

# Exploratory Genome-Wide Interaction Analysis of Nonsteroidal Anti-inflammatory Drugs and Predicted Gene Expression on Colorectal Cancer Risk



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## ABSTRACT

**Background:** Regular use of nonsteroidal anti-inflammatory drugs (NSAID) is associated with lower risk of colorectal cancer. Genome-wide interaction analysis on single variants ( $G \times E$ ) has identified several SNPs that may interact with NSAIDs to confer colorectal cancer risk, but variations in gene expression levels may also modify the effect of NSAID use. Therefore, we tested interactions between NSAID use and predicted gene expression levels in relation to colorectal cancer risk.

**Methods:** Genetically predicted gene expressions were tested for interaction with NSAID use on colorectal cancer risk among 19,258 colorectal cancer cases and 18,597 controls from 21 observational studies. A Mixed Score Test for Interactions (MiSTi) approach was used to jointly assess  $G \times E$  effects which are modeled via fixed interaction effects of the weighted burden within each gene set (burden) and residual  $G \times E$  effects

(variance). A false discovery rate (FDR) at 0.2 was applied to correct for multiple testing.

**Results:** Among the 4,840 genes tested, genetically predicted expression levels of four genes modified the effect of any NSAID use on colorectal cancer risk, including *DPP10* ( $P_{G \times E} = 1.96 \times 10^{-4}$ ), *KRT16* ( $P_{G \times E} = 2.3 \times 10^{-4}$ ), *CD14* ( $P_{G \times E} = 9.38 \times 10^{-4}$ ), and *CYP27A1* ( $P_{G \times E} = 1.44 \times 10^{-3}$ ). There was a significant interaction between expression level of *RP11-89N17* and regular use of aspirin only on colorectal cancer risk ( $P_{G \times E} = 3.23 \times 10^{-5}$ ). No interactions were observed between predicted gene expression and nonaspirin NSAID use at  $FDR < 0.2$ .

**Conclusions:** By incorporating functional information, we discovered several novel genes that interacted with NSAID use.

**Impact:** These findings provide preliminary support that could help understand the chemopreventive mechanisms of NSAIDs on colorectal cancer.

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**Note:** Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (<http://cebp.aacrjournals.org/>).

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## Introduction

Long-term use of aspirin and other nonsteroidal anti-inflammatory drugs (NSAID) has been consistently found to significantly reduce the incidence and mortality of colorectal cancer in both randomized clinical trials and observational studies (1–3). Despite its promising chemopreventive effects, aspirin is recommended for colorectal cancer prevention in those who are at greater than 10% risk of cardiovascular disease; no broad recommendation for the general population is in place due to concerns about side effects such as gastrointestinal bleeding (4, 5). Therefore, it is important to identify subgroups for which the risk–benefit ratio is more favorable.

To provide more precise guidelines for population stratification, the chemopreventive mechanisms of NSAIDs on colorectal cancer need to be better understood. NSAIDs have been shown to inhibit the initiation and growth of colorectal tumors via the inhibition of prostaglandin synthase [PTGS-2; also known as COX-2 activity and subsequent formation of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>; refs. 6–8)]. Our previous genome-wide interaction analysis and other independent cohort studies suggested that NSAIDs may function, in part, through the PI3K signaling pathway for colorectal carcinogenesis (9, 10). Other population-based, case–control, or prospective cohort studies using candidate genes and pathway analysis suggested that genetic variants in the Wnt/ $\beta$ -catenin signaling pathway (11) and NF8B signaling pathway (12, 13) might modify the effect of NSAIDs on colorectal cancer risk. An *in vitro* study also showed that NSAIDs inhibited the ERK signaling pathway in colon cancer cell lines (14).

However, previous studies were mainly limited by focusing on genetic variation in candidate genes and/or pathways. Genome-wide interaction analyses evaluating single variants have limited statistical power due to small effect size and sample sizes. In addition, it is possible that the effect of NSAIDs on tumorigenesis is mediated through molecular changes other than genetic mutation. Functional genetic information, such as tissue-specific gene expression data, has recently become increasingly accessible. Utilizing such functional data for the specific tissues of interest (e.g., colon) allows us to assess molecular changes in biologically relevant tissues where NSAIDs may also exert their effects. Results from functional analysis may therefore guide more informative and biologically meaningful analyses for gene–environment interactions. In this study, we aimed to identify new genetic regions that interact with the effect of NSAIDs on colorectal cancer risk by incorporating functional information from a publicly available database.

## Materials and Methods

### Study participants

We used epidemiologic and genetic data from 19,258 colorectal cancer cases and 18,597 controls from 21 participating studies in three international colorectal cancer consortia: the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO), the Colorectal Transdisciplinary Study (CORECT), and the Colon Cancer Family Registry (CCFR). Full details of study design and these GWAS consortia have been published previously (15–18), and the demographic characteristics of study participants are summarized in Supplementary Table S1. In brief, 9,917 cases and 10,553 controls were included from GECCO from nested case–control studies in 7 cohorts and 6 case–control studies. Furthermore, 9,341 cases and 8,044 controls were included from CORECT from nested case–control studies in 3 cohorts and 5 case–control studies. Nested case–control studies from CCFR were analyzed as individual studies in GECCO and CORECT.

There was no overlap of participants between studies. Given small sample sizes and the potential for differential distribution of NSAID use across populations, participants with non-European ancestry were excluded from analyses.

### Ethics statement

We have obtained written informed consent from all participants, that the studies were conducted in accordance with recognized ethical guidelines (e.g., Declaration of Helsinki, CIOMS, Belmont Report, U.S. Common Rule) and participating studies were approved by their respective Institutional Review Boards. This study has been approved by the Fred Hutchinson Cancer Research Center (FHCR) Institutional Review Board.

### Genotype data

Details on genotyping and imputation have been reported previously (17–19). In brief, DNA was mostly obtained from blood samples, with some from buccal swabs. Several platforms, including Affymetrix 500K, Affymetrix Axiom, Illumina 1M, 1M duo, Illumina 300K, Illumina 550K, 550Kduo, 610K, Illumina OmniExpress, Illumina OmniExpressExome, Illumina Oncoarray, Illumina Oncoarray+custom iSelect, were used for genotyping (20, 21). Samples were excluded on the basis of sample call rate, heterozygosity, unexpected duplicates or relative pairs, gender discrepancy and principal component analysis (PCA) outliers. SNPs were excluded on the basis of inconsistency across platforms, call rate, and out of Hardy-Weinberg equilibrium (HWE) in controls (20). SNPs were imputed using Haplotype Reference Consortium (HRC version r1.0) reference panel (22), and restricted by imputation accuracy ( $R^2 > 0.3$  for SNPs with  $MAF > 1\%$ ,  $R^2 > 0.5$  for SNPs with  $MAF > 0.5\%$  and  $< 1\%$ , and  $R^2 > 0.99$  for SNPs with  $MAF < 0.05\%$ ).

### Predicted gene expression

Functional information was obtained using a public database (23), which used elastic net penalized regression to predict tissue-specific genetically regulated expression using *cis*-SNPs ( $\pm 1$  Mb from TSS/TES of a gene) jointly measured from 169 transverse colon tissue samples from the GTEx project (GTEx v6p). The heritability of gene expression explained by the *cis*-SNPs (predictive  $R^2$ ) was calculated using a mixed-effects model (23, 24). The estimated SNP-specific effect sizes and predictive  $R^2$  for each gene were downloaded from the publicly available PredictDB Repository (<http://hakyimlab.org/post/2020/predictdb/>). A total of 4,840 genes (predictive  $R^2 > 1\%$ , with at least 3 SNPs in our data) were included in subsequent colorectal cancer interaction analyses.

### Exposure assessment

Demographic and environmental risk factor information was collected either by in-person interview or via structured self-administered questionnaires, as detailed previously (17, 25). Briefly, all risk factors were collected at either study entry or blood draw for cohort studies, or for the 1–2 years before sample ascertainment (the “reference period”) for case–control studies. A multistep, data harmonization procedure was conducted to reconcile each study’s unique protocols and data collection instruments (15, 25). Multiple quality control checks were performed, and values of variables were truncated to the minimum or maximum value of an established range for each variable. Variables and their values were combined into a single dataset with common definition, standardized coding, and standardized permissible values (Supplementary Fig. S1). Age was defined as age at diagnosis for cases and age at selection for controls.

For regular use of NSAIDs (any type of NSAIDs, aspirin only, and nonaspirin NSAIDs only), measures of both frequency and duration of regular intake were defined using study-specific definitions of regular use of aspirin and/or nonaspirin NSAIDs, instead of one uniform definition due to variability in questions across studies (Supplementary Table S1). Use of aspirin included both low-dose aspirin (81 mg) and regular or extra-strength aspirin ( $\geq 325$  mg). Use of nonaspirin NSAIDs included ibuprofen, naproxen, or other pain relievers, based on each study. Regular use of any NSAID was defined as regular use of either aspirin or nonaspirin NSAIDs. All participating studies, except PHS, collected use of aspirin and nonaspirin NSAIDs separately. PHS only collected aspirin use, and was not included in the analysis of nonaspirin NSAID use.

### Statistical analysis

We used individual level genotype and environmental data for this interaction analysis. We used MiSTi (26), a mixed-effect score test, for gene-based interaction test of regular use of any NSAID on colorectal cancer risk. The MiSTi approach considers a hierarchical model of the gene-environmental interaction effects with two components, burden and variance components via fixed and random effects, respectively. The burden component tested interactions between predicted gene expression and regular use of NSAID by incorporating SNP-specific weights to predict gene expression in the transverse colon based on individual genotype data. The variance component captured the residual interaction effect of the genes likely through alternative mechanisms resulted beyond the predicted gene expression. We calculated *P* values for burden and variance interaction effects, respectively, after adjusting for age, sex, study, genotyping platform, and principal components to account for population stratification. A false discovery rate (FDR) of 0.2 is considered statistically significant. In addition, we test for interactions for regular use of aspirin only and non-aspirin NSAIDs separately.

If the set-based interaction is detected by the burden component test, we further evaluate the associations between standardized estimated gene expression levels and colorectal cancer risk, stratified by NSAID use. In addition, we also evaluate interaction for individual SNPs within each gene set to assess whether the observed set-based significant interaction is driving by specific SNPs. We perform all analyses in R version 3.3.4.

## Results

The characteristics of the 19,258 cases and 18,597 controls included in this analysis are summarized in Supplementary Table S1. Regular use of any NSAID was lower among colorectal cancer cases (31.3%) than controls (39.2%), and NSAIDs was associated with 26% lower risk of colorectal cancer [OR = 0.74; 95% confidence interval (CI): 0.69–0.80; **Fig. 1A**]. A slightly smaller effect was observed for aspirin only, where regular use of only aspirin was associated with a 23% lower risk of colorectal cancer (OR = 0.77; 95% CI, 0.71–0.83; **Fig. 1B**).

Among 4,840 genes tested, genetically predicted gene expressions of four genes had an interaction with regular use of any NSAIDs on colorectal cancer risk at FDR < 0.2 (**Table 1**). All four of these interactions were detected by the burden component test (**Fig. 2**). The most significant gene, *DPP10*, is located on chromosome 2q14.1 ( $P_{G \times E} = 1.96 \times 10^{-4}$ ), with 4.46% of expression predicted by 8 SNPs in the transverse colon. The *KRT16* gene, which had the second strongest signal, is located on chromosome 17q21.2 ( $P_{G \times E} = 2.32 \times 10^{-4}$ ), with 2.80% of the expression predicted by 10 SNPs. The gene *CD14*, located on chromosome 5q31.3, was observed to have an interaction with

regular use of any NSAIDs ( $P_{G \times E} = 9.38 \times 10^{-4}$ ). A total of 14 SNPs predict 2.90% of *CD14* expression. In addition, a set of 6 SNPs on chromosome 2q35 that predict 11.45% of *CYP27A1* gene expression interacted with any NSAID use on colorectal cancer risk ( $P_{G \times E} = 1.44 \times 10^{-3}$ ).

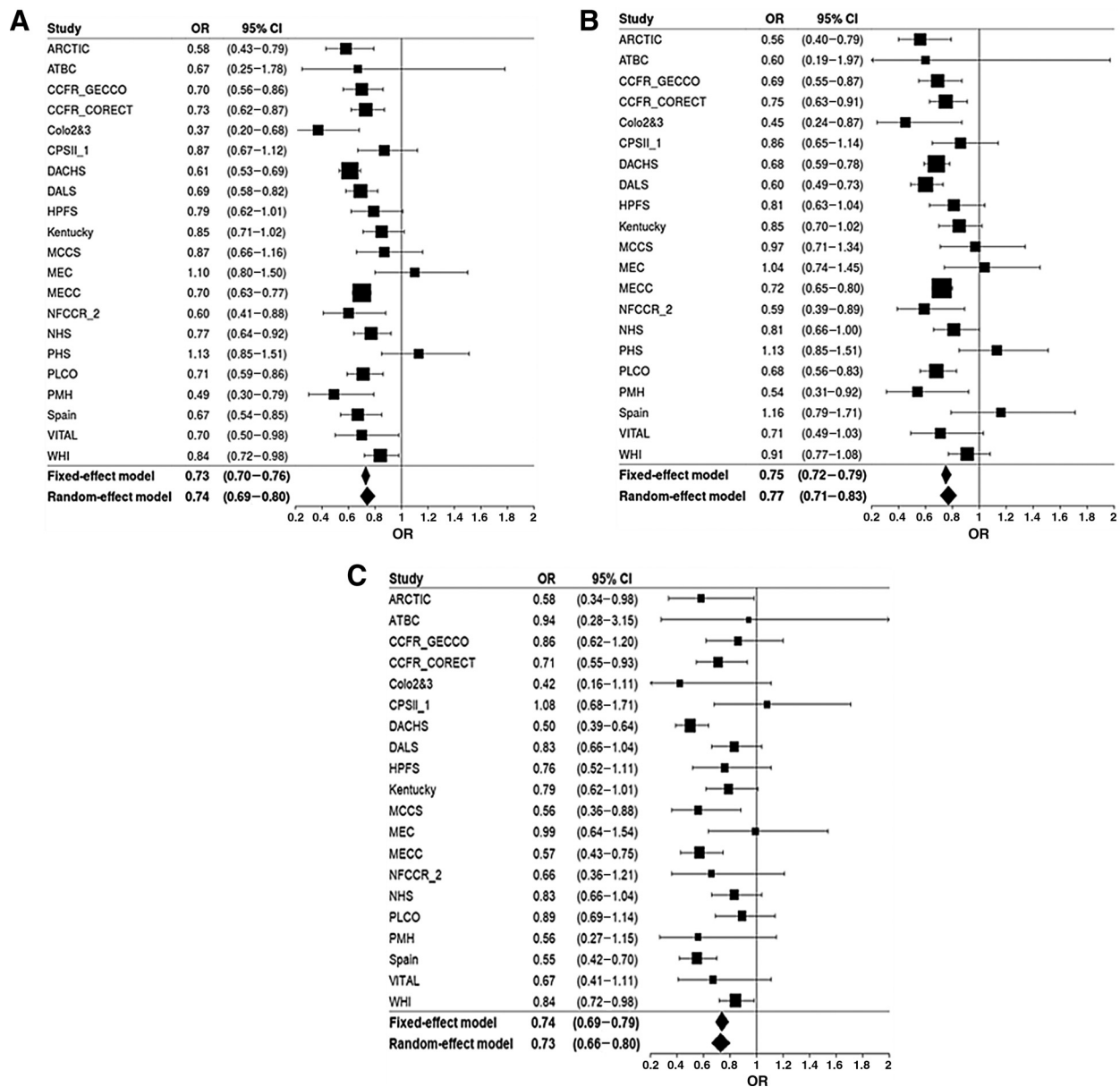
In the stratified analysis of these four genes detected by burden component tests, higher estimated expression levels of *DPP10* and *KRT16* was associated with higher risks of colorectal cancer among never or nonregular users of NSAIDs, but with lower risk of colorectal cancer among regular users of NSAIDs (**Table 2**). Higher expression of *CD14* was associated with lower risk of colorectal cancer among regular users of NSAIDs only. Higher estimated expressions of *CYP27A1* were associated with lower risk of colorectal cancer among never or nonregular users of NSAIDs, but with higher risk of colorectal cancer among regular users of NSAIDs. In the secondary analysis of these four genes, where we assessed the main effects and interactions between individual SNPs and regular use of any NSAIDs on colorectal cancer risk, we did not find that the observed interactions were driven by specific individual SNPs (Supplementary Table S2). Interaction analysis between these genes and different types of NSAID use (aspirin only, nonaspirin NSAIDs only) did not suggest the observed significant interactions were driven by either aspirin or nonaspirin NSAIDs (Supplementary Table S3). In sensitivity analysis, that only included participants from cohort studies, the estimated associations between predicted gene expression levels and colorectal cancer risk stratified by NSAID use remain in the same directions for all four genes.

Finally, using the random effects test, we observed the predicted expression of a long noncoding RNA gene *RP11-89N17* interacted with regular use of aspirin only use on colorectal cancer risk at FDR < 0.2 (variance component  $P_{G \times E} = 3.23 \times 10^{-5}$ ; **Fig. 3**). This gene is located on chromosome 7, with 60 SNPs explaining 3.93% of the expression (**Table 1**). Six of these SNPs were not available in our data. No genes significantly interacted with regular use of nonaspirin NSAIDs on colorectal cancer risk.

## Discussion

In this exploratory genome-wide investigation, we found statistical interactions between predicted gene expression of four genes in the transverse colon and regular use of NSAIDs on colorectal cancer risk (FDR < 0.2). In addition, we found that a gene encoding altered expression of a long noncoding RNA gene, as predicted beyond genetic variants, modified the effects of regular use of aspirin on colorectal cancer risk (FDR < 0.2).

The *DPP10* gene, encoding dipeptidyl-peptidase 10 (DPP10), was the most strongly associated gene with regular use of NSAIDs on colorectal cancer risk. DPP10 is structurally similar to DPP4, which is involved in cell-extracellular matrix interactions, apoptosis, and immunomodulation, and differential expression levels have been reported in various cancer cell lines (27). Genetic variants of *DPP10* were previously associated with increased eosinophilic inflammation and higher risk of aspirin-exacerbated respiratory disease (28). In addition, our results suggested that higher expression levels of *DPP10* is associated with higher risk of colorectal cancer among never or nonregular users of NSAIDs, while DPP10 was associated with lower colorectal cancer risks among NSAID users. This could be due to interrelation between anti-platelet and anti-inflammatory mechanisms (7). Serum DPP10 levels were significantly correlated with serum 15-hydroxyeicosatetraenoic acid (15-HETE levels), the major metabolic product of arachidonic acid (29), of which the production can be largely inhibited by aspirin via platelet COX-1-related pathway (30).



**Figure 1.** Main effect of regular use of any NSAID (A), aspirin (B), or nonaspirin NSAIDs (C) on colorectal cancer risk. A, Regular use of any NSAID ( $N = 39,290$ ).  $I^2 = 45.7\%$ ;  $P_{\text{heterogeneity}} = 0.001$ . B, Regular use of aspirin only ( $N = 38,784$ ).  $I^2 = 44.4\%$ ;  $P_{\text{heterogeneity}} = 0.001$ . C, Regular use of nonaspirin NSAIDs only ( $N = 37,645$ ).  $I^2 = 2.2\%$ ;  $P_{\text{heterogeneity}} = 0.012$ .

The second gene that was shown to interact with NSAID use is *KRT16*, encoding cytokeratin 16, which is associated with activation and hyperproliferation of keratinocytes (31) and can be regulated by growth factors (32) and oxidative stress (33). An *in vitro* study found that dysfunction of mitochondrial respiration chain and endoplasmic reticulum stress resulted in robust induction of *KRT16* expression in the HCT116 colon carcinoma cell line, but not in cell lines transformed *in vitro* (34), suggesting stimulation of *KRT16* is involved in tumor progression. We also found that higher expression levels of the *KRT16* gene is associated with higher colorectal cancer risk among never or nonregular users of NSAIDs; however, our study further suggested that this association is no longer observed among regular users of NSAIDs,

suggesting NSAIDs may partially act through the inhibition of *KRT16*-related tumorigenesis pathways.

We also found that *CD14* and *CYP27A1* interacted with NSAID use. The CD14 protein is a surface antigen that mediates immune response to bacterial lipopolysaccharide (35), which has been related to accelerated growth of human colorectal carcinoma cells through NF $\kappa$ B and PTGS-2 activation (36). The binding between lipopolysaccharide and CD14 protein was found to induce cytokines such as TNF $\alpha$  and interleukins, which were further related to the enrichment of inflammatory response in the colorectum (35, 36). The frequency of CD14+CD169+ circulating monocytes and infiltrating macrophages was found to be significantly increased in patients with advanced stage

**Table 1.** Characteristics of the genes that significantly interacted with regular use of NSAIDs or aspirin on colorectal cancer risk at FDR < 0.2.

Risk factors	Gene name	Chromosome	SNPs	R <sup>2</sup>	P <sub>G×E</sub>
Any NSAID	<i>DPP10</i>	2q14.1	8	4.46%	1.96 × 10 <sup>-4</sup>
	<i>KRT16</i>	17q21.2	10	2.80%	2.32 × 10 <sup>-4</sup>
	<i>CD14</i>	5q31.3	14	2.90%	9.38 × 10 <sup>-4</sup>
	<i>CYP27A1</i>	2q35	6	11.45%	1.44 × 10 <sup>-3</sup>
Aspirin	<i>RP11-89N17</i>	7p14.3	60	3.93%	3.23 × 10 <sup>-5</sup>

<sup>a</sup>R<sup>2</sup>: The variance of the gene expressions explained by the SNPs in each gene set, downloaded from the publicly available PredictDB Repository (<http://hakyimlab.org/post/2020/predictdb/>).

colorectal cancer compared to patients with earlier stage colorectal cancer and healthy controls, and is highly correlated with level of plasma carcinoembryonic antigen, suggesting that *CD14* expression levels may be associated with colorectal cancer progression (37). Our results support the involvement of *CD14* expression levels in the chemopreventive mechanisms of NSAIDs, and further suggested that regular use of NSAIDs possibly inhibit the oncogenic effects of *CD14*.

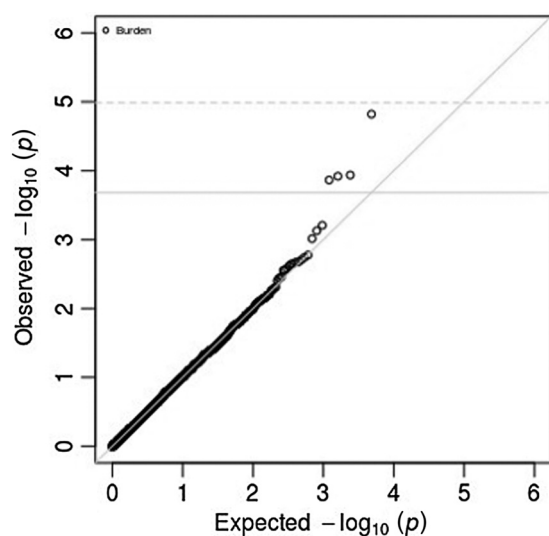
The *CYP27A1* is a member of the cytochrome P450 enzyme family that converts cholesterol into 27-hydroxycholesterol (27HC) in the acidic pathway of bile acid synthesis (38). *In vitro* studies have shown that nucleic *CYP27A1* expression level is decreased in colorectal cancer, compared with adjacent nonmalignant tissues (39, 40). Survival analysis has also shown that low expression of *CYP27A1* was associated with improved survival among patients with colorectal cancer (41). Limited evidence has shown direct relationship between *CYP27A1* expression and NSAID use, but *CYP27A1* overexpression in hematopoietic cells has been indicated to reduce diet-induced hepatic inflammation in mice, independent of other oxysterols (42), suggesting a potential role of *CYP27A1* expression levels in inflammation-related pathways. However, we found that higher expression levels of *CYP27A1* were only associated with lower risk of colorectal cancer

among never or nonregular users of NSAIDs, where the risk of colorectal cancer is higher with higher *CYP27A1* expression levels among regular users of NSAIDs. Future studies are needed for further evaluation.

In addition, we observed an interaction signal from the variance component for *RP11-89N17* interacting with regular use of aspirin only on colorectal cancer risk. Evidence has shown that long non-coding RNAs (lncRNA) play important roles in tumor malignancies by regulating gene expressions that affect cell proliferation, migration, and survival (43). In addition, lncRNAs may also be involved in chemopreventive effects and clinical outcomes (44, 45). In colorectal cancer cell lines, aspirin has been shown to induce the expression of a lncRNA, *OLA1P2*, which may suppress cancer cell proliferation and metastasis (46). However, little is known about the *RP11-89N17* gene in relation to colorectal cancer risk. One study found that *RP11-89N17* was downregulated in lung tumor tissues, compared with normal tissues, using 272 human serial analyses of gene expression (SAGE) libraries (47). It is possible that aspirin might share similar chemopreventive mechanisms through the expression of *RP11-89N17* in colorectal cancer.

Our study has several strengths as the largest study to-date that investigates interaction between genes and NSAID use on colorectal cancer risk. First of all, we focused on biologically relevant analyses on genes that are expressed and well predicted in transverse colons. This functional analysis may provide more informative findings on the molecular chemopreventive mechanisms of NSAIDs on colorectal tumorigenesis. In addition, we have well-imputed genotype data in all participating studies, which allow us to estimate gene expression of 4,840 genes across the genome. In addition, we collected information on regular use of aspirin and other NSAIDs, and used an iterative harmonization process across all studies. Furthermore, we tested for both fixed and random effects in the interaction testing, which allowed us to observe which component drives each gene-NSAID interaction: either the genetic effects linked to predicted gene expression or effects that are beyond that has been genetically predicted. Finally, we found that the observed interactions between these genes and regular use of any NSAID were not driven by any individual SNPs within each gene set, suggesting that our set-based testing using MiSTi may have increased our statistical power to detect interactions by aggregating the signals when single variant interaction effects were small.

There are also limitations to our study. We did not have directly measured expression levels of the genes in our study subjects. However, currently no observational studies have direct gene expression data measured in normal colon tissues, whereas studies with tissue-specific gene expression measurements have limited sample sizes. By using external information to predict gene expression, we were able to investigate the genetic effect of gene expression on NSAID-colorectal

**Figure 2.**

Quantile-quantile plot of *P* values of the fixed effects in gene-any NSAID use interaction. Number of genes = 4,840; dotted line (---), *P* value threshold of Bonferroni correction (1 × 10<sup>-5</sup>); solid line (—), *P* value threshold for FDR at 0.2.

**Table 2.** Association between predicted gene expressions and colorectal cancer risk stratified by status of regular use of any NSAIDs.

Gene name	Mean (SD)	Never or nonregular NSAID users		Regular NSAID users	
		OR <sup>a</sup> (95% CI)	P	OR <sup>a</sup> (95% CI)	P
<i>DPP10</i>	-0.18 (0.13)	1.04 (1.01-1.06)	0.005	0.94 (0.91-0.97)	<0.001
<i>KRT16</i>	-0.22 (0.07)	1.04 (1.01-1.06)	0.004	0.96 (0.93-1.00)	0.031
<i>CD14</i>	0.16 (0.16)	1.01 (0.99-1.04)	0.421	0.96 (0.92-0.99)	0.014
<i>CYP27A1</i>	0.24 (0.18)	0.96 (0.94-0.99)	0.002	1.05 (1.01-1.08)	0.009

<sup>a</sup>OR per SD increase in estimated gene expression level.

cancer associations within our large sample size. However, the statistical power to test for interactions might still have been limited by the accuracy of genetically predicted gene expression which were estimated using a small sample size of 169 colon transverse tissues. The SNP-expression effects of estimated gene expression levels are also relatively small. In addition, as gene expression levels changes depend on the subsites of colon, we used the transverse colon, the middle section of the entire colon, to avoid bias introduced from the extremes of colonic locations (48–50). However, the transverse colon tissue samples from the GTEx project included the whole colonic wall. As such, the GTEx samples not only included the epithelial cells of the mucosa, from which colorectal cancer originates and is more relevant, but also all other tissue layers, such as muscularis propria and serosa. We also did not use gene expression models for the sigmoid colon tissues, because the GTEx samples for this region were dominantly muscularis. Our subjects clustered primarily to European ancestry, due to limited sample sizes in other race/ethnicity groups. Because expression levels of different genes may differ across populations, our results may not be necessarily generalizable to other ancestry groups. Finally, we acknowledge that we used a liberal FDR threshold instead of Bonferroni correction for multiple testing, as this analysis is exploratory in

nature using set-based testing approaches. Independent replications using the same gene expression prediction models are warranted.

In summary, by incorporating functional information in set-based testing, we discovered several genes that statistically interacted with NSAID use among participants. These findings provide preliminary support for biological insight to chemopreventive mechanisms of NSAIDs on colorectal cancer risk. Follow-up studies are needed to confirm the function of these genes in relation to NSAID use and colorectal cancer development. Independent replication is also warranted.

**Disclosure of Potential Conflicts of Interest**

S.B. Gruber has ownership interest (including patents) in Brogent International LLC. A.T. Chan is a consultant for Bayer Pharma AG and Pfizer Inc. and reports receiving a commercial research grant from Bayer Pharma AG. No potential conflicts of interest were disclosed by the other authors.

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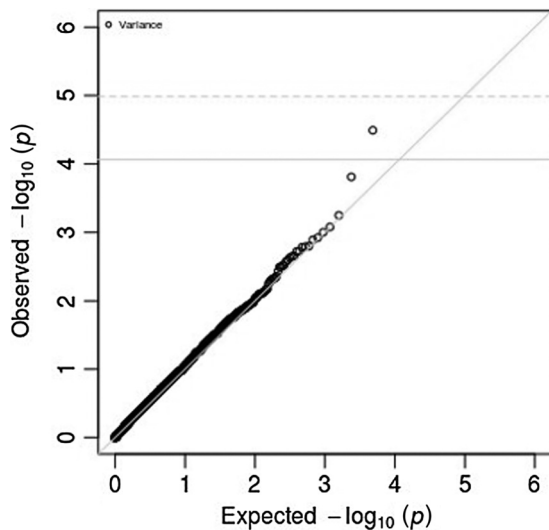
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**Figure 3.** Quantile-quantile plot of P values of the random effects in gene-aspirin use interaction. Number of genes = 4,840; dotted line (---), P value threshold of Bonferroni correction ( $1 \times 10^{-5}$ ); solid line (—), P value threshold for FDR at 0.2.

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