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C-reactive protein genotypes and haplotypes, polymorphisms in NSAID-metabolizing enzymes, and risk of colorectal polyps

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Abstract

Introduction—C-reactive protein (CRP) is a non-specific marker of inflammation linked to cardiovascular disease and possibly colon cancer. Polymorphisms in *CRP* have been associated with differential CRP concentrations among healthy adults, with some evidence for functional effects on CRP expression.

Methods—A linkage-disequilibrium-based tagSNP-selection algorithm identified six tagSNPs for Europeans (−821A>G, −390C>T/A 90A>T 838G>C 2043G>A and 4363C>A), defining 6 haplotypes >1% frequency. In a case-control study of adenomatous (n=491) or hyperplastic (n=184) polyps *vs.* polyp-free controls (n=583) we investigated these SNPs in relation to colorectal polyp risk.

Results—Individuals with 838 GC or CC genotypes had a modestly, although not statistically significantly, increased risk of adenomas (OR=1.4 95% CI 0.9-2.1) and a nearly 2-fold increased risk of concurrent adenomas and hyperplastic polyps (OR=2.0 95% CI 1.1-3.6). Increased risk for concurrent adenomas and hyperplastic polyps was also observed for haplotype ACACAC. No other main associations were detected. Risk of adenomas associated with 2043G>A differed with NSAID use. Among NSAID non-users, there was a suggestion that the GA or AA genotypes were associated with decreased risk of adenomas; this was not seen among NSAID users (p-interaction = 0.03). We also observed interactions between *UGT1A1* [TA](7) promoter repeat polymorphism and *CRP* tagSNPs −390C>T/A and 90A>T, in which only the homozygous variant *CRP* genotype was associated with increased adenoma risk among those with the *UGT1A1* 6rpt/6rpt genotype (p-interaction= 0.02 and 0.04 for −390C>T/A and 90A>T, respectively).

Conclusions—These results provide limited support for associations between genetic variation in *CRP* and colorectal polyp risk. The observed interactions should be evaluated further.

Keywords

CRP; UGT; CYP2C9; colorectal cancer; colorectal polyps; NSAIDs; aspirin

Introduction

C-reactive protein (CRP) is a non-specific acute-phase protein secreted by the liver in response to pro-inflammatory cytokines such as IL-6. Elevated CRP levels have been associated with increased risk of colorectal cancer in four prospective studies [1–4], but not

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in two others [5, 6]. Genetic polymorphisms in *CRP* have been associated with changes in CRP serum or plasma concentrations [7–15]. However, several of these polymorphisms are in intronic or untranslated regions of the *CRP* gene and associations with CRP concentrations may be attributable to a linked, but not yet identified, polymorphism.

Non-steroidal anti-inflammatory drugs (NSAIDs), including COX-2 specific NSAIDs (coxibs), have been consistently associated with a reduced risk of colorectal neoplasia (reviewed in [16]) and have been shown in clinical trials to prevent polyp recurrence [17–21]. NSAIDs have also been shown in some, but not all, studies to reduce CRP concentrations (reviewed in [22]). Thus, the reduction of risk in colorectal neoplasia afforded by NSAIDs may be partly through or marked by their effects on CRP concentrations (see Figure).

NSAIDs are primarily metabolized by oxidation or by glucuronidation. The former is achieved primarily by cytochrome P540 2C9 (CYP2C9) [23] and the latter by the UDP-glucoronosyltransferases (UGTs), such as UGT1A1, UGT1A6, UGT1A9, UGT2B4, and UGT2B7 [24]. Polymorphisms in several of these enzymes have been shown to alter drug metabolism [25–29]. Additionally, polymorphisms in *CYP2C9* and *UGT1A6* have been associated with risk of colorectal polyps or cancer and shown to interact with NSAID use in some, but not all, studies [30–33]. Because functional polymorphisms in NSAID-metabolism genes may alter the ability of NSAIDs to reduce CRP concentrations, we hypothesized that polymorphisms in NSAID metabolism may be effect modifiers of the relationship between genetic variation in *CRP* and risk of colorectal neoplasia (see Figure).

We selected tagSNPs to capture common *CRP* haplotypes (≥ 1% haplotype frequency); these haplotypes were analyzed in a colonoscopy-based case-control study of colorectal polyps. We also investigated whether *CRP* genotype or haplotype associations differed by NSAID use or by polymorphisms in genes encoding NSAID-metabolizing enzymes (*UGT*s and *CYP2C9*).

Materials and Methods

Participant recruitment has been previously described [30, 34, 35]. Briefly, adenoma and hyperplastic polyp cases and polyp-free controls were recruited through a large multiclinic gastroenterological practice in the Twin cities area of Minnesota from April 1991-April 1994. Eligibility criteria have been described elsewhere [34]; participants were aged 30-74 years, English-speaking residents of the Twin Cities metropolitan area with no known genetic syndrome associated with increased risk of colon neoplasia and no individual history of cancer (except non-melanoma skin cancer), prior colorectal polyps, or inflammatory bowel disease. Information on use of aspirin or other NSAIDs, diet, physical activity, anthropometrics, demographics, and medical history was obtained via questionnaire. The participation rate for all colonoscoped patients was 68%.

TagSNP selection

The *CRP* coding region and 2KB 5’ and 3’ of the gene was resequenced by the University of Washington-Fred Hutchinson Cancer Research Center Variation Discovery Resource (UW-FHCRC VDR) [36]. TagSNPs were selected using the LD Select algorithm developed by Carlson and colleagues [37] at the UW-FHCRC VDR. However, we used more stringent criteria specifically, a minor allele frequency of 4% (i.e. any variant that occurred twice in the resequencing effort) and an r^2 value of 0.90. This resulted in the selection of six tagSNPs estimated by the Genome Variation Server (http://gvs.gs.washington.edu/GVS/index.jsp) to cover 85% of the variation in the *CRP* locus: −821A>G (rs2794521), −390C>T/A

(rs3091244), 90A>T (rs1417938), 838G>C (L184L, rs1800947), 2043G>A (rs1205), and 4363C>A (rs3093075).

Genotyping

The selected *CRP* tagSNPs were detected by allelic discrimination using the 5' nuclease assay on a 7900HT sequence detection system (Applied Biosystems, Foster City, CA). The 5'nuclease genotyping assays were validated by genotyping 92 individuals by both 5' nuclease assay and RFLP or sequencing. There were no discrepancies between the two assays. The 20μl genotyping reactions contained 1x Taqman Core Reagents (Applied Biosystems), except for −821A>G for which the universal master mix was used, 0.5 units AmpliTaq DNA polymerase, 0.2 units AmpErase UNG, primers, probes, and 4ng genomic DNA. Primers, probes, Mg^{2+} concentrations, and cycling conditions are listed in Table 1. The triallelic polymorphism at −390 (−390C>T/A) was run as a real-time assay and all the other polymorphisms as end-point assays. Positive controls for all the genotypes as well as four negative controls were included on each plate. For quality control purposes, genotyping for 94 randomly selected samples was repeated. There were no discrepancies.

Genotyping of *CYP2C9*, *UGT1A1*, and *UGT1A6* has been described previously [27, 30]. Briefly, polymorphisms in *CYP2C9* and *UGT1A6* were genotyped by restriction fragment length polymorphism and oligonucleotide ligation assay. The *UGT1A1* polymorphism was genotyped by PCR.

Haplotypes

Haplotypes were inferred using proc haplotype in SAS Genetics v.9 (SAS Institute, Cary, NC). All haplotypes predicted to occur with more than 1% frequency were analyzed separately and the haplotypes with lower frequency were grouped together for analyses. The most common haplotype among the controls was used as the referent group.

Statistical Methods

Three cases groups were defined: adenomas (n=477, hyperplastic polyps (n=177, and those with concurrent adenomas and hyperplastic polyps (n=112). Cases with concurrent adenomas and hyperplastic polyps were included in the adenoma and hyperplastic analyses.

Unconditional logistic regression was used to estimate odds ratios (ORs) and corresponding 95% confidence intervals (CI) for the associations between *CRP* genotypes and haplotypes and polyp risk. For SNPs with a minor allele frequency <10%, we grouped the homozygous variant genotypes with the heterozygous genotypes. A logistic regression model using GEE was used to analyze haplotype effects, using the haplotype probabilities as weights and clustering the haplotypes for each individual [38]. Global tests of haplotype associations were calculated using score tests in the genmod procedure. Covariates considered in our models included age, sex, body mass index, dietary intakes of fiber, alcohol, and energy, postmenopausal hormone use, and smoking. Effect modification by NSAID use was evaluated by the inclusion of multiplicative interaction terms in logistic regression models. Because use of NSAIDs may be associated with other known risk factors for colorectal neoplasia, we adjusted our NSAID-interaction analyses for the variables listed above. All statistical analyses were carried out using SAS v.9.

Results

A total of 1719 subjects were recruited into this study, of which 1217 had DNA available for genotyping. Characteristics of the study population have been described previously [30, 34, 35]. Briefly, the study population was mostly Caucasian (97.2%) and tagSNPs were selected

within that group; adenoma cases tended to be older than hyperplastic polyp cases and controls. Both sets of cases were more likely to be male than controls. Genotype frequencies for all polymorphisms were in Hardy-Weinberg Equilibrium among the controls.

Risk of colon polyps associated with *CRP* genotypes and haplotypes are presented in Table 2 and in Supplemental Table S1 online. There were no differences in risk of adenomas or hyperplastic polyps for any of the genotypes, except possibly 838G>C, for which there was a non-significant increase in risk of adenoma (OR 1.4, 95%CI 0.9-2.1) and a marginally significant increased risk of concurrent adenomatous and hyperplastic polyps associated with the C allele (OR 2.0, 95%CI 1.1-3.6). This was no longer significant when Bonferroni correction for multiple testing was applied. Similarly, global tests of the haplotype associations were statistically non-significant (see Tables 2 and S1). There were no associations with adenoma risk observed for any of the individual imputed haplotypes; however the ACACAC haplotype and the grouping of all rare haplotypes were both associated with increased risk of concurrent adenomas and hyperplastic polyps. Multivariate adjustment for BMI, fiber intake, total energy intake, alcohol, hormone use (women), and smoking did not alter odds ratio estimates; thus results adjusted for age and sex are presented. Tests for heterogeneity of odds ratios, using the contrast statement in multinomial regression in SAS (the logistic procedure with the glogit link specified) in adenoma models vs. concurrent adenomas and hyperplastic polyps were statistically non-significant (p=0.86).

We detected a significant interaction between regular aspirin or other NSAID use and the 2043G>A polymorphism (Table 3). Among those with the common allele for 2043G>A (i.e. GG), NSAID use was associated with a decreased risk of adenoma (OR 0.4, 95% CI 0.3-0.7), whereas among those with at least one A allele, NSAID use was not associated with a further decrease in adenoma risk (AG: OR 0.6, 95% CI 0.4-0.9; AA: OR 0.5, 95% CI 0.4-1.2; p-interaction = 0.03). However, when Bonferroni correction is applied for multiple testing, the p-value for this interaction is 0.18. No other NSAID interactions were observed for *CRP* tagSNPs (see Table S2 online). For *CRP* haplotypes, no interactions with NSAID use were observed (p-interaction=0.63 for adenomas). Because BMI is an important predictor of CRP levels, we also investigated potential interactions between BMI and *CRP* genotypes. No statistically significant interactions were observed (data not shown).

Statistically significant interactions were observed between two *CRP* polymorphisms (−390C>T/A and 90A>T) and the *UGT1A1* promoter TA repeat polymorphism. Among those who were homozygous variant for either *CRP* genotype, the most frequent *UGT1A1* genotype (6rpt/6rpt) was associated with increased risk of adenoma, whereas having an increased number of *UGT1A1* repeats was associated with decreased risk (Table 4). No other interactions between *CRP* and *UGT* or *CYP2C9* polymorphisms were observed (see Tables S3–S5 online).

Because *CYP2C9* and *UGT1A6* are the major biotransformation enzymes involved in the metabolism of NSAIDs and polymorphisms in these genes have been previously found to alter associations of NSAID use with colorectal neoplasia [30–33], we investigated whether non-synonymous SNPs in *CYP2C9* (*2 and *3 alleles) and *UGT1A6* (T181A + R184S or R184S alone) combined with NSAID use interacted with *CRP* SNPs. For the *UGT1A6* polymorphism, the variant alleles are associated with slower drug metabolism [39], thus regular NSAID users with one or more variant alleles are likely to have the highest NSAID concentrations and, perhaps, the lowest risk of colorectal polyps [30]. Based on this hypothesis, we defined a three-level variable based on putative NSAID exposure: those who were wildtype and who didn't use NSAIDs (=high risk) were in one group, those with any variant allele and who were regular NSAID users (=low risk) in a second group and a third group contained all other combinations (=intermediate). A statistically significant interaction

was observed for *UGT1A6*/NSAID use and *CRP* −390C>T/A. Among those who were wildtype for *UGT1A6* and did not use NSAIDs, there was no association with the *CRP* −390C>T/A polymorphism. However, among those with at least one variant *UGT1A6* allele who regularly used NSAIDs, the lowest risk of adenoma was among those with the *CRP* homozygous variant alleles (Table 5; p-interaction = 0.03, Bonferroni correction p=0.18). Similar results were observed for hyperplastic polyps, although the interaction was not statistically significant. We followed a similar procedure for *CYP2C9*; however no interactions were observed (see Tables S6 and S7 online).

Discussion

There is growing interest in the role of CRP in colorectal neoplasia. Studies of plasma or serum CRP measures and colorectal neoplasia risk generally suggest that increasing CRP concentrations are associated with increased risk of colorectal cancer [1–4], although these results are not entirely consistent [5, 6]. Our findings suggest that genetic variability in *CRP* is unlikely to play a role in colorectal neoplasia risk, but that polymorphisms in *CRP* may be relevant to the pharmacogenetics of NSAIDs. Out of six tagSNPs tested for an association with risk of colorectal polyps, only one, 838G>C, was associated with increased risk of concurrent adenomatous and hyperplastic polyps. This SNP is a synonymous polymorphism in exon 2 of the *CRP* gene (L184L) and the G allele has been previously, although not consistently, associated with higher plasma CRP levels [8, 12, 13, 40, 41].

To our knowledge only one previous study has examined polymorphisms in *CRP* and risk of colorectal neoplasia [4]. In that study, no association was observed between three tagSNPs (rs1130864, rs1205, and rs3093068) and colorectal cancer risk. One of these SNPs, rs1205, was included in our study; we also found no association. The other two SNPs in that study are in LD ($r^2 \geq 90\%$) with two SNPs that we included in our study (rs1130864 is in LD with 90A>T and rs3093068 is in LD with 4363C>A); we also found no associations with these SNPs. No previous studies have examined potential interactions between variation in *CRP* and regular NSAID use. However, given the importance of aspirin and other NSAIDs for chemoprevention of cardiovascular disease and colorectal neoplasia, and the increasing use of CRP as a biomarker for inflammation, we suggest that potential interactions between NSAID use and *CRP* genetic variation should be considered in future etiologic studies of the associations between CRP and colorectal cancer risk.

In this exploratory study, we observed several interactions with NSAID use, NSAID-metabolizing enzymes, or the combination, suggesting that research into the role of CRP in cancer risk should take NSAID use into account. NSAID use has been reported to reduce CRP levels (reviewed in [22]). For the NSAID interaction, the OR among the homozygous variant NSAID users was higher than would be expected in NSAID use and *CRP* genotype were independent risk factors (expected: 0.7*0.4=0.28 observed: 0.5, p-0.05). This observed interaction suggests that NSAID use may not be beneficial to all, but rather may be more effective among people with higher underlying inflammation. We have previously reported on NSAID-gene interactions in genes relevant to prostaglandin synthesis [42–44] and functional polymorphisms in NSAID-metabolizing enzymes *UGT1A6* and *CYP2C9* have also been reported to alter the NSAID-colorectal neoplasia association [30–33]. Although our findings require confirmation, the current results add to the evidence that pharmacogenetic studies are necessary to truly understand which patients are most likely to benefit from NSAID chemoprevention and which are most likely to experience side effects.

Because we chose tagSNPs rather than candidate functional SNPs for this study, we had no prior hypotheses as to the association between CRP polymorphisms and colorectal polyp risk. However, several of these tagSNPs have been previously associated with plasma or

serum CRP levels, indicating that at least one of them has functional effects. Most consistently, the T and A alleles of −390C>T/A have been previously associated with increased plasma CRP concentrations [13–15, 45, 46]. Our results do not support a role for this polymorphism in polyp risk, but we did observe an interaction between −390C>T/A and the combination of *UGT1A6* genotype and regular NSAID use. In this interaction, those with two variant -390 alleles (i.e. AA, TT or AT) had a decreased risk in combination with having *UGT1A6* variant alleles, which are associated with slower drug metabolism, and NSAID use. If the *CRP* alleles are associated with increased CRP production, then this is the group in which NSAID use, particularly among slow metabolizers, would be expected to be the most effective. This and other reported interactions require confirmation in other studies.

This was an initial exploratory study of the association between *CRP* SNPs and colorectal neoplasia. We performed 48 tests for interaction; at α=0.05, by chance, we would expect at least 2 to be significant. We recognize that our sample size was relatively small and that our findings may be false positives. However, because this study was meant to inform further investigations of *CRP* variability and neoplasia risk, we decided to report all significant and near-significant findings.

In summary, we found limited evidence for an association between genetic variability in *CRP* and colorectal polyp risk, yet these polymorphisms may interact with NSAID use or NSAID-metabolizing enzymes. Further research is required to confirm these findings in larger studies of colorectal neoplasia. A comprehensive investigation of the genetics of biologic pathways relevant to inflammation, including prostaglandin synthesis and pro- and anti-inflammatory cytokines will further our understanding of the role of inherited susceptibility in colorectal cancer risk. Clearly such studies require information on use of NSAIDs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Erlinger TP, Platz EA, Rifai N, Helzlsouer KJ. C-reactive protein and the risk of incident colorectal cancer. JAMA. 2004; 291:585–90. [PubMed: 14762037]
2. Otani T, Iwasaki M, Sasazuki S, Inoue M, Tsugane S. Plasma C-reactive protein and risk of colorectal cancer in a nested case-control study: Japan Public Health Center-based prospective study. Cancer Epidemiol Biomarkers Prev. 2006; 15:690–5. [PubMed: 16614110]
3. Gunter MJ, Stolzenberg-Solomon R, Cross AJ, Leitzmann MF, Weinstein S, Wood RJ, et al. A prospective study of serum C-reactive protein and colorectal cancer risk in men. Cancer Res. 2006; 66:2483–7. [PubMed: 16489056]
4. Siemes C, Visser LE, Coebergh JW, Splinter TA, Witteman JC, Uitterlinden AG, et al. C-reactive protein levels, variation in the C-reactive protein gene, and cancer risk: the Rotterdam Study. J Clin Oncol. 2006; 24:5216–22. [PubMed: 17114654]
5. Zhang SM, Buring JE, Lee IM, Cook NR, Ridker PM. C-reactive protein levels are not associated with increased risk for colorectal cancer in women. Ann Intern Med. 2005; 142:425–32. [PubMed: 15767620]

6. Ito Y, Suzuki K, Tamakoshi K, Wakai K, Kojima M, Ozasa K, et al. Colorectal cancer and serum C-reactive protein levels: a case-control study nested in the JACC Study. J Epidemiol. 2005; 15(Suppl 2):S185–9. [PubMed: 16127232]
7. Szalai AJ, McCrory MA, Cooper GS, Wu J, Kimberly RP. Association between baseline levels of C-reactive protein (CRP) and a dinucleotide repeat polymorphism in the intron of the CRP gene. Genes Immun. 2002; 3:14–9. [PubMed: 11857055]
8. Zee RY, Ridker PM. Polymorphism in the human C-reactive protein (CRP) gene, plasma concentrations of CRP, and the risk of future arterial thrombosis. Atherosclerosis. 2002; 162:217–9. [PubMed: 11947917]
9. Brull DJ, Serrano N, Zito F, Jones L, Montgomery HE, Rumley A, et al. Human CRP gene polymorphism influences CRP levels: implications for the prediction and pathogenesis of coronary heart disease.[see comment]. Arterioscler Thromb Vasc Biol. 2003; 23:2063–9. [PubMed: 12842840]
10. Russell AI, Cunninghame Graham DS, Shepherd C, Roberton CA, Whittaker J, Meeks J, et al. Polymorphism at the C-reactive protein locus influences gene expression and predisposes to systemic lupus erythematosus. Hum Mol Genet. 2004; 13:137–147. [PubMed: 14645206]
11. Obisesan TO, Leeuwenburgh C, Phillips T, Ferrell RE, Phares DA, Prior SJ, et al. C-reactive protein genotypes affect baseline, but not exercise training-induced changes, in C-reactive protein levels. Arterioscler Thromb Vasc Biol. 2004; 24:1874–9. [PubMed: 15271790]
12. Suk HJ, Ridker PM, Cook NR, Zee RY. Relation of polymorphism within the C-reactive protein gene and plasma CRP levels. Atherosclerosis. 2005; 178:139–45. [PubMed: 15585211]
13. Kovacs A, Green F, Hansson LO, Lundman P, Samnegard A, Boquist S, et al. A novel common single nucleotide polymorphism in the promoter region of the C-reactive protein gene associated with the plasma concentration of C-reactive protein. Atherosclerosis. 2005; 178:193–8. [PubMed: 15585218]
14. Szalai AJ, Wu J, Lange EM, McCrory MA, Langefeld CD, Williams A, et al. Single-nucleotide polymorphisms in the C-reactive protein (CRP) gene promoter that affect transcription factor binding, alter transcriptional activity, and associate with differences in baseline serum CRP level. J Mol Med. 2005; 83:440–7. [PubMed: 15778807]
15. Carlson CS, Aldred SF, Lee PK, Tracy RP, Schwartz SM, Rieder M, et al. Polymorphisms within the C-reactive protein (CRP) promoter region are associated with plasma CRP levels. Am J Hum Genet. 2005; 77:64–77. [PubMed: 15897982]
16. Bosetti C, Gallus S, La Vecchia C. Aspirin and cancer risk: an updated quantitative review to 2005. Cancer Causes Contr. 2006; 17:871–88.
17. Baron JA, Cole BF, Sandler RS, Haile RW, Ahnen D, Bresalier R, et al. A randomized trial of aspirin to prevent colorectal adenomas. New Engl J Med. 2003; 348:891–9. [PubMed: 12621133]
18. Sandler RS, Halabi S, Baron JA, Budinger S, Paskett E, Keresztes R, et al. A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. New Engl J Med. 2003; 348:883–90. [PubMed: 12621132]
19. Baron JA, Sandler RS, Bresalier RS, Quan H, Riddell R, Lanas A, et al. A randomized trial of rofecoxib for the chemoprevention of colorectal adenomas. Gastroenterology. 2006; 131:1674–82. [PubMed: 17087947]
20. Arber N, Eagle CJ, Spicak J, Racz I, Dite P, Hajer J, et al. Celecoxib for the prevention of colorectal adenomatous polyps. New Engl J Med. 2006; 355:885–895. [PubMed: 16943401]
21. Bertagnolli MM, Eagle CJ, Zauber AG, Redston M, Solomon DH, Kim K, et al. Celecoxib for the prevention of sporadic colorectal adenomas. New Engl J Med. 2006; 355:873–874. [PubMed: 16943400]
22. Prasad K. C-reactive protein (CRP)-lowering agents. Cardiovasc Drug Rev. 2006; 24:33–50. [PubMed: 16939632]
23. Miners JO, Birkett DJ. Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. Br J Clin Pharmacol. 1998; 45:525–38. [PubMed: 9663807]
24. Kuehl GE, Lampe JW, Potter JD, Bigler J. Glucuronidation of nonsteroidal anti-inflammatory drugs (NSAIDs): identifying the enzymes responsible in human liver microsomes. Drug Metabol Dispos. 2005; 33:1027–35.

25. Takahashi H, Kashima T, Nomoto S, Iwade K, Tainaka H, Shimizu T, et al. Comparisons between in-vitro and in-vivo metabolism of (S)-warfarin: catalytic activities of cDNA-expressed CYP2C9, its Leu359 variant and their mixture versus unbound clearance in patients with the corresponding CYP2C9 genotypes. Pharmacogenetics. 1998; 8:365–73. [PubMed: 9825828]
26. Rettie AE, Wienkers LC, Gonzalez FJ, Trager WF, Korzekwa KR. Impaired (S)-warfarin metabolism catalysed by the R144C allelic variant of CYP2C9. Pharmacogenetics. 1994; 4:39–42. [PubMed: 8004131]
27. Lampe JW, Bigler J, Horner NK, Potter JD. UDP-glucuronosyltransferase (UGT1A1*28 and UGT1A6*2) polymorphisms in Caucasians and Asians: relationships to serum bilirubin concentrations. Pharmacogenetics. 1999; 9:341–9. [PubMed: 10471066]
28. Iyer L, Das S, Janisch L, Wen M, Ramirez J, Karrison T, et al. UGT1A1*28 polymorphism as a determinant of irinotecan disposition and toxicity. Pharmacogenomics Journal. 2002; 2:43–7. [PubMed: 11990381]
29. Sparks R, Ulrich CM, Bigler J, Tworoger SS, Yasui Y, Rajan KB, et al. UDP-glucuronosyltransferase and sulfotransferase polymorphisms, sex hormone concentrations, and tumor receptor status in breast cancer patients. Breast Cancer Res. 2004; 6:R488–98. [PubMed: 15318931]
30. Bigler J, Whitton J, Lampe JW, Fosdick L, Bostick RM, Potter JD. CYP2C9 and UGT1A6 genotypes modulate the protective effect of aspirin on colon adenoma risk. Cancer Res. 2001; 61:3566–9. [PubMed: 11325819]
31. Chan AT, Tranah GJ, Giovannucci EL, Hunter DJ, Fuchs CS. Genetic variants in the UGT1A6 enzyme, aspirin use, and the risk of colorectal adenoma. J Natl Cancer Inst. 2005; 97:457–60. [PubMed: 15770010]
32. Samowitz WS, Wolff RK, Curtin K, Sweeney C, Ma KN, Andersen K, et al. Interactions between CYP2C9 and UGT1A6 polymorphisms and nonsteroidal anti-inflammatory drugs in colorectal cancer prevention. Clin Gastroenterol Hepatol. 2006; 4:894–901. [PubMed: 16797247]
33. Hubner RA, Muir KR, Liu JF, Logan RF, Grainge M, Armitage N, et al. Genetic variants of UGT1A6 influence risk of colorectal adenoma recurrence. Clin Cancer Res. 2006; 12:6585–9. [PubMed: 17085674]
34. Potter JD, Bostick RM, Grandits GA, Fosdick L, Elmer P, Wood J, et al. Hormone replacement therapy is associated with lower risk of adenomatous polyps of the large bowel: the Minnesota Cancer Prevention Research Unit Case-Control Study. Cancer Epidemiol Biomarkers Prev. 1996; 5:779–84. [PubMed: 8896888]
35. Morimoto LM, Newcomb PA, Ulrich CM, Bostick RM, Lais CJ, Potter JD. Risk factors for hyperplastic and adenomatous polyps: evidence for malignant potential? Cancer Epidemiol Biomarkers Prev. 2002; 11:1012–8. [PubMed: 12376501]
36. UW-FHCRC Variation Discovery Resource. http://pga.gs.washington.edu/
37. Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. Am J Hum Genet. 2004; 74:106–20. [PubMed: 14681826]
38. Liang K-Y, Zeger S. Longitudinal data analysis using generalized linear models. Biometrika. 1986; 73:13–22.
39. Ciotti M, Marrone A, Potter C, Owens IS. Genetic polymorphism in the human UGT1A6 (planar phenol) UDP-glucuronosyltransferase: pharmacological implications. Pharmacogenetics. 1997; 7:485–95. [PubMed: 9429234]
40. Eklund C, Lehtimaki T, Hurme M. Epistatic effect of C-reactive protein (CRP) single nucleotide polymorphism (SNP) +1059 and interleukin-1B SNP +3954 on CRP concentration in healthy male blood donors. Int J Immunogenet. 2005; 32:229–32. [PubMed: 16026589]
41. Davey, Smith G.; Lawlor, DA.; Harbord, R.; Timpson, N.; Rumley, A.; Lowe, GD., et al. Association of C-reactive protein with blood pressure and hypertension: life course confounding and mendelian randomization tests of causality. Arterioscler Thromb Vasc Biol. 2005; 25:1051–6. [PubMed: 15731495]

42. Ulrich CM, Bigler J, Sparks R, Whitton J, Sibert JG, Goode EL, et al. Polymorphisms in PTGS1 (=COX-1) and risk of colorectal polyps. Cancer Epidemiol Biomarkers Prev. 2004; 13:889–893. [PubMed: 15159324]

43. Ulrich CM, Whitton J, Yu JH, Sibert J, Sparks R, Potter JD, et al. PTGS2 (COX-2) −765G > C promoter variant reduces risk of colorectal adenoma among nonusers of nonsteroidal anti-inflammatory drugs. Cancer Epidemiol Biomarkers Prev. 2005; 14:616–9. [PubMed: 15767339]

44. Poole E, Bigler J, Whitton J, Potter J, Sibert J, Ulrich C. Prostacyclin synthase and arachidonate 5-lipoxygenase polymorphisms and risk of colorectal polyps. Cancer Epidemiol Biomarkers Prev. 2006; 15:502–508. [PubMed: 16537708]

45. Kathiresan S, Larson MG, Vasan RS, Guo CY, Gona P, Keaney JF Jr, et al. Contribution of clinical correlates and 13 C-reactive protein gene polymorphisms to interindividual variability in serum C-reactive protein level. Circulation. 2006; 113:1415–23. [PubMed: 16534007]

46. Crawford DC, Sanders CL, Qin X, Smith JD, Shephard C, Wong M, et al. Genetic variation is associated with C-reactive protein levels in the Third National Health and Nutrition Examination Survey. Circulation. 2006; 114:2458–65. [PubMed: 17101857]

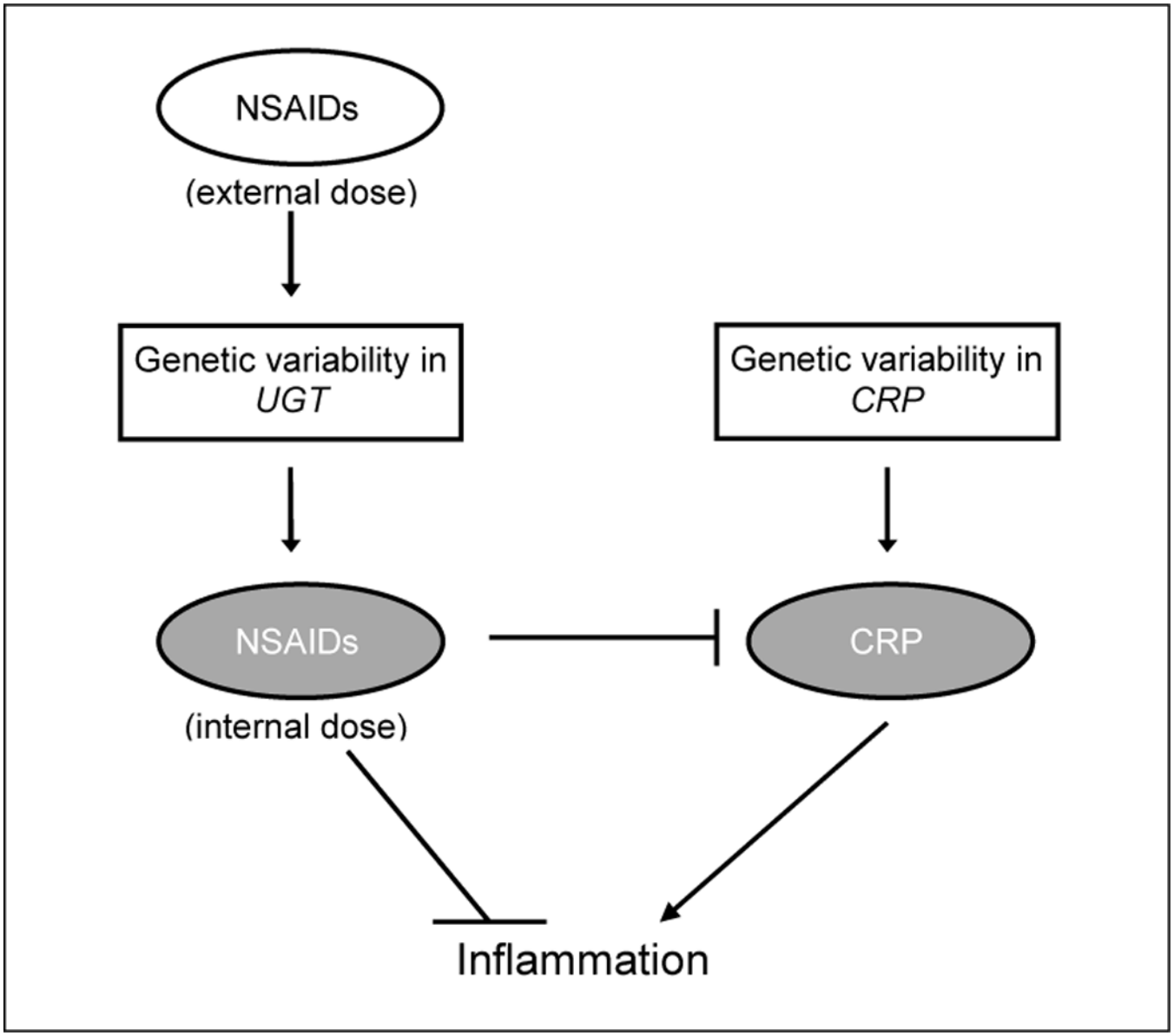

Figure.
CRP and NSAID use in inflammation.

Table 1

PCR Conditions

Polymorphism	PCR	Primers/Probes	[Mg^{2+}]	[Primers, Probes]	Amplicon	Cycling
−821A>G	FP	5'GGCCGTCATTTAGTGCCAA3'		200nM		50°C, 2min, 95°C, 10min, 40x 94°C, 30sec; 58°C, 45sec; 72°C, 1min 72°C, 5min
	RP	5'GTGCTGCACCCATTAACTCATC3'		200nM		
	A-allele	5'6FAM-CACCGC**A**TGTTCT-3'MGB	2.5mM	100nM	250bp	
	G-allele	5'VIC-CACCGC**G**TGTTCT-3'MGB		100nM		
−390C>T/A	FP	5'TCAGATTTCCTTTGTCAAACTCTATGA3'		200nM		50°C, 2min, 95°C, 10min, 40x 95°C, 15sec; 60°C, 90sec
	RP	5'TCCACTTTGGCTATCTATCCTGC3'		200nM		
	C-allele	5'VIC-AACATATTAAAC**G**AGTGGCCAT-3'MGB	4mM	100nM	138bp	
	T-allele	5'TET-CATATTAAAC**A**AGTGGCCATC-3'MGB		100nM		
	A-allele	5'6FAM-TAACATATTAAAC**T**AGTGGCCATC-3'MGB		100nM		
90A>T	FP	5'TGGCCAGACAGGTAAGGGC3'		200nM		50°C ,2min, 95°C, 10min, 40x 95°C, 15sec; 59°C, 90sec
	RP	5'ACCATGAAGGATGCTCCACTG3'		200nM		
	A-allele	5'VIC-TCAGATCAAA**T**CTCTCCCAT-3'MGB	4mM	100nM	141bp	
	T-allele	5'6FAM-TCAGATCAAA**A**CTCTCCCATA-3'MGB		100nM		
838G>C	FP	5'TGGGAGACATTGGAAATGTGAAC3'		200nM		50°C, 2min, 95°C, 10min, 40x 95°C, 15sec; 59°C, 1 min
	RP	5'CCGCCAAGATAGATGGTGTTAAT3'		200nM		
	G-allele	5'VIC-TTGTGCT**G**TCACCAGA-3'MGB	3mM	100nM	76bp	
	C-allele	5'6FAM-TTTGTGCT**C**TCACCAGA-3'MGB		100nM		
2043G>A	FP	5'GCCATCTTGTTTGCCACATG3'		200nM		50°C, 2min, 95°C, 10min, 40X 95°C, 15sec; 60°C, 1 min
	RP	5'CCCTTGGCTCCTCCACTTC3'		200nM		
	G-allele	5'VIC-TGTCCTCA**C**AGTCTC-3'MGB	5mM	100nM	70bp	
	A-allele	5'6-FAM-TGTCCTCA**T**AGTCTCT-3'MGB		150nM		
4363C>A	FP	5'AACCTAAAATCTCCCTGTGTCAGAA3'		200nM		50°C, 2min, 95°C, 10min, 40x 95°C, 15sec; 61°C, 2min 30sec
	RP	5'CTACTTACTTTGTCAGCTGGGACTCC3'		200nM		
	C-allele	5'VIC-TTTCCATCA**G**GTCCCA-3'MGB	4mM	100nM	263bp	
	A-allele	5'6-FAM-TCCATCA**T**GTCCCAGC-3'MGB		100nM		

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Table 2

Risk of colorectal polyps associated with *CRP* genotypes[1]

	Controls (N=562)	Adenomas (N=477)		Concurrent adenomas and hyperplastic polyps (N=112)	
	N	N	OR (95% CI)	N	OR (95% CI)
***CRP* Genotype**					
−821A>G rs2794521					
AA	294	245	1.0 (ref.)	61	1.0 (ref.)
AG	217	180	1.0 (0.7-1.3)	41	0.8 (0.5-1.3)
GG	51	53	1.4 (0.9-2.1)	11	1.2 (0.5-2.5)
−390C>T/A rs3091244 (all genotypes)					
CC	221	196	1.0 (ref.)	43	1.0 (ref.)
CT	215	181	1.0 (0.7-1.3)	43	1.1 (0.6-1.8)
CA	56	37	0.9 (0.6-1.5)	12	1.5 (0.7-3.2)
TT	42	42	1.1 (0.7-1.8)	10	1.1 (0.5-2.4)
AA	4	1	0.2 (0.1-1.4)	0	0 (--)
TA	25	20	0.9 (0.4-1.6)	4	0.8 (0.3-2.7)
−390C>T/A rs3091244 (grouped by putative phenotype as in [15])					
CC	221	196	1.0 (ref.)	43	1.0 (ref.)
CT or CA	271	218	1.0 (0.7-1.3)	55	1.2 (0.7-1.7)
TT, AA, or TA	71	63	1.0 (0.6-1.4)	14	0.9 (0.4-1.8)
90A>T rs1417938					
AA	281	236	1.0 (ref.)	57	1.0 (ref.)
AT	239	200	1.0 (0.8-1.3)	46	1.0 (0.6-1.5)
TT	43	42	1.1 (0.6-1.8)	10	1.0 (0.4-2.1)
838G>C rs1800947 (L184L)					
GG	504	414	1.00(ref.)	94	1.0 (ref.)
GC or CC	59	64	1.4 (0.9-2.1)	19	**2.0 (1.1-3.6)**
2043G>A rs1205					
GG	241	218	1.0 (ref.)	50	1.0 (ref.)
GA	262	210	0.9 (0.7-1.2)	51	1.0 (0.6-1.7)
AA	60	50	0.9 (0.6-1.4)	12	1.1 (0.5-2.2)

	Controls (N=562)	Adenomas (N=477)		Concurrent adenomas and hyperplastic polyps (N=112)	
	N	N	OR (95% CI)	N	OR (95% CI)
4363C>A rs3093075					
CC	477	419	1.0 (ref.)	96	1.0 (ref.)
CA or AA	86	57	0.9 (0.6-1.3)	16	1.1 (0.6-2.0)
***CRP*haplotype[2](−821A>G; −390C>T/A; 90A>T; 838G>C; 2043G>A; 4363C>A)**					
GCAGGC	28.6	29.3	1.0 (ref.)	26.9	1.0 (ref.)
ATTGGC	28.4	29.7	1.0 (0.9-1.2)	30.0	1.1 (0.8-1.4)
ACAGGC	1.3	1.9	1.2 (0.8-1. 8)	2.6	1.5 (0.8-2.8)
ACAGAC	28.1	25.1	0.9 (0.8-1.1)	24.3	1.0 (0.7-1.3)
ACACAC	5.2	7.1	1.2 (0.9-1.6)	7.8	**1.6 (1.0-2.5)**
AAAGGA	8.1	6.3	0.9 (0.7-1.2)	7.0	1.1 (0.7-1.78)
all rare haplotypes	0.4	0.8	1.5 (0.7-3.1)	1.3	**2.0 (1.0-3.8)**
			global p=0.63		global p=0.49

[1]Age and sex adjusted.

[2]Percents rather than total N are reported for haplotypes since they are inferred rather than determined.

Table 3

Association between *CRP* 2043G>A and risk of adenoma, stratified by NSAID use*

	Common Referent Group						Separate Referent Groups					
	Aspirin or other NSAID use						Aspirin or other NSAID use					
	No			Yes			No			Yes		
	Controls (N)	Cases (N)	OR (95% CI)	Controls (N)	Cases (N)	OR (95% CI)	Controls (N)	Cases (N)	OR (95% CI)	Controls (N)	Cases (N)	OR (95% CI)
2043G>A rs1205												
GG	124	145	1.0 (ref.)	117	73	0.4 (0.3-0.7)	124	145	1.0 (ref.)	117	73	1.0 (ref.)
GA	154	125	0.6 (0.4-0.9)	108	85	0.6 (0.4-0.9)	154	125	0.6 (0.4-0.9)	108	85	1.4 (0.9-2.3)
AA	34	33	0.7 (0.4-1.4)	26	17	0.5 (0.2-1.1)	34	33	0.7 (0.4-1.4-)	26	17	1.2 (0.5-2.6)
												p-interaction = 0.03

*Adjusted for age, sex, BMI, intakes of total calories, alcohol, and fiber, smoking (pack-years) and hormone use (females).

Table 4

CRP genotype, *UGT1A1* genotype, and risk of colorectal adenoma[1]

	Common Referent Group						Separate Referent Groups					
	UGT1A1 genotype						UGT1A1 genotype					
	6/6 (wildtype)			6/7, 6/8, 7/7, 7/8			6/6 (wildtype)			6/7, 6/8, 7/7, 7/8		
***CRP* Genotype**	**Controls (N)**	**Cases (N)**	**OR (95% CI)**	**Controls (N)**	**Cases (N)**	**OR (95% CI)**	**Controls (N)**	**Cases (N)**	**OR (95% CI)**	**Controls (N)**	**Cases (N)**	**OR (95% CI)**
−390C>T/A rs3091244 (grouped by putative phenotype)												
CC	101	92	1.0 (ref.)	117	103	1.1 (0.7-1.7)	101	92	1.0 (ref.)	117	103	1.0 (ref.)
CT or CA	114	91	1.0 (0.7-1.6)	154	126	1.1 (0.7-1.6)	114	91	1.0 (0.7-1.6)	154	126	1.0 (0.7-1.4)
TT, AA, or TA	25	32	2.1 (1.0-4.2)	45	30	0.7 (0.4-1.2)	25	32	2.1 (1.0-4.2)	45	30	0.6 (0.3-1.1)
					p-interaction = 0.02				p-trend=0.08			p-trend=0.19
90A>T rs1417938												
AA	120	105	1.0 (ref.)	156	130	1.1 (0.7-1.6)	120	105	1.0 (ref.)	156	130	1.0 (ref.)
AT	104	86	1.1 (0.7-1.7)	133	112	1.0 (0.7-1.6)	104	86	1.1 (0.7-1.7)	133	112	1.0 (0.7-1.4)
TT	16	25	2.6 (1.2-5.6)	27	17	0.7 (0.3-1.4)	16	25	2.6 (1.2-5.6)	27	17	0.6 (0.3-1.2)
					p-interaction = 0.04				p-trend=0.07			p-trend=0.31

[1] Adjusted for age, sex, BMI, intakes of total calories, alcohol, and fiber, current NSAID use, smoking (pack-years) and hormone use (females).

Table 5a

CRP genotype, *UGT1A6* genotype and NSAID use and risk of colorectal adenoma (common referent group)[1]

	***UGT1A6* genotype/regular NSAID use**								
	wt and no NSAIDs			**All others**			**181A + 184S or 184S and NSAID use**		
	Controls (N)	**Cases (N)**	**OR (95% CI)**	**Controls (N)**	**Cases (N)**	**OR (95% CI)**	**Controls (N)**	**Cases (N)**	**OR (95% CI)**
***CRP* genotype**									
−390C>T/A rs3091244 (grouped by putative phenotype)									
CC	61	56	1.0 (ref.)	111	98	1.0 (0.6-1.7)	49	43	1.0 (0.5-1.8)
CT or CA	68	55	1.0 (0.6-1.8)	132	118	1.1 (0.7-1.8)	70	46	0.8 (0.5-1.5)
TT, AA, or TA	12	17	1.9 (0.7-5.0)	32	37	1.5 (0.7-2.9)	27	9	0.3 (0.1-0.7)
									p-interaction = 0.03

[1]Adjusted for age, sex, BMI, intakes of total calories, alcohol, and fiber, smoking (pack-years) and hormone use (females).

Table 5b

CRP genotype, *UGT1A6* genotype and NSAID use and risk of colorectal adenoma (separately by strata of UGT1A6 genotype and NSAID use)[1]

	***UGT1A6* genotype/regular NSAID use**								
	wt and no NSAIDs			**All others**			**181A + 184S or 184S and NSAID use**		
	Controls (N)	**Cases (N)**	**OR (95% CI)**	**Controls (N)**	**Cases (N)**	**OR (95% CI)**	**Controls (N)**	**Cases (N)**	**OR (95% CI)**
***CRP* genotype**									
−390C>T/A rs3091244 (grouped by putative phenotype)									
CC	61	56	1.0 (ref.)	111	98	1.0 (ref.)	49	43	1.0 (ref.)
CT or CA	68	55	1.0 (0.6-1.8)	132	118	1.0 (0.7-1.6))	70	46	0.9 (0.5-1.7)
TT, AA, or TA	12	17	1.9 (0.7-5.0)	32	37	1.4 (0.8-2.7)	27	9	0.3 (0.1-0.7)
			p-trend=0.28			p-trend=0.32			p-trend=0.02

[1]Adjusted for age, sex, BMI, intakes of total calories, alcohol, and fiber, smoking (pack- years) and hormone use (females).