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Genetic variation in prostaglandin synthesis and related pathways, NSAID use and colorectal cancer risk in the Colon Cancer Family Registry

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Although use of non-steroidal anti-inflammatory drugs (NSAIDs) generally decreases colorectal cancer (CRC) risk, inherited genetic variation in inflammatory pathways may alter their potential as preventive agents. We investigated whether variation in prostaglandin synthesis and related pathways influences CRC risk in the Colon Cancer Family Registry by examining associations between 192 single nucleotide polymorphisms (SNPs) and two variable nucleotide tandem repeats (VNTRs) within 17 candidate genes and CRC risk. We further assessed interactions between these polymorphisms and NSAID use on CRC risk. Using a case-unaffected-sibling-control design, this study included 1621 primary invasive CRC cases and 2592 sibling controls among Caucasian men and women aged 18–90. After adjustment for multiple comparisons, two intronic SNPs were associated with rectal cancer risk: rs11571364 in *ALOX12* [$OR_{het/hzv}$ = 1.87, 95% confidence interval (CI) = 1.19–2.95, *P* = 0.03] and rs45525634 in *PTGER2* ($OR_{het/hzv}$ = 0.49, 95% CI = 0.29–0.82, *P* = 0.03). Additionally, there was an interaction between NSAID use and the intronic SNP rs2920421 in *ALOX12* on risk of CRC (*P* = 0.03); among those with heterozygous genotypes, risk was reduced for current NSAID users compared with never or former users (OR_{het} = 0.60, 95% CI = 0.45–0.80), though not among those with homozygous wild-type or variant genotypes. The results of this study suggest that genetic variation in *ALOX12* and *PTGER2* may affect the risk of rectal cancer. In addition, this study suggests plausible interactions between NSAID use and variants in *ALOX12* on CRC risk. These results may aid in the development of genetically targeted cancer prevention strategies with NSAIDs.

Introduction

Inflammation has long been recognized as an important contributor to colorectal cancer (CRC) risk (1–3). For example, chronic inflammatory conditions, such as inflammatory bowel disease, are associated with increased CRC risk and prior studies suggest that both inflammatory bowel disease-associated and sporadic CRC share mechanisms linked to inflammation (3–5). Although the exact mechanisms that contribute to this process are still unclear (3,4), inflammation may enable carcinogenesis by contributing to cell proliferation and survival, angiogenesis and genetic mutations (4,6,7). Use of non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit inflammation, decreases CRC risk (8–12); however, inherited variation in genes that affect NSAID metabolism may alter their potential as preventive agents.

NSAIDs inhibit the cyclooxygenase (COX) enzymes, which control the conversion of arachidonic acid (AA) to prostaglandins and thromboxane (Figure 1) (2,12). COX1 and COX2 (encoded by *PTGS1* and *PTGS2*) are the main targets of traditional NSAIDs (such as aspirin and ibuprofen) as well as COX2-specific inhibitors. They are the main enzymes responsible for reducing AA to prostaglandin H_2, which is metabolized to a substrate for thromboxane synthase, prostacyclin synthase (PGI) and prostaglandin synthase (PG). Via these synthases, prostaglandin H_2 is metabolized into thromboxane A_2, PGI_2, prostaglandin E_2 (PGE_2) and prostaglandin D_2, which modulate important cellular functions by binding to their respective receptors: TP to thromboxane A_2, IP to PGI_2, EP (EP1-4) to PGE_2 and DP to prostaglandin D_2 (12–14). Arachidonate lipoxygenases (ALOXs) convert AA into leukotrienes and other eicosanoids that regulate inflammatory processes (15,16). Additionally, eicosanoid levels differ between normal and colon cancer tissues, with PGE_2 levels increased and PGI_2 levels decreased in colon cancer samples (17).

Prior studies, including ours, reported associations between polymorphisms in genes involved in inflammation pathways and CRC risk and mortality (18–24). Likewise, numerous epidemiologic studies and randomized trials have shown that NSAID use reduces CRC risk and mortality (8–11,25–27). However, as we have shown previously, genetic variation in NSAID-metabolizing pathways (upstream and downstream) may affect the capacity of NSAIDs to serve as chemopreventive agents (28). Therefore, we investigated whether variation in genes involved in prostaglandin synthesis or related pathways might impact CRC risk. Using data from the population-based Colon Cancer Family Registry (CCFR) (29), we assessed whether single nucleotide polymorphisms (SNPs) and variable nucleotide tandem repeats (VNTRs) were independently associated with CRC risk and whether these associations were modified by pre-diagnostic use of NSAIDs.

Materials and methods

Study population

The study population has been described previously (29). Briefly, cases were men and women recruited from six registry centres and aged 18–90 when diagnosed with primary invasive CRC from 1998–2002. Cases included probands and any affected relatives, and were interviewed within 5 years of diagnosis. CRC included distal (left-sided), proximal (right-sided) and not otherwise specified colon cancers as well as rectal cancers. Controls were siblings not diagnosed with CRC at the time of ascertainment. Individuals whose self-reported sex did not match that determined by genotyping were excluded, as were cases that did not have at least one matched unaffected sibling control. Further, this study included only participants ascertained through population-based recruitment who self-reported as Caucasian, with no known familial adenomatous polyposis and no personal history of ulcerative colitis or Crohn's disease. Other racial/ethnic groups and clinic-based populations were excluded as sample sizes were too small to examine associations.

Epidemiologic data were collected from CCFR study participants using standard questionnaires on demographic characteristics, medical history, NSAID use, family history of cancer, smoking history, diet, physical activity, height and weight. Blood and tissue samples were collected using standardized procedures across CCFR sites and DNA was extracted from peripheral blood

Abbreviations: AA, arachidonic acid; bp, base pair; ALOX, arachidonate lipoxygenase; CI, confidence interval; COX, cyclooxygenase; CRC, colorectal cancer; LD, linkage disequilibrium; NSAID, non-steroidal anti-inflammatory drug; OR, odds ratio; PGE_2, prostaglandin E_2; PGI, prostacyclin synthase; SNP, single nucleotide polymorphism; VNTR, variable nucleotide tandem repeat.

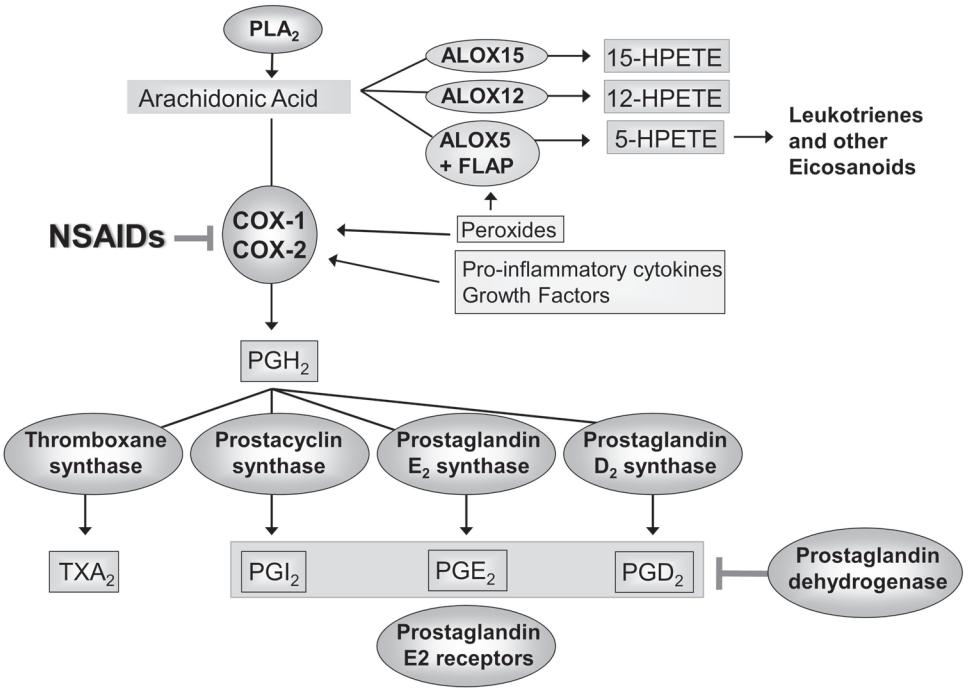

Fig. 1. Prostaglandin synthesis pathway. The COX enzymes COX1 and COX2, which reduce arachidonic acid to prostaglandin H_2 (PGH_2), are the main targets of COX2-specific inhibitors and NSAIDs. PGH_2 becomes a substrate for thromboxane, prostacyclin and prostaglandin synthases, which are converted into thromboxane A_2 (TXA_2), PGI_2, PGE_2 and prostaglandin D_2 (PGD_2). Arachidonic lipoxygenesases and hydroperoxides (HPETEs) convert arachidonic acid into leukotrienes and other inflammation-mediating eicosanoids.

leucocytes. Participants were provided a list of common aspirin and non-aspirin NSAIDs brands and reported their average weekly consumption. Current NSAID use was defined as use two or more times per week for at least 1 month in the 2 years prior to study enrollment. The Institutional Review Boards at each CCFR site approved this study and informed consent was obtained from all study participants.

Polymorphism selection

We selected a total of 220 candidate and tagging SNPs (tagSNPs) and three functional VNTRs in 17 candidate genes involved in prostaglandin synthesis and related pathways. The following genes were included: *ALOX12*, *ALOX5*, *ALOX5AP*, *CRP*, *EGFR*, *GIPC1*, *HPGD*, *PARP12*, *PKN1*, *PLA2G1B*, *PTGER1*, *PTGER2*, *PTGER3*, *PTGER4*, *PTGES*, *PTGIS* and *TBXAS1*. The procedure for tagSNP selection has previously been published (30). Briefly, using Haploview Tagger on publicly available HapMap 2 data (31), tagSNPs were selected based on a pairwise $r^2 > 0.95$, a minor allele frequency >5% and distance from the closest SNP of >60 base pairs (bp). Within linkage disequilibrium (LD) blocks for each gene, the 5′- and 3′-untranslated regions (UTR) were extended to include the most up- or downstream SNP (~10 kb upstream and 5 kb downstream). In regions of no or low LD, SNPs with a minor allele frequency >5% at a density of ~1 per kb were selected from either HapMap (31) or dbSNP (32). When we designed the study using HapMap 2 as a reference panel, tagSNPs genotyped by the CCFR were estimated to cover at least 80% of the variation in these loci. With the HapMap 3 release, ~50% of these loci are covered.

Genotyping assays

SNPs were genotyped using the Illumina BeadXpress GoldenGate at Translational Genomics Research Institute (TGen, Phoenix, AZ). Replicate aliquots were included for 5% of samples to assess reliability. Concordance among duplicates was at least 99.8% for all SNPs. Monomorphic SNPs, SNPs with call rates <90% and SNPs with Hardy–Weinberg Equilibrium *P* values < 0.001 among unrelated Caucasian controls were excluded (*N* SNPs excluded = 28), resulting in 192 SNPs available for analysis.

Three VNTRs were also genotyped: the 9-base microsatellite polymorphism at nt –3 in the *PTGIS* (*CYP8A1*) 5′-untranslated region, which is a repeat of 4–7 copies of a putative Sp1 binding site (33), the *ALOX5* VNTR promoter polymorphism [−176(GGGCGG)2–8], and the *ALOX5AP* (*FLAP*) poly(A) promoter repeat [−169poly(A)]. The *PTGIS* VNTR was genotyped by GeneScan assay as described previously (34). Briefly, the *PTGIS* (*CYP8A1*) promoter region was PCR amplified with a fluorescently tagged primer and size separated by capillary electrophoresis using an ABI 3130xl Sequence Analyzer (Life Technologies). Amplicon length was analysed using Genotyper software. The DNA input was 40 ng with a total reaction volume of 5 µl. The *ALOX5* and *ALOX5AP* VNTRs were genotyped using GeneScan on an ABI 3130xl, based on previously published protocols (34,35). Briefly, multiplex PCR reactions consisted of 40 ng genomic template DNA, 1.5 mmol/l MgCl2, 1x PCR Gold Buffer (Applied Biosystems), 0.5 units of Amplitaq Gold Polymerase (Applied Biosystems), 200 nmol/l each of oligonucleotide primers ALOX5sp1-F 5′-6FAM-AGGAA CAGACACC TCGCTG AGGAGAG-3′, ALOX5sp1-R 5′GAGCAGCGAGCGCCGGGAGCCTCGGC-3′, -F: 5′ VIC-CGTGCTCCTCTGCCAAGCCCTGCTTC- 3′ and ALOX5AP-R02: 5′-GCTCT GCCTCCAGCTGCACAACCTG-3′, 150 µmol/l each of dATP, dCTP and dTTP, 75 µmol/l each of dGTP and 7-deaza-2V-dGTP (Roche Diagnostics GmbH, Mannheim, Germany) and 8% (vol/vol) DMSO (Sigma, St Louis, MO) in 5 µl. Cycling was as follows: 96°C for 7 min, 30 cycles of: 94°C for 30 s, 58°C for 30 s, 72°C for 30 s, 72°C for 7 min. The observed 6FAM-labeled *ALOX5* amplicon lengths ranged from 254 bp (26-bp repeats) to 290 bp (86-bp repeats) and VIC-labeled *ALOX5AP* amplicon was either 212 bp (19A) or 216 bp (23A). However, we excluded the *ALOX5AP* VNTR from analysis as it had a Hardy–Weinberg Equilibrium *P* value < 0.001 among Caucasian controls.

Statistical methods

Because colon and rectal cancer could have unique etiological mechanisms, we chose *a priori* to assess separately the association between each polymorphism and colon and rectal cancer risk as well as combined CRC risk using conditional logistic regression to compute odds ratios (OR) and 95% confidence intervals (CI). Conditional logistic regression was also used to examine interactions between polymorphisms and current NSAID use for CRC, colon and rectal cancer risk. For interaction analyses, ORs and 95% CI were computed within categories of NSAID use in order to compare genotypes. Likelihood ratio tests were used to calculate *P* values for polymorphism main effects and polymorphism–NSAID interactions. Statistical significance of main effects was determined using minP permutation tests (36) with 10 000 replications to obtain multiple comparison corrected *P* values. Statistical significance of interactions was determined using Bonferroni correction (37). VNTR genotypes were analysed categorically based on the number of repeats (≥6/≥6, <6/≥6 and <6/<6 for the *PTGIS* repeat and ≥5/≥5, <5/≥5 and <5/<5 for the *ALOX5* repeat). We used co-dominant models for each polymorphism, except where genotype cell counts were <10 in either cases or controls in which case we used a dominant model. We did not estimate associations where genotype cell counts were less than five after combining heterozygotes and homozygous

minor allele carriers. All models used sibling kinship as a matching variable and were adjusted for continuous linear age and sex. Polymorphism–NSAID interactions were further adjusted for body mass index (continuous), cigarette smoking in pack–years (continuous) and physical activity [categorized from average metabolic equivalent of task hours into inactive (0–6), less active (6.1–20), active (20.1–44) and very active (>44)]. All analyses were performed using SAS 9.3 or R version 2.13.2.

Results

Distributions of demographic characteristics were mostly similar by case–control status (Table I). Although slightly more cases than controls were male, there were no substantial differences by age, study center, NSAID use, physical activity, body mass index or cigarette smoking.

After correcting for multiple comparisons, no polymorphisms reached statistical significance for associations with CRC or colon cancer risk. However, two SNPs were associated with rectal cancer risk: rs11571364 in *ALOX12* and rs45525634 in *PTGER2* (Table II). Individuals carrying the variant allele of rs11571364 (G>A) in *ALOX12* were at an almost 90% increased risk of rectal cancer ($OR_{het/hzv}$ = 1.87, 95% CI = 1.19–2.95). In contrast, those carrying the variant allele of rs45525634 (G>T) in *PTGER2* had an ~50% decreased risk of rectal cancer ($OR_{het/hzv}$ = 0.49, 95% CI = 0.29–0.82). All other associations with CRC, colon and rectal cancer risk are presented in Supplementary Table 1, available at *Carcinogenesis* Online.

Although current NSAID use was not associated with a statistically significant reduced risk of CRC in this study population when compared with never or former use (OR = 0.86, 95% CI = 0.71–1.04, P = 0.11), after correcting for multiple comparisons we observed an interaction (P = 0.03) between NSAID use and the intronic SNP rs2920421 (G>A) in *ALOX12* on the risk of CRC (Table III). Among those with heterozygous genotypes for rs2920421, current NSAID users had an ~40% decreased risk of CRC (OR_{het} = 0.60, 95% CI = 0.45–0.80) compared with never or former users, whereas this association was null among individuals with homozygous wild-type genotypes (OR_{hzw} = 1.12, 95% CI = 0.86–1.46) and not significantly reduced among homozygous variant genotypes (OR_{hzv} = 0.83, 95% CI = 0.45–1.51). We observed similar effect sizes for colon and rectal cancer risk separately, though these associations were not statistically significant after multiple comparison adjustment (Supplementary Table 2a and b, available at *Carcinogenesis* Online). Results for all other potential interactions for CRC, colon and rectal cancer risk are presented in Supplementary Table 2a and b, available at *Carcinogenesis* Online.

Table I. Selected characteristics of colorectal cancer cases and unaffected sibling controls

	Controls (N = 2592)		Cases (N = 1621)	
	N	%	*N*	%
Age				
Years (mean ± SD)	53.9 ± 11.7		53.6 ± 10.8	
Sex				
Male	1158	44.7	833	51.4
Center				
Ontario	499	19.3	301	18.6
Los Angeles	461	17.8	335	20.7
Australia	594	22.9	327	20.2
Hawaii	8	0.3	7	0.4
Mayo	474	18.3	251	15.5
Seattle	556	21.5	400	24.7
NSAID use				
Never/former	1974	76.8	1265	78.3
Current	595	23.2	351	21.7
Physical activity				
Inactive	598	24.5	383	24.8
Less active	702	28.8	433	28.0
Active	582	23.9	389	25.2
Very active	558	22.9	340	22.0
BMI				
kg/m^2 (mean ± SD)	26.7 ± 5.2		27.2 ± 5.5	
Cigarette smoking				
Pack–years (mean ± SD)	11.7 ± 19.4		12.9 ± 19.7	
Tumor site				
Colon				
Right	—	—	542	33.4
Left	—	—	466	28.7
NOS	—	—	76	4.7
Rectal	—	—	537	33.1

BMI, body mass index; NOS, not otherwise specified.

Discussion

We examined the association between CRC risk, NSAID use, and 192 candidate and tagSNPs and two VNTRs within 17 candidate genes involved in prostaglandin synthesis and related pathways. We observed that variants in two intronic SNPs, rs11571364 in *ALOX12* and rs45525634 in *PTGER2*, were associated with rectal cancer risk. Our observation that these SNPs were associated with rectal cancer risk and not colon or combined colorectal cancer risk reflects the likelihood that colon and rectal cancer have unique etiological mechanisms (38,39). A post-hoc case–case comparison suggested a marginally significant difference in relative risk between colon and rectal cancers for rs11571364 in *ALOX12* (P = 0.05), yet less so for rs45525634 in *PTGER2* (P = 0.09) (data not shown). We observed an interaction between current NSAID use and rs2920421 in *ALOX12* in relation to risk of CRC. A post-hoc case–case comparison of the interaction between this SNP and current NSAID use indicated there was no difference in the association between colon and rectal cancers (P = 0.20) (data not shown). The SNPs for which we observed statistically significant associations in *ALOX12* (rs2920421 and rs11571364) and *PTGER2* (rs45525634) were not in high LD with any of the other genotyped SNPs within these genes among controls (all pairwise $r^2 < 0.65$).

We observed that current NSAID users had a decreased risk of CRC compared with never or former users among those with heterozygous genotypes for rs2920421 in *ALOX12*. We conducted a post-hoc analysis with NSAID use categorized as never, former and current. When compared with never users the risk of CRC was reduced among current NSAID users (OR = 0.88, 95% CI = 0.72–1.07) and null among former users (OR = 1.07, 95% CI = 0.88–1.29), though the overall association between NSAID use and CRC risk was not statistically significant (global P = 0.22). When examining the interaction between NSAID use and rs2920421, we observed that current NSAID users with heterozygous genotypes had an ~40% decreased risk of CRC (OR_{het} = 0.61, 95% CI = 0.45–0.83) while there was no association for former NSAID users with heterozygous genotypes (OR_{het} = 1.07, 95% CI = 0.79–1.44) when compared with never users. Among individuals with homozygous wild-type genotypes, this association was null when comparing former users (OR_{hzw} = 1.14, 95% CI = 0.86–1.50) and current users (OR_{hzw} = 1.17, 95% CI = 0.89–1.54) to never users. Among individuals with homozygous variant genotypes, this association was modestly, although not statistically significantly, reduced when comparing former users (OR_{hzv} = 0.84, 95% CI = 0.46–1.52) and current users (OR_{hzv} = 0.79, 95% CI = 0.43–1.47) to never users. Overall, our results suggest that the expected protective association with NSAIDs is present only among subjects with variant genotypes. Variants in *ALOX12* could modify the effect of NSAIDs on CRC risk, with this association likely being driven by current NSAID use. However, larger sample sizes are needed to verify these results.

ALOX12 is one of three main lipoxygenases responsible for mediating inflammatory responses and converting AA into leukotrienes and other eicosanoids (16,40–42). Further, *ALOX12* is up-regulated in CRC tumour tissues and promotes vascular endothelial

Table II. Statistically significant[a] associations between polymorphisms and rectal cancer risk

		Rectal cancer						
		Controls		Cases		OR[b]	95% CI	*P*[c]
		N	%	*N*	%			
ALOX12								
rs11571364	G/G	762	87.8	429	83.3	ref	—	0.03
Intron	G/A or A/A	106	12.2	86	16.7	1.87	1.19–2.95	
PTGER2								
rs45525634	G/G	764	87.9	468	91.2	ref	—	0.03
Intron	G/T or TT	105	12.1	45	8.8	0.49	0.29–0.82	

[a]Statistical significance considered a minP *P* value ≤ 0.05.
[b]Adjusted for continuous linear age and sex.
[c]Multiple comparisons corrected *P* value from a minP permutation test with 10 000 replications.

Table III. Statistically significant[a] polymorphism–NSAID use interactions and colorectal cancer risk

		NSAID use								
		Never/former				Current[b]				*P*[d]
		Controls	Cases	OR[c]	95% CI	Controls	Cases	OR[c]	95% CI	
ALOX12										
rs2920421	G/G	920	586	ref	—	257	183	1.12	0.86–1.46	0.03
Intron	G/A	818	541	ref	—	268	132	0.60	0.45–0.80	
	A/A	203	124	ref	—	59	29	0.83	0.45–1.51	
rs2920421[e]	G/G	920	586	ref	—	257	183	1.12	0.86–1.46	0.03
Intron	G/A	818	541	1.16	0.95–1.43	268	132	0.70	0.51–0.95	
	A/A	203	124	1.09	0.77–1.53	59	29	0.90	0.49–1.65	

BMI, body mass index.
[a]Statistical significance considered a Bonferroni-adjusted *P* value ≤ 0.05.
[b]Current NSAID use defined as use two or more times per week for at least 1 month in the 2 years prior to study enrollment.
[c]Adjusted for continuous linear age, BMI, and smoking as well as sex and physical activity (categorized from average MET hours into inactive, less active, active and very active).
[d]Bonferroni-adjusted interaction *P* value.
[e]Results presented using a common reference group.

growth factor production in tumour stroma, which contributes to angiogenesis and the motility of cancer cells (43,44). Of interest, the SNP in *ALOX12* for which we observed an interaction between current NSAID use and CRC risk (rs2920421) is in high LD (r^2 = 1.0) with another SNP (rs2440129) that has been associated with microRNA expression levels in adipose tissue and body mass index in genome-wide association studies (45). Although genome-wide association studies have not observed associations between CRC risk and variants in *ALOX12*, results from previous candidate-gene studies suggest plausible associations. In a recent case–control study of colon and rectal cancer risk (*N* colon cases = 1574, *N* rectal cases = 791, *N* controls = 2,969), there was evidence that fatty acid intake and aspirin use modified the associations between two variants in *ALOX12*, the intronic SNP rs11571339 and the coding SNP rs312462 (L634L), and the risk of rectal cancer (46). Another study of 2300 Chinese CRC cases and controls observed that the coding SNP rs1126667 (G261A) in *ALOX12* was associated with an increased risk of CRC, and further observed an interaction between this SNP and rs689466 in *PTGS2* on CRC risk (47). A study assessing the association of variants in *ALOX12* and *ALOX5* with CRC risk among 162 cases and 211 controls reported a borderline association between rs1126667 and CRC risk (48). However, two other studies reported no association between this SNP and CRC risk (15,49). In the current study, rs1126667 was associated with a decreased risk of CRC ($OR_{AA\ versus\ wtGG}$ = 0.71, 95% CI 0.55–0.93); however, this association was not statistically significant after correcting for multiple comparisons. Nonetheless, our study presents novel results for variants in *ALOX12*, as no prior studies have reported an association between rs11571364 and rectal cancer risk or an interaction between NSAID use and rs2920421 on the risk of CRC.

This study is also the first to report an association between rectal cancer risk and the intronic SNP rs45525634 in *PTGER2*. Based on data from the Encyclopedia of DNA Elements (ENCODE) Consortium (50), rs45525634 may regulate transcription. *PTGER2* encodes the receptor EP2 for PGE_2 (21,51–55), which is thought to be critical in CRC development as it promotes angiogenesis as well as the survival, motility and invasiveness of cancer cells (52–57). In a recent study, overexpression of *PTGER2* was associated with high microsatellite instability in CRC tumours (58). EP2 also plays an important role in colorectal carcinogenesis, having been shown to contribute to intestinal polyps and to affect tumour growth in mice (59–61). Genome-wide association studies have not identified SNPs in *PTGER2* as associated with CRC risk, though some candidate-gene studies have reported associations. In a study of 1225 CRC cases and 2032 controls, the intergenic SNP rs17831718 was associated with a roughly 30% decreased risk of CRC (OR = 0.73, 95% CI: 0.58–0.91) (62). Moreover, this study observed a statistically significant inverse association (*P* = 0.006) between CRC risk and the *PTGER2* GGG haplotype based on the SNPs rs17831718, rs17125362 and rs1254580. Another recent large study observed an increased polyp risk for rs17125318, an intergenic SNP close to *PTGER2* (*P* = 0.008) (63). In our prior study examining adenoma cases and polyp-free controls, we observed the intronic SNP rs33993630 in *PTGER2* to be associated with a reduced risk of colorectal adenoma (OR = 0.71, 95% CI: 0.52–0.99) (21). In the present study, those carrying the variant allele for rs33993630 had a

reduced risk of rectal cancer (OR = 0.49, 95% CI: 0.29–0.82, $P = 0.02$); however, this association was not statistically significant after correcting for multiple comparisons ($P = 0.07$). Likewise, we observed no evidence of an interaction between rs33993630 and NSAID use on CRC, colon or rectal cancer risk.

This study is the first to examine associations and NSAID use interactions between CRC risk and the functional VNTRs in *ALOX5* and *PTGIS*. Our previous study observed an increased risk of colorectal polyps associated with the *PTGIS* VNTR ($OR_{<6/<6 \text{ versus } 6/6}$ = 1.90, 95% CI: 1.09–3.30), though not with the *ALOX5* VNTR (34). In the current study, neither the *ALOX5* nor *PTGIS* VNTR was associated with CRC risk, nor did we observe evidence of interactions with NSAID use.

Although we observed an association only between rectal cancer risk and variants in *ALOX12* and *PTGER2*, previous studies have shown variants in other genes involved in prostaglandin synthesis and related pathways to be associated with CRC risk. For example, prior studies have suggested associations between CRC risk and variants in the cytokines *IL6* and *TNF* (64–66) as well as *PTGS2* (*COX2*), including rs689466, rs20417 and rs4648298 (47,67). Further, a prior study of variants in *ALOX5*, *ALOX12*, *PTGS1* and *PTGS2* observed two SNPs both in the flanking 5′-untranslated region of *ALOX5*, rs6413416 and rs4986832, to be associated with colon cancer risk among Caucasians (15). We assessed 21 SNPs in *ALOX5* in this study, including rs4986832, but none was significantly associated with CRC, colon or rectal cancer risk after adjustment for multiple comparisons.

There were some potential limitations to this study. We observed that current NSAID use was associated with a modestly and non-significantly reduced risk of CRC compared with never or former use (OR = 0.86, 95% CI: 0.71–1.04; $P = 0.11$); therefore, we may have been limited in our ability to detect interactions between current NSAID use and the polymorphisms analysed. It is possible that we missed relevant variation in the genes we examined due to incomplete coverage, though this is unlikely given our thorough candidate and tagSNP approach. We may also have missed variation by not examining deletions, copy number variation or additional variants in repeat regions; however, we were unable to examine some of this variation given our genotyping methods. Finally, although assays may have misclassified or failed to detect variation in the genes examined for this study, this was not likely an issue since we had high concordance among duplicate samples.

There were several strengths to this study. Our use of a combined candidate and tagSNP approach enabled comprehensive coverage of most of our genes of interest, including an assessment of VNTRs in *ALOX5* and *PTGIS*. By using data from the CCFR, this study had large numbers of cases and controls as well as access to extensive data on demographic and lifestyle factors, including NSAID use. These design features allowed us to examine the association between polymorphism–NSAID interactions and CRC, colon and rectal cancer risk. Further, although this study made a number of comparisons, all results were interpreted after correcting for multiple comparisons.

Results from this study suggest that genetic variation in *ALOX12* and *PTGER2* may be associated with the risk of rectal cancer. In addition, this study suggests that current use of NSAIDs may interact with variants in *ALOX12* to modify CRC risk. As these results may aid in the development of genetically targeted cancer prevention strategies with NSAIDs, further studies are warranted that examine the association between CRC risk, NSAID use, and polymorphisms in genes involved in prostaglandin synthesis and related pathways.

Supplementary material

Supplementary Tables 1 and 2 can be found at http://carcin.oxfordjournals.org/

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References

1. Terzić,J. *et al.* (2010) Inflammation and colon cancer. *Gastroenterology*, **138**, 2101–2114.e5.
2. Ulrich,C.M. *et al.* (2006) Non-steroidal anti-inflammatory drugs for cancer prevention: promise, perils and pharmacogenetics. *Nat. Rev. Cancer*, **6**, 130–140.
3. Rizzo,A. *et al.* (2011) Intestinal inflammation and colorectal cancer: a double-edged sword? *World J. Gastroenterol.*, **17**, 3092–3100.
4. Kraus,S. *et al.* (2009) Inflammation and colorectal cancer. *Curr. Opin. Pharmacol.*, **9**, 405–410.
5. Rhodes,J.M. *et al.* (2002) Inflammation and colorectal cancer: IBD-associated and sporadic cancer compared. *Trends Mol. Med.*, **8**, 10–16.
6. Hanahan,D. *et al.* (2011) Hallmarks of cancer: the next generation. *Cell*, **144**, 646–674.
7. Mantovani,A. (2009) Cancer: inflaming metastasis. *Nature*, **457**, 36–37.
8. Sandler,R.S. *et al.* (2003) A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. *N. Engl. J. Med.*, **348**, 883–890.
9. Baron,J.A. *et al.* (2003) A randomized trial of aspirin to prevent colorectal adenomas. *N. Engl. J. Med.*, **348**, 891–899.
10. Baron,J.A. *et al.*; APPROVe Trial Investigators. (2006) A randomized trial of rofecoxib for the chemoprevention of colorectal adenomas. *Gastroenterology*, **131**, 1674–1682.
11. Baron,J.A. (2009) Aspirin and NSAIDs for the prevention of colorectal cancer. *Recent Results Cancer Res.*, **181**, 223–229.
12. Wang,D. *et al.* (2010) The role of COX-2 in intestinal inflammation and colorectal cancer. *Oncogene*, **29**, 781–788.
13. Greenhough,A. *et al.* (2009) The COX-2/PGE2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. *Carcinogenesis*, **30**, 377–386.
14. Cebola,I. *et al.* (2012) Epigenetic deregulation of the COX pathway in cancer. *Prog. Lipid Res.*, **51**, 301–313.
15. Goodman,J.E. *et al.* (2004) Arachidonate lipoxygenase (ALOX) and cyclooxygenase (COX) polymorphisms and colon cancer risk. *Carcinogenesis*, **25**, 2467–2472.
16. Shureiqi,I. *et al.* (2001) Lipoxygenase modulation to reverse carcinogenesis. *Cancer Res.*, **61**, 6307–6312.
17. Rigas,B. *et al.* (1993) Altered eicosanoid levels in human colon cancer. *J. Lab. Clin. Med.*, **122**, 518–523.
18. Boland,C.R. (2010) Chronic inflammation, colorectal cancer and gene polymorphisms. *Dig. Dis.*, **28**, 590–595.
19. Coghill,A.E. *et al.* (2011) Genetic variation in inflammatory pathways is related to colorectal cancer survival. *Clin. Cancer Res.*, **17**, 7139–7147.
20. Poole,E.M. *et al.* (2007) Genetic variability in prostaglandin synthesis, fish intake and risk of colorectal polyps. *Carcinogenesis*, **28**, 1259–1263.
21. Poole,E.M. *et al.* (2010) Genetic variation in prostaglandin E2 synthesis and signaling, prostaglandin dehydrogenase, and the risk of colorectal adenoma. *Cancer Epidemiol. Biomarkers Prev.*, **19**, 547–557.
22. Poole,E.M. *et al.* (2012) Genetic variability in IL23R and risk of colorectal adenoma and colorectal cancer. *Cancer Epidemiol.*, **36**, e104–e110.

23. Ulrich,C.M. *et al.* (2004) Polymorphisms in PTGS1 (=COX-1) and risk of colorectal polyps. *Cancer Epidemiol. Biomarkers Prev*., **13**, 889–893.
24. Ulrich,C.M. *et al.* (2005) PTGS2 (COX-2) -765G > C promoter variant reduces risk of colorectal adenoma among nonusers of nonsteroidal anti-inflammatory drugs. *Cancer Epidemiol. Biomarkers Prev*., **14**, 616–619.
25. Algra,A.M. *et al.* (2012) Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials. *Lancet Oncol*., **13**, 518–527.
26. Flossmann,E. *et al.*; British Doctors Aspirin Trial and the UK-TIA Aspirin Trial. (2007) Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. *Lancet*, **369**, 1603–1613.
27. Dubé,C. *et al.*; U.S. Preventive Services Task Force. (2007) The use of aspirin for primary prevention of colorectal cancer: a systematic review prepared for the U.S. Preventive Services Task Force. *Ann. Intern. Med*., **146**, 365–375.
28. Bigler,J. *et al.* (2001) CYP2C9 and UGT1A6 genotypes modulate the protective effect of aspirin on colon adenoma risk. *Cancer Res*., **61**, 3566–3569.
29. Newcomb,P.A. *et al.*; Colon Cancer Family Registry. (2007) Colon Cancer Family Registry: an international resource for studies of the genetic epidemiology of colon cancer. *Cancer Epidemiol. Biomarkers Prev*., **16**, 2331–2343.
30. Levine,A.J. *et al.* (2010) A candidate gene study of folate-associated one carbon metabolism genes and colorectal cancer risk. *Cancer Epidemiol. Biomarkers Prev*., **19**, 1812–1821.
31. Thorisson,G.A. *et al.* (2005) The International HapMap Project Web site. *Genome Res*., **15**, 1592–1593.
32. Sherry,S.T. *et al.* (2001) dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res*., **29**, 308–311.
33. Chevalier,D. *et al.* (2001) Characterization of new mutations in the coding sequence and 5'-untranslated region of the human prostacylcin synthase gene (CYP8A1). *Hum. Genet*., **108**, 148–155.
34. Poole,E.M. *et al.* (2006) Prostacyclin synthase and arachidonate 5-lipoxygenase polymorphisms and risk of colorectal polyps. *Cancer Epidemiol. Biomarkers Prev*., **15**, 502–508.
35. Sayers,I. *et al.* (2003) Promoter polymorphism in the 5-lipoxygenase (ALOX5) and 5-lipoxygenase-activating protein (ALOX5AP) genes and asthma susceptibility in a Caucasian population. *Clin. Exp. Allergy*, **33**, 1103–1110.
36. Dudoit,S. *et al.* (2004) Multiple testing. Part I. Single-step procedures for control of general type I error rates. *Stat. Appl. Genet. Mol. Biol*., **3**, Article13.
37. Murcray,C.E. *et al.* (2009) Gene-environment interaction in genome-wide association studies. *Am. J. Epidemiol*., **169**, 219–226.
38. Slattery,M.L. *et al.* (2005) Associations between apoE genotype and colon and rectal cancer. *Carcinogenesis*, **26**, 1422–1429.
39. Slattery,M.L. *et al.* (2007) IL6 genotypes and colon and rectal cancer. *Cancer Causes Control*, **18**, 1095–1105.
40. Wecksler,A.T. *et al.* (2009) Mechanistic investigations of human reticulocyte 15- and platelet 12-lipoxygenases with arachidonic acid. *Biochemistry*, **48**, 6259–6267.
41. Sears,D.D. *et al.* (2009) 12/15-lipoxygenase is required for the early onset of high fat diet-induced adipose tissue inflammation and insulin resistance in mice. *PLoS One*, **4**, e7250.
42. Cole,B.K. *et al.* (2012) 12/15-Lipoxygenase signaling in the endoplasmic reticulum stress response. *Am. J. Physiol. Endocrinol. Metab*., **302**, E654–E665.
43. Szefel,J. *et al.* (2011) Eicosanoids in prevention and management of diseases. *Curr. Mol. Med*., **11**, 13–25.
44. Klampfl,T. *et al.* (2012) Up-regulation of 12(S)-lipoxygenase induces a migratory phenotype in colorectal cancer cells. *Exp. Cell Res*., **318**, 768–778.
45. Parts,L. *et al.*; MuTHER Consortium. (2012) Extent, causes, and consequences of small RNA expression variation in human adipose tissue. *PLoS Genet*., **8**, e1002704.
46. Habermann,N. *et al.* (2013) PTGS1, PTGS2, ALOX5, ALOX12, ALOX15, and FLAP SNPs: interaction with fatty acids in colon cancer and rectal cancer. *Genes Nutr*., **8**, 115–126.
47. Tan,W. *et al.* (2007) Associations of functional polymorphisms in cyclooxygenase-2 and platelet 12-lipoxygenase with risk of occurrence and advanced disease status of colorectal cancer. *Carcinogenesis*, **28**, 1197–1201.
48. Gong,Z. *et al.* (2007) Common polymorphisms in 5-lipoxygenase and 12-lipoxygenase genes and the risk of incident, sporadic colorectal adenoma. *Cancer*, **109**, 849–857.
49. Küry,S. *et al.* (2008) Low-penetrance alleles predisposing to sporadic colorectal cancers: a French case-controlled genetic association study. *BMC Cancer*, **8**, 326.
50. Rosenbloom,K.R. *et al.* (2012) ENCODE whole-genome data in the UCSC Genome Browser: update 2012. *Nucleic Acids Res*., **40**(Database issue), D912–D917.
51. Obermajer,N. *et al.* (2011) Positive feedback between PGE2 and COX2 redirects the differentiation of human dendritic cells toward stable myeloid-derived suppressor cells. *Blood*, **118**, 5498–5505.
52. Kamei,D. *et al.* (2003) Potential role of microsomal prostaglandin E synthase-1 in tumorigenesis. *J. Biol. Chem*., **278**, 19396–19405.
53. Amano,H. *et al.* (2009) Roles of a prostaglandin E-type receptor, EP3, in upregulation of matrix metalloproteinase-9 and vascular endothelial growth factor during enhancement of tumor metastasis. *Cancer Sci*., **100**, 2318–2324.
54. Kopp,K.L. *et al.* (2010) COX-2-dependent PGE(2) acts as a growth factor in mycosis fungoides (MF). *Leukemia*, **24**, 1179–1185.
55. Wang,D. *et al.* (2010) Eicosanoids and cancer. *Nat. Rev. Cancer*, **10**, 181–193.
56. Sheng,H. *et al.* (2001) Prostaglandin E2 increases growth and motility of colorectal carcinoma cells. *J. Biol. Chem*., **276**, 18075–18081.
57. Wang,D. *et al.* (2004) Prostaglandin E(2) promotes colorectal adenoma growth via transactivation of the nuclear peroxisome proliferator-activated receptor delta. *Cancer Cell*, **6**, 285–295.
58. Baba,Y. *et al.* (2010) PTGER2 overexpression in colorectal cancer is associated with microsatellite instability, independent of CpG island methylator phenotype. *Cancer Epidemiol. Biomarkers Prev*., **19**, 822–831.
59. Sonoshita,M. *et al.* (2001) Acceleration of intestinal polyposis through prostaglandin receptor EP2 in Apc(Delta 716) knockout mice. *Nat. Med*., **7**, 1048–1051.
60. Seno,H. *et al.* (2002) Cyclooxygenase 2- and prostaglandin E(2) receptor EP(2)-dependent angiogenesis in Apc(Delta716) mouse intestinal polyps. *Cancer Res*., **62**, 506–511.
61. Yang,L. *et al.* (2003) Cancer-associated immunodeficiency and dendritic cell abnormalities mediated by the prostaglandin EP2 receptor. *J. Clin. Invest*., **111**, 727–735.
62. Hoeft,B. *et al.* (2010) Polymorphisms in fatty-acid-metabolism-related genes are associated with colorectal cancer risk. *Carcinogenesis*, **31**, 466–472.
63. Edwards,T.L. *et al.* (2012) A study of prostaglandin pathway genes and interactions with current nonsteroidal anti-inflammatory drug use in colorectal adenoma. *Cancer Prev. Res. (Phila)*., **5**, 855–863.
64. Landi,S. *et al.*; Bellvitge Colorectal Cancer Study Group. (2003) Association of common polymorphisms in inflammatory genes interleukin (IL)6, IL8, tumor necrosis factor alpha, NFKB1, and peroxisome proliferator-activated receptor gamma with colorectal cancer. *Cancer Res*., **63**, 3560–3566.
65. Gunter,M.J. *et al.* (2006) Inflammation-related gene polymorphisms and colorectal adenoma. *Cancer Epidemiol. Biomarkers Prev*., **15**, 1126–1131.
66. Theodoropoulos,G. *et al.* (2006) Relation between common polymorphisms in genes related to inflammatory response and colorectal cancer. *World J. Gastroenterol*., **12**, 5037–5043.
67. Cox,D.G. *et al.*; Bellvitge Colorectal Cancer Study Group. (2004) Polymorphisms in prostaglandin synthase 2/cyclooxygenase 2 (PTGS2/COX2) and risk of colorectal cancer. *Br. J. Cancer*, **91**, 339–343.

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