 2	Tertile 3	p-value
Female sex, n (%)	60 (57.7)	198 (52.2)	85 (55.6)	53 (58.9)	0.58	137 (52.5)	104 (54.7)	155 (56.4)	0.67
Age, years, mean (SD)	58.9 (8.4)	59.2 (7.8)	58.2 (8.0)	59.3 (7.6)	0.80	58.8 (7.9)	59.6 (8.1)	58.6 (7.8)	0.75
Current smoking, n (%)	27 (26.0)	74 (19.5)	33 (21.6)	16 (17.8)	0.46	62 (23.8)	32 (16.8)	56 (20.4)	0.20
University degree, n (%)	15 (14.4)	66 (17.4)	21 (13.7)	16 (17.8)	0.68	43 (16.5)	30 (15.8)	45 (16.4)	0.98
Physically inactive, n (%)	4 (3.8)	43 (11.3)	18 (11.8)	10 (11.1)	0.14	23 (8.8)	19 (10.0)	33 (12.0)	0.47
Height, cm, mean (SD)	165 (9.2)	166 (8.8)	166 (9.0)	166 (9.0)	0.76	166 (9.3)	166 (8.5)	166 (8.9)	0.51
Body mass index, kg/m2, mean (SD)	26.9 (4.0)	26.4 (3.5)	26.3 (4.2)	26.2 (3.7)	0.21	26.6 (3.8)	26.4 (3.5)	26.3 (3.9)	0.39
Waist circumference, cm, mean (SD)	89.3 (11.5)	88.7 (11.4)	87.9 (13.3)	88.1 (11.9)	0.39	89.2 (11.6)	88.5 (11.6)	88.0 (12.4)	0.23
Alcohol intake, g/day, mean (SD)	11.5 (17.4)	12.7 (18.2)	13.2 (18.1)	13.4 (20.1)	0.45	12.1 (17.5)	12.7 (18.0)	13.3 (19.2)	0.46
Fiber, g/day, mean (SD)	22.1 (7.1)	23.2 (7.9)	23.3 (8.2)	21.8 (7.0)	0.82	22.4 (7.4)	23.8 (8.4)	22.6 (7.6)	0.75
Energy, kcal/day, mean (SD)	1999 (681)	2072 (620)	2082 (660)	2022 (565)	0.81	2035 (638)	2080 (632)	2062 (625)	0.62
Fruits and vegetables, g/day, mean (SD)	435 (256)	448 (262)	451 (260)	437 (292)	0.95	425 (262)	472 (261)	446 (267)	0.36
Red and processed meat, g/day, mean (SD)	74.0 (45.1)	77.7 (43.9)	76.3 (50.9)	79.4 (44.7)	0.56	77.4 (44.3)	76.5 (45.2)	77.2 (47.3)	0.97
Fish, g/day, mean (SD)	30.6 (29.6)	30.2 (29.6)	32.9 (33.6)	28.4 (28.8)	0.95	31.8 (31.3)	27.9 (27.9)	31.2 (31.1)	0.84
CRP, mg/L, median (IQR)	1.85 (0.82- 3.44)	2.19 (1.04- 4.45)	2.33 (1.09- 4.89)	2.91 (1.66- 5.40)	0.0008	1.84 (0.83- 3.65)	2.39 (1.11- 4.74)	2.76 (1.41- 5.46)	<0.0001
C-peptide (ng/mL), median (IQR)	3.44 (2.62- 4.93)	3.52 (2.69- 5.21)	3.27 (2.53- 4.79)	3.71 (2.79- 5.60)	0.35	3.34 (2.70- 4.97)	3.74 (2.71- 5.60)	3.44 (2.59- 4.87)	0.35
HbA1c (%), median (IQR)	5.70 (5.50- 5.90)	5.70 (5.50- 6.00)	5.70 (5.40- 6.00)	5.80 (5.40- 6.10)	0.75	5.70 (5.50- 6.00)	5.80 (5.50- 6.00)	5.70 (5.50- 6.00)	0.41
IGF1 (ng/mL), median (IQR)	193.9 (172.5- 241.9)	214.8 (170.7- 261.6)	219.0 (165.9- 263.3)	214.4 (173.4- 308.0)	0.68	208.8 (174.0- 264.7)	210.5 (163.9- 259.6)	216.7 (167.6- 266.7)	0.61
Total cholesterol (mmol/L), median (IQR)	6.48 (5.69- 7.13)	6.32 (5.60- 7.15)	6.46 (5.68- 7.38)	6.36 (5.56- 7.08)	0.66	6.37 (5.61- 7.05)	6.37 (5.67- 7.25)	6.41 (5.57- 7.21)	0.70
LDL cholesterol (mmol/L), median (IQR)	4.28 (3.67- 5.06)	4.35 (3.69- 5.12)	4.48 (3.75- 5.31)	4.27 (3.87- 5.11)	0.67	4.32 (3.66- 5.00)	4.49 (3.81- 5.29)	4.36 (3.75- 5.17)	0.35

HDL cholesterol (mmol/L), median (IQR)	1.29 (1.14- 1.57)	1.34 (1.14- 1.62)	1.40 (1.20- 1.64)	1.31 (1.11- 1.56)	0.25	1.33 (1.13- 1.62)	1.34 (1.15- 1.62)	1.37 (1.15- 1.61)	0.61
Triglycerides (mmol/L), median (IQR)	1.40 (0.93- 2.35)	1.41 (0.96- 2.01)	1.38 (0.95- 1.94)	1.42 (0.99- 1.78)	0.90	1.41 (0.93- 2.06)	1.44 (0.98- 2.04)	1.38 (0.97- 1.84)	0.60
Total adiponectin (µg/mL), median (IQR)	6.55 (4.78- 9.19)	6.40 (4.89- 8.95)	6.67 (4.93- 9.32)	6.97 (5.35- 8.71)	0.61	6.65 (4.85- 9.08)	6.37 (4.80- 8.97)	6.79 (5.05- 8.84)	0.38
HMW adiponectin (µg/mL), median (IQR)	3.29 (2.08- 5.01)	3.14 (2.09- 4.87)	3.55 (2.42- 5.04)	3.50 (2.38- 5.14)	0.50	3.30 (2.10- 5.03)	3.12 (2.06- 4.67)	3.48 (2.36- 5.04)	0.28
Leptin (ng/mL), median (IQR)	10.15 (4.67-20.90)	9.02 (4.08-19.90)	9.24 (4.31-18.10)	10.55 (4.60-20.80)	0.83	9.70 (4.24-20.10)	8.26 (3.71-19.40)	9.26 (4.44-19.80)	0.82
Soluble Leptin Receptor (ng/mL), median (IQR)	20.15 (16.80-25.20)	21.65 (17.70-26.50)	22.10 (18.30-26.50)	20.40 (16.40-26.30)	0.20	20.90 (17.70-25.70)	21.70 (17.30-26.95)	21.65 (17.80-26.40)	0.71
25-hydrxoy vitamin D (nmol/L), median (IQR)	55.15 (44.50-72.40)	59.70 (42.50-77.80)	59.20 (44.90-78.35)	61.50 (46.70-81.60)	0.63	57.70 (44.30-75.00)	61.00 (42.10-78.80)	59.60 (44.50-78.80)	0.93

SD, standard deviation; IQR, inter-quartile range ; CRP, C-reactive protein; , HbA1c, glycated hemoglobin; LDL, low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol; HMW, high-molecular weight

P-values were determined by Chi-Square test for categorical variables, by analysis of variance for variables expressed as means, and by Kruskal-Wallis test for variables expressed as medians

Supplemental Table 2 Percent difference in CRP associated with frequent CRP haplotypes in controls (n=727)

CRP haplotypes	Estimated frequency (SE)	% difference in plasma CRP (mg/L)	95% CI
C-G-C-T-C	0.07 (0.005)	0.0	(Reference)
C-G-C-C-A	0.28 (0.008)	-71.5	(-116.7, -26.3)
C-G-T-T-A	0.26 (0.008)	-85.0	(-130.7, -39.4)
C-A-C-T-A	0.32 (0.009)	-44.6	(-89.3, 0.1)
G-G-T-T-A	0.06 (0.005)	-128.3	(-188.8, -67.7)
rare haplotypes		-168.2	(-298.9, -37.4)

Models were unadjusted.

Haplotype frequencies were estimated from the CRP SNPs rs1800947, rs1130864, rs1205, rs2808630 and rs3093077.

Supplemental Table 3 Association between frequent CRP haplotypes and risk of colorectal cancer (n=1,454)

		Colorectal cancer		Men		Women		Colon cancer		Rectal cancer	
No. of cases/controls		727/727		331/331		393/393		483/483		244/244	
CRP haplotypes	% difference in CRP[a]	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
C-G-C-T-C	0.0	1.00	(Ref.)	1.00	(Ref.)	1.00	(Ref.)	1.00	(Ref.)	1.00	(Ref.)
C-G-C-C-A	-71.5	1.10	(0.60, 2.01)	0.97	(0.39, 2.45)	1.18	(0.53, 2.63)	1.16	(0.55, 2.44)	0.96	(0.34, 2.74)
C-G-T-T-A	-85.0	0.71	(0.38, 1.30)	0.63	(0.24, 1.65)	0.76	(0.34, 1.68)	0.57	(0.27, 1.23)	1.00	(0.36, 2.83)
C-A-C-T-A	-44.6	1.30	(0.71, 2.37)	1.04	(0.41, 2.64)	1.55	(0.71, 3.42)	1.20	(0.57, 2.53)	1.48	(0.53, 4.14)
G-G-T-T-A	-128.3	0.95	(0.43, 2.11)	0.76	(0.22, 2.62)	1.14	(0.40, 3.27)	1.06	(0.39, 2.91)	0.77	(0.21, 2.88)
rare haplotypes	-168.2	2.58	(0.48, 13.94)	9.92	(0.64, 154.28)	0.76	(0.08, 7.71)	2.22	(0.32, 15.21)	4.21	(0.12, 152.73)

[a] based on unadjusted mixed model

OR, odds ratio; models were conditional logistic regression models, controlled for matching factors, without further adjustment.

Supplemental Figure 1. Estimates of the association of circulating (observational) and genetically raised (instrumental variable analysis) CRP levels with risk of colorectal cancer by sex and cancer subsites

† Observational OR (95% CI) from conditional logistic regression adjusted for smoking status (never, former, current or missing data), education (no school degree/primary school, technical/professional school, secondary school, university degree, or missing data), alcohol consumption (nondrinker or g/day), and physical activity (inactive, moderately inactive, moderately active, active, or missing data), body mass index and waist circumference

‡ Instrumental variable analysis by 2-stage least-squares regression, adjusted for matching factors

F-values (indicator of instrument strength) derived from first-stage regression.

rs2808630 not displayed because of insufficient instrument strength (F-value=0.46)

*OR per 2-fold higher CRP concentration on the original scale corresponds to a difference in log-transformed CRP concentrations of log 2.

	Ca/Co	F-value	OR (95% CI)*
Colorectal cancer, men			
Observed (full dataset)†	551/551	-	1.10 (1.01-1.20)
Observed (present dataset)†	331/331	-	1.06 (0.94-1.18)
Instrumental variable analysis ‡			
unweighted CRP-score	329/330	35.1	1.64 (0.81, 3.32)
weighted CRP-score	329/330	38.4	1.69 (0.89, 3.23)
CRP Haplotypes	331/331	8.1	1.23 (0.67, 2.26)
rs1205	329/329	21.6	2.74 (0.81, 9.28)
rs1800947	328/330	12.9	1.27 (0.27, 6.00)
rs1130864	328/329	13.2	1.53 (0.61, 3.84)
rs3093077	329/326	9.1	1.00 (0.40, 2.46)
Colorectal cancer, women			
Observed (full dataset)†	445/445	-	1.03 (0.94-1.12)
Observed (present dataset)†	396/396	-	1.04 (0.94-1.15)
Instrumental variable analysis ‡			
unweighted CRP-score	392/396	35.1	1.84 (0.91, 3.71)
weighted CRP-score	392/396	38.4	1.46 (0.75, 2.84)
CRP Haplotypes	396/396	8.1	1.56 (0.86, 2.84)
rs1205	392/396	21.6	2.25 (0.99, 5.12)
rs1800947	392/396	12.9	1.05 (0.45, 2.45)
rs1130864	392/396	13.2	4.21 (0.88, 20.28)
rs3093077	392/396	9.1	0.23 (0.02, 2.84)
Colon cancer			
Observed (full dataset)†	696/696	-	1.09 (1.01, 1.18)
Observed (present dataset)†	483/483	-	1.03 (0.94, 1.12)
Instrumental variable analysis ‡			
unweighted CRP-score	480/483	35.1	2.08 (0.99, 4.38)
weighted CRP-score	480/483	38.4	1.76 (0.90, 3.45)
CRP Haplotypes	483/483	8.1	1.32 (0.71, 2.45)
rs1205	480/482	21.6	4.70 (1.33, 16.56)
rs1800947	480/483	12.9	0.90 (0.35, 2.32)
rs1130864	480/483	13.2	2.47 (0.73, 8.28)
rs3093077	480/482	9.1	0.56 (0.11, 2.69)
Rectal cancer			
Observed (full dataset)†	400/400	-	0.99 (0.89, 1.11)
Observed (present dataset)†	244/244	-	1.07 (0.92, 1.25)
Instrumental variable analysis ‡			
unweighted CRP-score	241/243	35.1	1.47 (0.76, 2.84)
weighted CRP-score	241/243	38.4	1.42 (0.76, 2.65)
CRP Haplotypes	244/244	8.1	1.32 (0.74, 2.36)
rs1205	240/243	21.6	1.33 (0.65, 2.73)
rs1800947	240/243	12.9	2.05 (0.46, 9.13)
rs1130864	240/242	13.2	2.45 (0.81, 7.43)
rs3093077	241/240	9.1	0.61 (0.21, 1.83)