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ORIGINAL PAPER

COX-1 (*PTGS1*) and COX-2 (*PTGS2*) polymorphisms, NSAID interactions, and risk of colon and rectal cancers in two independent populations

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Abstract
Purpose Nonsteroidal anti-inflammatory drugs (NSAIDs) target the prostaglandin H synthase enzymes, cyclooxygenase (COX)-1 and COX-2, and reduce colorectal cancer risk. Genetic variation in the genes encoding these enzymes may be associated with changes in colon and rectal cancer risk and in NSAID efficacy.
Methods We genotyped candidate polymorphisms and tag SNPs in *PTGS1* (COX-1) and *PTGS2* (COX-2) in a population-based case–control study (Diet, Activity and Lifestyle Study, DALS) of colon cancer ($n = 1{,}470$ cases/1,837 controls) and rectal cancer ($n = 583/775$), and independently among cases and controls from the Colon Cancer Family Registry (CCFR; colon $n = 959/1{,}535$, rectal $n = 505/839$).
Results In *PTGS2*, a functional polymorphism (−765G>C; rs20417) was associated with a twofold increased rectal cancer risk ($p = 0.05$) in the DALS. This association replicated with a significant nearly fivefold increased risk of rectal cancer in the CCFR study ($OR_{CC\ vs.\ GG} = 4.88$; 95 % CI 1.54–15.45; $OR_{GC\ vs.\ GG} = 1.36$; 95 %CI 0.95–1.94). Genotype–NSAID interactions were observed in the DALS for *PTGS1* and rectal cancer risk and for *PTGS2* and colon cancer risk, but were no longer significant after correcting for multiple comparisons and did not replicate in the CCFR. No significant associations between *PTGS1* polymorphisms and colon or rectal cancer risk were observed.

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Conclusions These findings suggest that polymorphisms in *PTGS2* may be associated with rectal cancer risk and impact the protective effects of NSAIDs.

Keywords Colorectal cancer · PTGS · COX · Genetic association · NSAID · Aspirin · Polymorphism

Introduction

Inflammation is thought to play a major role in the development and progression of colorectal cancer. The use of nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, reduces the risk of colorectal cancer [1]. NSAIDs inhibit the prostaglandin H synthase (PTGS) enzymes, which convert arachidonic acid into prostaglandins. Several prostaglandins, primarily PGE_2, have been implicated in colorectal carcinogenesis [2]. Although both PTGS isoforms, cyclooxygenase (COX)-1 and COX-2, catalyze the same reactions and share approximately 60 % amino acid identity, they are encoded by distinct genes and differ substantially in their expression and regulation [3, 4]. COX-1 is constitutively expressed and is important for "housekeeping" functions, whereas COX-2 is typically an inducible enzyme expressed in cells responding to inflammatory or proliferative stimuli [3].

Several lines of evidence indicate that COX-2 facilitates colorectal carcinogenesis. COX-2 is overexpressed in up to 90 % of colon carcinomas and 40 % of a precursor lesion, colorectal adenoma [5–7]. Aspirin decreases the risk of colorectal cancers that express high levels of COX-2 but has little effect on the risk of tumors that have little or no COX-2 [8]. Further, in Min mice, selective inhibition of COX-2 or deletion of the *PTGS2* gene results in a substantial reduction in polyp development and tumorigenesis, providing evidence for COX-2 involvement in carcinogenesis that is already at the stage of precursor lesions [9]. COX-2 has also been shown to activate co-carcinogens through oxidation [14].

There is also accumulating evidence to support the proposal [10] that COX-1, specifically the platelet enzyme, is involved in colorectal tumorigenesis. First, there is the decreased incidence and mortality of colorectal cancer that are associated with low doses of aspirin [11], doses that selectively and persistently inhibit COX-1 in anucleate platelets [12]. Oral low-dose aspirin (80–100 mg) produces a transient pulse of the drug in the blood that peaks at only 1–3 μM, with a $t_{1/2}$ of ~20 min [13, 14]. Given that aspirin's IC_{50} for human COX-2 is ~15 μM [15, 16], low-dose aspirin is likely to give little if any prolonged inhibition of COX-2 activity in nucleated cells, which readily replace any acetylated COX-2 protein. A second observation linking COX-1 to colorectal carcinogenesis is that knockout of the *PTGS1* gene markedly decreases the incidence of polyposis in Min mice [17]. Thus, COX-1 and COX-2 appear to have distinct roles in colorectal carcinogenesis, and polymorphisms in the genes encoding these enzymes (*PTGS1* and *PTGS2*) might plausibly affect cancer risk. We have previously shown that polymorphisms related to prostaglandin synthesis affect the risk of colorectal adenoma and may modify the preventive associations with NSAID use [1, 18–22]. In the current analysis, we investigated *PTGS1* and *PTGS2* polymorphisms in relation to the risk of colon and rectal cancers and their potential interactions with NSAID use in a large population-based study of colon and rectal cancer risk and validated those findings in a second, independent, study. The results indicate that a *PTGS2* functional promoter variant is reproducibly associated with a two- to fourfold increased risk of rectal, but not colon, cancer.

Materials and methods

Study design and data collection

The analyses are based on a case/unrelated-control study of colon and rectal cancers and a population-based case/unaffected-sibling-control study, here restricted to non-Hispanic whites (NHW). Methods, described in detail elsewhere [23–27], are described briefly here. Study population characteristics are showed in Table 1.

Diet, Activity and Lifestyle Study (DALS) of colon and rectal cancer populations (discovery study)

NHW colon cancer cases ($n = 1{,}470$) and controls ($n = 1{,}837$) and rectal cancer cases ($n = 583$) and controls ($n = 775$) were recruited from Utah, the Northern California Kaiser Permanente Medical Care Program (KPMCP), and metropolitan Minneapolis–St. Paul, Minnesota (colon cases only). Eligible participants were aged 30–79 years with no previous diagnosis of colorectal cancer, familial adenomatous polyposis, Crohn's disease, or ulcerative colitis. Colon cancer cases were diagnosed between 1991 and 1994 [23], and rectal cancer cases between 1997 and 2001 [24, 25]. Diet, physical activity,

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Table 1 Characteristics of the DALS and CCFR study populations

	DALS									
	Colon cancer				*p*	Rectal cancer				*p*
	Controls		Cases			Controls		Cases		
	(*n* = 1,837)		(*n* = 1,470)			(*n* = 775)		(*n* = 583)		
	N	%	*N*	%		*N*	%	*N*	%	
Age					NA[b]					NA[b]
Mean ± SD	65.1 ± 10.3	65.1 ± 9.7		62.6 ± 10.5	62.3 ± 10.8					
Location					NA					NA
Distal			724	49.3						
Proximal			710	48.3						
Rectal								583	100.0	
Sex					NA[b]					NA[b]
Female	868	47.3	642	43.7		347	44.8	237	40.7	
Male	969	52.7	828	56.3		428	55.2	346	59.3	
Study site					<0.001					0.51
Kaiser Northern California	695	37.8	662	45.0		449	57.9	349	59.9	
Minnesota	800	43.5	566	38.5						
Utah	342	18.6	242	16.5		326	42.1	234	40.1	
Current NSAID use[a]					<0.001					0.002
Yes	762	41.7	477	32.7		359	46.6	221	38.1	
No	1,065	58.3	980	67.3		412	53.4	359	61.9	

	CCFR									
	Colon cancer				*p*	Rectal cancer				*p*
	Controls		Cases			Controls		Cases		
	(*n* = 1,535)		(*n* = 959)			(*n* = 839)		(*n* = 505)		
	N	%	*N*	%		*N*	%	*N*	%	
Age					NA[b]					NA[b]
Mean ± SD	54.6 ± 11.8	54.1 ± 11.0		52.7 ± 11.3	51.8 ± 10.1					
Location					NA					NA
Distal			444	46.3						
Proximal			515	53.7						
Rectal								505	100.0	
Sex					NA[b]					NA[b]
Female	871	56.7	505	52.7		446	53.2	216	42.8	
Male	664	43.3	454	47.3		393	46.8	289	57.2	
Study site					0.12					0.16
Ontario	339	22.1	193	20.1		130	15.5	79	15.6	
Los Angeles	269	17.5	189	19.7		125	14.9	85	16.8	
Australia	277	18.0	164	17.1		252	30.0	130	25.7	
Hawaii	3	0.2	3	0.3		4	0.5	3	0.6	
Mayo foundation	314	20.5	169	17.6		151	18.0	76	15.0	
Seattle	333	21.7	241	25.1		177	21.1	132	26.1	
Current NSAID use[a]					0.07					0.08
Yes	373	24.5	218	22.9		173	20.8	92	18.3	
No	1,148	75.5	736	77.1		659	79.2	412	81.7	

Numbers may not total to 100 % due to rounding and missing values

[a] Current NSAID use is defined as current, regular use three times per week for at least 1 month in the DALS and as current, regular use of at least two pills per week for at least 1 month for the CCFR study

[b] NA—this was a matching factor

smoking, anthropometry, medical history, NSAID use, family history of cancer, demographics, race/ethnicity, and reproductive history data were obtained by questionnaire [23, 24, 26, 28–32]. The referent period for the study was 2 years prior to diagnosis for cases and 2 years prior to selection for controls. NSAID use was defined as aspirin/NSAID use at least three times per week for 1 month or more. The colon and rectal cancer populations were recruited separately at different time periods, but are collectively referred to in this manuscript as the DALS as they were parallel study designs.

Colon Cancer Family Registry study (validation study)

Participants were recruited to the Colon Cancer Family Registry (CCFR) from six registry centers: University of Hawaii, Honolulu, Hawaii, USA; Fred Hutchinson Cancer Research Center, Seattle, Washington, USA; Mayo Clinic; University of Southern California Consortium (Dartmouth Medical School, University of Southern California, University of Colorado, University of Arizona, Cleveland Clinic Foundation, University of North Carolina, and University of Minnesota); Cancer Care Ontario, Toronto, Ontario, Canada; and the University of Melbourne, Victoria, Australia. Both population-based and clinic-based ascertainment strategies were used [27], with some centers recruiting all incident cases from population-based cancer registries (i.e., population-based recruiting), whereas others oversampled cases with a family history of colorectal cancer or cases who were diagnosed at a young age (i.e., family-based recruiting), as described in detail previously [27]. The current study includes only population-based participants. All cases were interviewed within 5 years of diagnosis; 73 % of cases were interviewed within 2 years of diagnosis. Standardized questionnaires were used to collect epidemiologic data from study participants on demographic characteristics, race/ethnicity, medical history, NSAID use, family history of cancer, smoking history, selected diet, physical activity, height and weight, and, in women only, reproductive history and hormone use. "Regular NSAID use" was defined as use of aspirin or ibuprofen at least twice per week for 1 month or more [27, 33, 34]. The CCFR study used a case/unaffected-sibling-control design restricted to NHW. Analyses of population-based families included 1,464 cases and 2,374 unaffected siblings after exclusion criteria were applied (see below). Cases included probands and affected relatives diagnosed with primary invasive colorectal cancer from 1998 to 2002. Controls were siblings of cases without a colorectal cancer diagnosis at the time of ascertainment. There were 1,534 sibships in our study. Because some sibships have multiple cases and/or controls, the number of sibships can exceed the total number of cases.

Sibships lacking either a case or an unaffected sibling and cases for whom time-to-interview was more than 5 years were excluded. Also excluded were individuals whose samples were not available for genotyping, who did not have epidemiologic data and duplicate samples, or who had missing genotypes. Informed consent was obtained from all participants. This study was approved by the Institutional Review Board at each CCFR site.

Genotyping

For the DALS, a linkage-disequilibrium (LD)-based tag SNP-selection algorithm [35] was used to identify tag SNPs ($r^2 = 0.90$, MAF > 4 %) representing common genetic variation in *PTGS1* and *PTGS2* in the CEPH population (Utah residents with ancestry from northern and western Europe) [36]. We genotyped 19 polymorphisms in *PTGS1*, including 13 tag SNPs, five candidate SNPs: R8 W (rs1236913), P17L (rs3842787), R149L (rs10306140), L237M (rs5789), and R108Q (rs5787), and one deletion polymorphism (L15–L16del) identified previously through sequencing [37]. *PTGS2* polymorphisms included 15 tag SNPs and two candidate SNPs from the promoter region: −765 G>C (rs20417) and −163 C>G (rs5270). The targeted polymorphisms are shown in Supplementary Table 1. Genotype quality control and exclusion criteria were as described [21]. To ensure adequate gene coverage, multiple SNPs were genotyped from LD bins containing a large number of SNPs. After genotyping was completed, redundant SNPs were removed from the analysis based on LD value ($r^2 > 0.9$) among NHW controls (Supplementary Figure 1). The CCFR study was genotyped for six *PTGS1* and eight *PTGS2* SNPs to provide independent validation of findings from the DALS. SNPs were chosen for the validation study if preliminary analyses in the discovery dataset resulted in an unadjusted p value < 0.10. In addition, all candidate SNPs were genotyped in the CCFR study unless they were monomorphic in the DALS (Supplementary Table 1). The p value cutoff was determined during the preliminary analyses of the DALS dataset and chosen to minimize the number of false-negative SNPs from DALS while reducing the number of SNPs to be tested in the CCFR.

We used the Illumina™ GoldenGate assay to genotype blood-derived DNA in both the DALS and CCFR studies. *PTGS1* SNPs rs5789 (L237M) and rs1236913 (R8W) were confirmed by Taqman allelic discrimination assay in the DALS colon cancer study. The −765 G>C polymorphism in *PTGS2* (rs20417) was genotyped in the CCFR study using a Taqman allelic discrimination assay [18]. *PTGS1* rs3842787 (P17L) and the L15–L16del were genotyped by Sanger sequencing in all studies. Two *PTGS1* tag SNPs that failed QC and three candidate SNPs with a

MAF < 0.2 %: R149L (rs10306140), R108Q (rs5787), and −163 C>G (rs5270), were excluded from subsequent analysis (Supplementary Table 1).

Statistical analysis

Main effects of single SNP

Odds ratios (ORs) and 95 % confidence intervals (CIs) were calculated in the DALS using unconditional logistic regression. Because of the case/unaffected-sibling-control design for the CCFR, conditional logistic regression was used with each sibling set treated as a matched set. All models were restricted to NHW, as they represented >90 % of all study populations and the tag SNP-selection algorithm used was based on the LD structure of the CEPH population, which has ancestry from northern and western Europe. All models were adjusted for continuous age and sex. DALS models were also adjusted for study site. "Main effect" analyses examined the association between each individual SNP and colon or rectal cancer risk. For each of these analyses, likelihood ratio tests (LRTs) were from a 2-degree of freedom (*df*) test where genotypes were modeled using indicator variables for the heterozygous and the homozygous variant genotypes (codominant models) and from a 1-*df* test where homozygous variant and heterozygous genotypes were grouped for analysis (dominant models), which was the case only for SNPs where fewer than ten cases or controls had the homozygous variant genotype. If fewer than five cases or controls had the heterozygous variant genotype, the statistical model was not run. Significance was assessed using LRTs. All tests of statistical significance used a two-sided p value and $\alpha = 0.050$.

Interaction analyses

Interactions were evaluated by taking the product of indicator variables for NSAID use (current vs. never/former) and for genotypes. For SNP–NSAID interactions, a 2-*df* test was used to evaluate the multiplicative interaction term for codominant SNPs and binary NSAID use (current vs. never/former) and a 1-*df* test was used to evaluate the multiplicative interaction term for dominant SNPs and binary NSAID use. Because use of NSAIDs may be associated with other known risk factors for CRC, NSAID interactions were adjusted for additional variables within each study. DALS interactions were additionally adjusted for the following continuous variables: BMI, smoking (cigarettes/day), physical activity (hours/week), dietary calcium (mg/day), calories (kilocalories/day), and dietary fiber (g/day). CCFR interactions were additionally adjusted for BMI (continuous), smoking in pack-years (continuous), and physical activity (categorized from average MET hours into inactive, less active, active, and very active). Aspirin use was also investigated independently. To avoid small-cell counts, the dominant model was used if there were less than ten homozygous variant cases or controls in either NSAID category. If fewer than five cases or controls had the heterozygous variant genotype in either NSAID category, the statistical model was not run. Significance was assessed using likelihood ratio tests (LRTs). Table 3 includes only SNPs genotyped in both studies with a p interaction less than 0.05 in at least one study prior to any multiple-testing correction. The p values presented in Tables 2 and 3 are prior to correction for multiple testing. All analyses were performed using SAS 9.3 or R version 2.13.2.

Multiple-testing corrections

The DALS was treated as the discovery dataset, and multiple-comparison corrected p values were attained for all polymorphisms using minP permutation tests with 10,000 replications [38]. Candidate functional polymorphisms have prespecified hypotheses to impact cancer risk; therefore, multiple-testing corrections are not necessarily applicable to those polymorphisms. The CCFR study served as an independent validation dataset and was not subject to multiple-comparison correction in the primary analyses. A secondary, post hoc multiple-testing correction was performed in the CCFR colon cancer study for genotype–NSAID interactions (Table 3).

Results

Genetic associations

Characteristics (age, sex, site, and NSAID use) of the DALS and CCFR study populations are presented in Table 1. In our analysis, the DALS served as the discovery dataset, and the CCFR study was an independent validation dataset. Table 2 includes only SNPs that were genotyped in both studies. After correcting for multiple comparisons, there were no statistically significant (minP ≤ 0.05) associations between SNPs in *PTGS1* and risk of colon or rectal cancer in the DALS discovery dataset (Table 2). The rare L15–L16 deletion did show a trend toward increased risk in both the DALS and CCFR colon and rectal studies, consistent with previous observations for adenoma [19], but this did not reach statistical significance. Post hoc analyses combining colon and rectal cancers within each study also did not reach significance for this polymorphism (data not shown).

In *PTGS2*, we observed a nearly twofold increase in risk of rectal cancer in the DALS for individuals with the

Table 2 Association between selected *PTGS1* and *PTGS2* polymorphisms and risk of colon and rectal cancers

	Colon cancer							
	Controls		Cases		OR	95 % CI		p^c
	N	%	*N*	%				
PTGS1 candidate polymorphisms								
rs1236913 (R8W C>T)								
DALS[a]								
C/C	1,608	87.6	1,273	86.6	Ref	–	–	0.35
C/T + T/T	227	12.4	197	13.4	1.10	0.90	1.35	
CCFR[b]								
C/C	1,306	87.4	808	87.1	Ref	–	–	0.91
C/T + T/T	188	12.6	120	12.9	1.02	0.72	1.44	
L15–L16 deletion								
DALS[a]								
No deletion	1,770	98.9	1,411	98.7	Ref	–	–	0.63
Deletion	19	1.1	18	1.3	1.17	0.61	2.25	
CCFR[b]								
No deletion	1,478	98.9	918	98.9	Ref	–	–	0.21
Deletion	16	1.1	10	1.1	2.19	0.61	7.83	
rs3842787 (P17L C>T)								
DALS[a]								
C/C	1,546	86.6	1,231	86.2	Ref	–	–	0.94
C/T	228	12.8	187	13.1	1.04	0.84	1.28	
T/T	12	0.7	10	0.7	1.01	0.44	2.36	
CCFR[b]								
C/C	1,308	87.6	792	85.3	Ref	–	–	0.09
C/T + T/T	176	12.4	136	14.6	1.41	0.95	2.09	
rs5789 (L237M C>A)								
DALS[a]								
C/C	1,738	94.7	1,394	94.8	Ref	–	–	0.82
C/A + A/A	97	5.3	76	5.2	0.96	0.71	1.32	
CCFR[b]								
C/C	1,410	93.9	883	94.4	Ref	–	–	0.17
C/A + A/A	92	6.1	52	5.6	0.72	0.44	1.15	
PTGS1 tag SNPs								
rs10306135 (4,331 A>T)								
DALS[a]								
A/A	1,295	73.5	1,009	71.7	Ref	–	–	0.55
A/T	428	24.3	367	26.1	1.09	0.93	1.29	
T/T	40	2.3	32	2.3	1.02	0.64	1.64	
CCFR[b]								
A/A	1,113	74.1	700	74.4	Ref	–	–	0.42
A/T	352	23.4	223	23.7	0.95	0.73	1.24	
T/T	38	2.5	18	1.9	0.61	0.29	1.29	
rs6478565 (15,268 A>G)								
DALS[a]								
A/A	1,197	67.9	972	68.8	Ref	–	–	0.77
A/G	507	28.8	398	28.2	0.96	0.82	1.12	
G/G	58	3.3	42	3.0	0.89	0.59	1.34	

Table 2 continued

	Colon cancer							
	Controls		Cases		OR	95 % CI		p^c
	N	%	*N*	%				
CCFR[b]								
A/A	1,073	69.9	664	69.2	Ref	–	–	0.77
A/G	418	27.2	272	28.4	0.97	0.76	1.23	
G/G	44	2.9	23	2.4	0.79	0.42	1.48	
PTGS2 candidate SNP								
rs20417 (−765 G>C)								
DALS[a]								
G/G	1,232	69.6	979	68.9	Ref	–	–	0.95
G/C	495	28.0	404	28.5	1.02	0.87	1.20	
C/C	44	2.5	37	2.6	1.04	0.67	1.63	
CCFR[b]								
G/G	1,059	71.0	648	69.8	Ref	–	–	0.67
G/C	393	26.3	258	27.8	1.10	0.85	1.43	
C/C	40	2.7	23	2.5	0.89	0.42	1.88	
PTGS2 tag SNPs								
rs4648250 (−1,740 A>G)								
DALS[a]								
A/A	1,715	97.9	1,376	98.6	Ref	–	–	0.09
A/G	37	2.1	19	1.4	0.63	0.36	1.10	
CCFR[b]								
A/A	1,343	99.2	841	98.6	Ref	–	–	0.13
A/G	11	0.8	12	1.4	2.32	0.75	7.15	
rs689466 (−1,195 A>G)								
DALS[a]								
A/A	1,198	67.5	910	64.0	Ref	–	–	0.15
A/G	509	28.7	455	32.0	1.16	1.00	1.36	
G/G	67	3.8	57	4.0	1.10	0.76	1.58	
CCFR[b]								
A/A	958	63.2	619	65.9	Ref	–	–	0.38
A/G	496	32.7	287	30.6	0.83	0.64	1.08	
G/G	63	4.2	33	3.5	0.84	0.49	1.44	
rs20424 (−196 C>G)								
DALS[a]								
C/C	1,726	97.2	1,379	97.2	Ref	–	–	0.91
C/G + G/G	49	2.8	40	2.8	1.02	0.67	1.57	
G/G	1	0.1	0	0.0				
CCFR[b]								
C/C	1,327	97.1	831	96.5	Ref	–	–	**0.03**
C/G + G/G	40	3.0	30	3.5	2.44	1.05	5.67	
rs2745557 (201 G>A)								
DALS[a]								
G/G	1,233	69.6	981	69.5	Ref	–	–	0.98
G/A	492	27.8	394	27.9	1.01	0.86	1.18	
A/A	47	2.7	36	2.6	0.96	0.62	1.50	
CCFR[b]								
G/G	1,048	68.4	649	67.7	Ref	–	–	0.41

Table 2 continued

	Colon cancer							
	Controls		Cases		OR	95 % CI		p^c
	N	%	*N*	%				
G/A	431	28.1	281	29.3	1.10	0.86	1.42	
A/A	54	3.5	29	3.0	0.78	0.42	1.44	
rs4648261 (418 G>A)								
DALS[a]								
G/G	1,664	94.0	1,332	93.9	Ref	–	–	0.90
G/A + A/A	107	6.1	86	6.1	1.02	0.76	1.37	
CCFR[b]								
G/G	1,432	93.3	904	94.3	Ref	–	–	**0.04**
G/A + A/A	103	6.7	55	5.7	0.58	0.35	0.98	
rs4648268 (2,284 G>A)								
DALS[a]								
G/G	1,443	81.2	1,148	80.7	Ref	–	–	0.14
G/A	322	18.1	253	17.8	0.99	0.82	1.19	
A/A	13	0.7	21	1.5	1.98	0.99	3.98	
CCFR[b]								
G/G	1,051	79.0	690	81.2	Ref	–	–	**0.01**
G/A	269	20.2	149	17.5	0.61	0.43	0.86	
A/A	11	0.8	11	1.3	1.39	0.50	3.87	
rs5275 (6,364 T>C)								
DALS[a]								
T/T	725	40.8	599	42.2	Ref	–	–	0.20
T/C	805	45.4	655	46.1	0.99	0.86	1.16	
C/C	245	13.8	166	11.7	0.82	0.66	1.03	
CCFR[b]								
T/T	588	43.4	370	43.2	Ref	–	–	0.71
T/C	601	44.3	378	44.1	0.90	0.69	1.18	
C/C	167	12.3	109	12.7	0.98	0.64	1.51	
	Rectal cancer							
	Controls		Cases		OR	95 % CI		p^c
	N	%	*N*	%				
PTGS1 candidate polymorphisms								
rs1236913 (R8W C>T)								
DALS[a]								
C/C	668	87.3	491	85.7	Ref	–	–	0.34
C/T + T/T	97	12.7	82	14.3	1.17	0.85	1.60	
CCFR[b]								
C/C	720	87.3	414	86.6	Ref	–	–	0.28
C/T + T/T	105	12.7	64	13.4	1.32	0.79	2.18	
L15–L16 deletion								
DALS[a]								
No deletion	752	98.3	559	97.9	Ref	–	–	0.55
Deletion	13	1.7	12	2.1	1.28	0.58	2.83	
CCFR[b]								
No deletion	816	98.9	472	98.7	Ref	–	–	0.22
Deletion	9	1.1	6	1.3	2.89	0.51	16.48	

Table 2 continued

	Rectal cancer							
	Controls		Cases		OR	95 % CI		p^c
	N	%	*N*	%				
rs3842787 (P17L C>T)								
DALS[a]								
C/C	668	87.2	490	85.4	Ref	–	–	0.35
C/T	98	12.8	84	14.7	1.16	0.85	1.59	
T/T	*		*		*			
CCFR[b]								
C/C	724	87.8	413	86.4	Ref	–	–	0.89
C/T + T/T	101	12.2	65	13.6	1.04	0.62	1.73	
rs5789 (L237M C>A)								
DALS[a]								
C/C	732	94.6	551	94.5	Ref	–	–	0.97
C/A + A/A	42	5.5	32	5.5	1.01	0.63	1.62	
CCFR[b]								
C/C	776	94.4	456	93.4	Ref	–	–	0.35
C/A + A/A	46	5.6	32	6.6	1.42	0.67	3.00	
PTGS1 tag SNPs								
rs10306135 (4,331 A>T)								
DALS[a]								
A/A	577	74.5	432	74.4	Ref	–	–	0.91
A/T	197	25.4	149	25.6	1.01	0.79	1.30	
T/T	*		*		*			
CCFR[b]								
A/A	595	72.3	355	72.4	Ref	–	–	0.27
A/T	208	25.3	121	24.7	1.03	0.72	1.48	
T/T	20	2.4	14	2.9	2.04	0.84	4.94	
rs6478565 (15,268 A>G)								
DALS[a]								
A/A	536	69.2	397	68.2	Ref	–	–	0.18
A/G	209	27.0	172	29.6	1.11	0.88	1.42	
G/G	30	3.9	13	2.2	0.60	0.31	1.17	
CCFR[b]								
A/A	580	69.1	343	67.9	Ref	–	–	0.30
A/G	233	27.8	143	28.3	1.07	0.76	1.52	
G/G	26	3.1	19	3.8	1.82	0.85	3.93	
PTGS2 candidate SNP								
rs20417 (−765 G>C)								
DALS[a]								
G/G	553	71.4	433	74.3	Ref	–	–	**0.05**
G/C	211	27.2	134	23.0	0.81	0.63	1.04	
C/C	11	1.4	16	2.7	1.95	0.89	4.26	
CCFR[b]								
G/G	576	70.4	314	65.4	Ref	–	–	**0.01**
G/C	232	28.4	151	31.5	1.36	0.95	1.94	
C/C	10	1.2	15	3.1	4.88	1.54	15.45	

Table 2 continued

	Rectal cancer							
	Controls		Cases		OR	95 % CI		p^c
	N	%	*N*	%				
PTGS2 tag SNPs								
rs4648250 (−1,740 A>G)								
DALS[a]								
A/A	748	98.6	575	99.7	–	–	–	ND
A/G	11	1.4	2	0.3	–	–	–	
CCFR[b]								
A/A	726	99.5	427	99.1	–	–	–	ND
A/G	4	0.5	4	0.9	–	–	–	
rs689466 (−1,195 A>G)								
DALS[a]								
A/A	509	65.7	376	64.7	Ref	–	–	0.87
A/G	237	30.6	185	31.8	1.06	0.83	1.33	
G/G	29	3.7	20	3.4	0.94	0.52	1.68	
CCFR[b]								
A/A	558	67.5	338	68.0	Ref	–	–	0.42
A/G	249	30.1	138	27.8	0.91	0.63	1.31	
G/G	20	2.4	21	4.2	1.46	0.66	3.25	
rs20424 (−196 C>G)								
DALS[a]								
C/C	748	96.6	556	95.4	Ref	–	–	0.22
C/G + G/G	26	3.3	27	4.6	1.41	0.81	2.45	
G/G	1	0.1	0	0.0				
CCFR[b]								
C/C	720	97.0	424	97.0	Ref	–	–	0.24
C/G + G/G	22	3.0	13	3.0	0.56	0.21	1.48	
rs2745557 (201 G>A)								
DALS[a]								
G/G	534	68.9	392	67.5	Ref	–	–	0.45
G/A	214	27.6	174	29.9	1.10	0.87	1.40	
A/A	27	3.5	15	2.6	0.75	0.39	1.42	
CCFR[b]								
G/G	556	66.3	322	63.9	Ref	–	–	0.19
G/A	258	30.8	162	32.1	1.28	0.90	1.84	
A/A	25	3.0	20	4.0	1.88	0.86	4.11	
rs4648261 (418 G>A)								
DALS[a]								
G/G	732	94.6	546	94.1	Ref	–	–	0.70
G/A + A/A	42	5.4	34	5.9	1.10	0.69	1.75	
CCFR[b]								
G/G	769	91.7	478	94.7	Ref	–	–	0.07
G/A + A/A	70	8.3	27	5.3	0.52	0.25	1.08	
rs4648268 (2,284 G>A)								
DALS[a]								
G/G	626	80.8	462	79.2	Ref	–	–	0.47
G/A	149	19.2	121	20.7	1.10	0.84	1.44	
A/A	*		*		*			

Table 2 continued

	Rectal cancer							
	Controls		Cases		OR	95 % CI		p^c
	N	%	*N*	%				
CCFR[b]								
G/G	581	81.0	344	80.4	Ref	–	–	0.41
G/A	136	19.0	84	19.7	1.22	0.76	1.95	
A/A	*		*		*			
rs5275 (6,364 T>C)								
DALS[a]								
T/T	342	44.2	252	43.2	Ref	–	–	0.91
T/C	344	44.4	265	45.5	1.05	0.84	1.32	
C/C	88	11.4	66	11.3	1.02	0.71	1.47	
CCFR[b]								
T/T	299	41.0	182	41.9	Ref	–	–	0.97
T/C	339	46.5	204	47.0	1.01	0.70	1.46	
C/C	91	12.5	48	11.1	1.08	0.59	1.97	

* Dominant model

Only SNPs genotyped in both DALS and CCFR are shown. Additional SNPs genotyped in DALS are in Supplementary Table 2. The dominant model was used when <10 cases or controls had the homozygous variant genotype; modeling was not run when fewer than five cases or controls were heterozygotes

[a] Adjusted for age, sex, and center

[b] Adjusted for age and sex

[c] Global p value from a likelihood ratio test prior to correction for multiple comparisons, with statistically significant p values ($p < 0.050$) shown in bold. No SNPs remained significant in the DALS after correcting for multiple comparisons using minP permutation tests (minP ≤ 0.05)

rs20417 CC genotype (−765 G>C, $OR_{CC\ vs.\ GG} = 1.95$; 95 % CI 0.89–4.26; LRT $p = 0.05$). Although this association was not significant after correcting for multiple testing (minP > 0.05), it did replicate in the independent CCFR study population, with a statistically significant (LRT $p = 0.01$) increased risk of rectal cancer for individuals with the GC or CC genotype (Table 2). Individuals with the CC genotype had an almost fivefold increase in rectal cancer risk ($OR_{CC\ vs.\ GG} = 4.88$; 95 % CI 1.54–15.45). A comparison of rectal cancer risk for the homozygous variant CC genotype to the GG common genotype resulted in p value of 0.09 in the DALS and a p value of 0.01 in the CCFR study (data not shown). In both study populations, the increased risk was limited to rectal cancer. A polytomous regression model found a significant difference between colon and rectal cancer risk (global $p < 0.0001$, data not shown) for this SNP in the DALS. There were no other statistically significant associations between *PTGS2* SNPs and risk of colon or rectal cancer in the DALS.

NSAID interactions

We observed nominally significant (LRT $p \leq 0.05$) genotype–NSAID interactions for SNPs in *PTGS1* in the DALS discovery study (Table 3). First, the benefit of regular NSAID use for reducing rectal cancer risk was limited to those with the PP (CC) genotype for P17L (rs3842787; LRT $p = 0.05$). This is consistent with our previous finding that the benefit of regular NSAID use for reducing adenoma risk was limited to those with the PP genotype [19]. Additionally, we observed that NSAID use was of greater benefit for reducing rectal cancer risk among those carrying the variant allele of either rs10306135 (4,331 A>T, LRT $p = 0.01$) or rs6478565 (15,268 A>G, LRT $p = 0.03$). There is modest linkage disequilibrium between these two SNPs, which may contribute to the similar findings ($r^2 = 0.56$, Supplementary Figure 1). These associations were no longer statistically significant after correcting for multiple testing (minP > 0.05) and did not replicate in the CCFR independent validation study. No significant genotype–NSAID interactions were observed in *PTGS1* in relation to colon cancer risk. Aspirin use alone also showed no significant interactions with *PTGS1* genotypes for colon or rectal cancer in either study (data not shown).

For *PTGS2*, one significant genotype–NSAID interaction was seen in the DALS colon cancer discovery dataset (rs20424; LRT $p = 0.01$), but it was no longer significant after correcting for multiple testing (minP > 0.05), and did

Table 3 Interactions between selected *PTGS1* and *PTGS2* SNPs, NSAID use, and risk of colon and rectal cancers

NSAID Use[a]	Colon cancer									
	Never/former					Current				p^d
	Controls	Cases	OR	95 % CI		Controls	Cases	OR	95 % CI	
PTGS1										
rs3842787 (P17L 50 C>T)										
DALS[b]										
C/C	914	824	Ref	–	–	623	396	0.65	0.55 0.77	0.40
C/T + T/T	128	132	1.16	0.89	1.51	111	63	0.63	0.45 0.87	
CCFR[c]										
C/C	969	605	Ref	–	–	327	182	0.83	0.64 1.07	0.26
C/T + T/T	147	104	1.34	0.85	2.11	37	32	1.66	0.83 3.32	
rs10306135 (4,331 A>T)										
DALS[b]										
A/A	723	662	Ref	–	–	565	337	0.62	0.52 0.73	0.65
A/T	266	247	1.03	0.84	1.27	160	117	0.75	0.57 0.98	
T/T	25	22	1.00	0.55	1.82	15	10	0.69	0.30 1.56	
CCFR[c]										
A/A	822	542	Ref	–	–	279	157	0.82	0.62 1.07	0.69
A/T + T/T	302	182	0.85	0.62	1.18	86	55	0.78	0.49 1.23	
rs6478565 (15,268 A>G)										
DALS[b]										
A/A	663	634	Ref	–	–	526	330	0.61	0.51 0.73	0.88
A/G	309	272	0.92	0.75	1.12	197	121	0.61	0.47 0.79	
G/G	36	28	0.88	0.53	1.48	22	14	0.58	0.29 1.17	
CCFR[c]										
A/A	801	514	Ref	–	–	260	149	0.86	0.65 1.14	0.89
A/G + G/G	347	222	0.85	0.63	1.14	113	69	0.71	0.46 1.08	
PTGS2										
rs20424 (−62 C>G)										
DALS[b]										
C/C	986	918	Ref	–	–	731	448	0.61	0.53 0.72	**0.01**
C/G + G/G	34	22	0.65	0.37	1.14	15	18	1.24	0.61 2.49	
CCFR[c]										
C/C	992	637	Ref	–	–	323	191	0.90	0.69 1.17	0.31
C/G + G/G	25	22	2.75	1.07	7.04	15	8	1.22	0.30 4.90	
NSAID use[a]	Rectal cancer									
	Never/former					Current				p^d
	Controls	Cases	OR	95 % CI		Controls	Cases	OR	95 % CI	
PTGS1										
rs3842787 (P17L 50 C>T)										
DALS[b]										
C/C	350	310	Ref	–	–	314	178	0.61	0.48 0.78	**0.05**
C/T + T/T	57	44	0.88	0.57	1.34	41	37	1.03	0.64 1.66	
CCFR[c]										
C/C	570	335	Ref	–	–	149	78	0.86	0.57 1.28	0.46
C/T + T/T	76	52	1.20	0.66	2.19	23	12	0.71	0.28 1.78	

Table 3 continued

NSAID use[a]	Rectal cancer									
	Never/former					Current				p^d
	Controls	Cases	OR	95 % CI		Controls	Cases	OR	95 % CI	
rs10306135 (4,331 A>T)										
DALS[b]										
A/A	318	256	Ref	–	–	255	174	0.80	0.62	1.04	**0.01**
A/T	93	101	1.33	0.96	1.85	104	47	0.55	0.38	0.82	
T/T	*	*				*	*				
CCFR[c]										
A/A	467	290	Ref	–	–	123	64	0.68	0.43	1.06	0.21
A/T + T/T	182	112	0.86	0.58	1.29	44	23	0.97	0.50	1.88	
rs6478565 (15,268 A>G)										
DALS[b]										
A/A	294	236	Ref	–	–	238	160	0.80	0.61	1.04	**0.03**
A/G	118	123	1.31	0.96	1.79	121	60	0.60	0.42	0.87	
G/G	*	*				*	*				
CCFR[c]										
A/A	456	281	Ref	–	–	120	61	0.75	0.48	1.16	0.61
A/G + G/G	203	131	1.00	0.68	1.48	53	31	0.91	0.48	1.75	
PTGS2										
rs20424 (−62 C>G)										
DALS[b]										
C/C	397	342	Ref	–	–	347	211	0.68	0.54	0.85	0.97
C/G + G/G	15	17	1.35	0.66	2.76	11	10	0.94	0.39	2.26	
CCFR[c]										
C/C	560	338	–	–	–	154	85	–	–	–	ND
C/G + G/G	17	11	–	–	–	5	2	–	–	–	

* Dominant model

Only SNPs with an interaction p value <0.05 in the DALS are shown. The dominant model was used when <10 cases or controls had the homozygous variant genotype; modeling was not run if fewer than five cases or controls were heterozygotes

[a] Current NSAID use is defined as current, regular use three times per week for at least 1 month in the DALS and as current, regular use of at least two pills per week for at least 1 month for the CCFR study

[b] Adjusted for age, sex, center, BMI, smoking, physical activity, calcium, calories, and dietary fiber

[c] Adjusted for age, sex, BMI, smoking, and physical activity

[d] Interaction p value from a likelihood ratio test prior to correction for multiple comparisons, with statistically significant p values ($p < 0.050$) shown in bold. No SNPs remained significant in the DALS after correcting for multiple comparisons using minP permutation tests (minP ≤ 0.05)

not replicate in the CCFR validation study. No other significant genotype–NSAID interactions were observed in *PTGS2* in the DALS.

Examination of aspirin use alone showed nominally significant associations with three SNPs in *PTGS2* in the DALS (Supplementary Table 3). However, two of the three interactions seen between *PTGS2* genotype and aspirin use did not replicate in the CCFR validation study. A third, rs2745557, had a significant interaction with aspirin use for rectal cancer in the DALS (int $p = 0.03$) and for colon cancer in the CCFR study (int $p = 0.001$), but there was no association for rectal cancer in DALS or colon cancer in the CCFR. In both studies, the variant allele carriers not currently taking aspirin were at increased risk compared to wild-type nonusers, but appeared to benefit more from aspirin than wild-type individuals. No observed genotype–NSAID interactions reached statistical significance in both the CCFR and DALS populations. Several genotype–NSAID interactions were observed in the CCFR validation study but not in the DALS discovery study (Supplementary Table 4). Associations observed in the CCFR validation study but not in the DALS discovery study may be due to chance, inadequately controlling for interactions in the DALS discovery study in the original analysis, or due to

the sib-pair design of the CCFR study [39]. To partly address this, we performed an exploratory post hoc stratification of DALS by family history, but were unable to replicate associations seen in the CCFR (data not shown). In addition, statistically significant findings were typically limited to either colon or rectal cancer, but not seen in both.

Discussion

We comprehensively assessed the importance of genetic variability in the two primary prostaglandin synthesis genes, *PTGS1* and *PTGS2*, using two independent study populations of colon and rectal cancer risk. We describe a significant association between the rs20417 variant C allele in *PTGS2* and increased risk of rectal cancer in DALS, a large population-based study, which replicated in a second, independent, large population-based study of rectal cancer from the CCFR. Genotype–NSAID interactions were observed in the DALS for *PTGS1* and rectal cancer risk, and for *PTGS2* and colon cancer risk; however, these interactions were no longer statistically significant after correcting for multiple comparisons and did not replicate in the CCFR validation study. Interactions between aspirin use alone and *PTGS2* genotypes were also inconsistent between the DALS and CCFR studies.

The first report of an association between *PTGS2* rs20417 and risk of colorectal cancer was in a Japanese study [40], but other studies in Caucasian populations did not confirm the finding [41, 42]. Recent meta-analyses have indicated that the variant C allele may be a risk factor for colorectal cancer in Asian but not in Caucasian populations [43–47]. Importantly, these earlier analyses did not examine colon and rectal cancers separately, and thus, it is unknown whether previous studies would have seen an association with rectal cancer risk in Caucasians. In addition, large genome-wide association studies generally have not genotyped rs20417 directly and also have not been stratified by colon and rectal cancers. As our studies were restricted to NHW, our results suggest that the rs20417 C allele may be a risk factor for this group, but only for rectal cancer and not colon cancer. We have previously reported a possible reduced risk of colorectal adenoma associated with this allele in Caucasians, although sample sizes were too small to distinguish between adenomas in colon and rectal sites [18].

We observed an interaction between this rs20417 SNP, NSAID use, and rectal cancer risk in the CCFR study, where the variant C allele carriers had a greater protective benefit from NSAID use. Observing statistically significant NSAID interactions in the CCFR and not in the DALS may be due to chance or may be due to differences in the study designs. As a case/sibling-control study in which shared genetics and environment are matched between siblings, the CCFR study is potentially more efficient for studying gene–environment interactions [39], which could be one reason why this interaction was observed in the CCFR but not in the DALS. Alternatively, there could be confounding factors in the DALS that were not adequately controlled for in our analysis. We did not observe an association between rs20417 and colon cancer risk in either study. A polytomous regression model indicated that the difference in risk between colon and rectal cancers for rs20417 in the DALS was significant, with a global $p < 0.0001$ for both main association and NSAID interaction models (data not shown). In general, we saw little reproducibility in statistically significant genetic associations between colon and rectal cancer risk.

These findings add further data to evidence that colon and rectal cancers have different etiologies. In addition, a significant interaction between *PTGS2* rs20417 and NSAID use suggests that, in contrast to colorectal adenoma [18], rs20417 interacts with NSAIDs for rectal cancer risk. The interaction was only seen in the CCFR, so this also could be a chance finding. A study of colorectal cancer from Rotterdam reported that NSAID users carrying the rs20417 C allele lived longer than nonusers with the wild-type G allele [48]. The Rotterdam study did not see an association between colorectal cancer risk and rs20417 genotype, but they did not analyze colon and rectal cancers separately.

COX-2, encoded by the *PTGS2* gene, catalyzes a key step in the conversion of arachidonic acid to bioactive prostaglandins. The *PTGS2* candidate polymorphism, rs20417 (−765 G>C), is known to affect gene expression and prostaglandin production [49, 50]. The functional impact of rs20417 has been studied by several groups; their studies suggest a proinflammatory effect of the CC genotype via increased prostanoids. A more than tenfold increase in PGE_2 and PGD_2 production was observed in monocytes from asthma patients homozygous for rs20417 CC compared to monocytes from GG homozygotes, with monocytes from heterozygotes displaying an intermediate phenotype of elevated PGE_2 and PGD_2 [50, 51]. This is consistent with the observation of increased urinary PGE_2 metabolites and biomarkers of monocyte/macrophage activation in stable coronary artery disease patients with the CC genotype [52]. Further, AML patients have been found to have increased *PTGS2* mRNA levels in bone marrow and increased COX-2 protein levels in serum [53]. There has been a report of a 30 % reduction in gene expression associated with the CC variant in an initial study with a reporter-gene system [49]. However, subsequent findings were mixed [52], suggesting that in vitro models of promoter activity do not fully capture the complex regulation of *PTGS2* transcription.

We previously reported an increased adenoma risk for carriers of the *PTGS1* L15–L16 deletion [19]. The current

analyses found similar trends for both colon and rectal cancers. However, the trend is not statistically significant, possibly because this deletion is rare and there is lack of power for validating the association. We also present a replication of our previously reported genotype–NSAID interaction for the *PTGS1* P17L polymorphism (rs3842787). Consistent with our findings in colorectal adenoma [19], we found in the DALS that the NSAID-associated risk reduction for rectal cancer was limited to the wild-type genotype for P17L. The functional impact of *PTGS1* P17L (rs3842787) may be direct, due to the amino acid change in the signal peptide, or indirect via the near-complete linkage disequilibrium in Caucasians between rs3842787 and seven 5′ polymorphisms [54].

In general, statistically significant genotype–NSAID interactions did not replicate between the DALS and CCFR studies. The one significant interaction seen in both studies, between aspirin use and *PTGS2* rs2745557, was inconsistent in that it was seen in rectal cancer in the DALS and in colon cancer in the CCFR study (Supplementary Table 3). This may be a chance finding or may be due to these studies' somewhat different study designs and definitions of NSAID use, different adjustment variables, limited sample size, and a weaker NSAID effect in the CCFR (Table 1). Both DALS and CCFR are large population-based case–control studies of colon and rectal cancer risk. The DALS uses population-based controls, and the CCFR uses unaffected siblings as controls. This is both the strength and a limitation of using the CCFR as a replication dataset for DALS. On the one hand, the CCFR sib-pair design helps avoid false positives that may result from population stratification and increases the power to detect gene–NSAID interactions. The CCFR sib-pair design, under which the shared genetics and environment are matched between siblings, can have greater power to detect gene–environment interactions than a case–control study design [39]. On the other hand, the family-based study design may have reduced the power of the main effect analyses. We felt that the potential benefit of the CCFR in replicating NSAID interactions outweighed the limitations in the main effect analysis. The main effects are adjusted for age and sex in both studies. DALS is further adjusted for center, which is not necessary in the CCFR due to the sib-pair design. For the NSAID analysis, both studies were also adjusted for the known CRC risk factors of BMI, physical activity, and smoking. We were not able to adjust the CCFR for dietary risk factors as such data are not available within the CCFR. However, the additional adjustments to the DALS did not substantially alter the results. Larger-scale investigations are needed to address NSAID, and particularly aspirin, pharmacogenetics with more certainty. Given the new results from randomized controlled trials of aspirin, which demonstrated strong cancer preventive effects [1, 11, 55], this issue deserves further attention.

Conclusions

One polymorphism in the *PTGS2* gene (−765 G>C; rs20417) was associated with a statistically significantly increased risk of rectal cancer in two large, independent, population-based studies in the USA. Our results suggest that the rs20417 C allele may be a risk factor for non-Hispanic whites, but only for rectal cancer, not colon cancer. No significant associations were observed between the targeted *PTGS1* polymorphisms and colon or rectal cancer risk. A number of genotype–NSAID interactions were noted; however, no genotype–NSAID interactions reached statistical significance in both the discovery and validation studies or for both colon and rectal cancers. An interaction between rs2745557 in *PTGS2* and aspirin use was suggestive—showing similar gene–aspirin interaction patterns and reaching significance in the DALS rectal cancer study and the CCFR colon cancer study, but not vice versa. These findings suggest that further validation is needed.

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Conflict of interest The authors declare that they have no conflict of interest.

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