HHS Public Access

Author manuscript

Cancer Epidemiol Biomarkers Prev. Author manuscript; available in PMC 2021 March 01.

Published in final edited form as:

Cancer Epidemiol Biomarkers Prev. 2020 September ; 29(9): 1800–1808.

doi:10.1158/1055-9965.EPI-19-1018.

Exploratory genome-wide interaction analysis of non-steroidal anti-inflammatory drugs and predicted gene expression on colorectal cancer risk

Xiaoliang Wang[1,2,*], **Yu-Ru Su**[1], **Paneen S. Petersen**[1,2], **Stephanie Bien**[1], **Stephanie L Schmit**[3], **David A. Drew**[4,5], **Demetrius Albanes**[6], **Sonja I Berndt**[6], **Hermann Brenner**[7,8,9], **Peter T Campbell**[10], **Graham Casey**[11], **Jenny Chang-Claude**[12,13], **Steven J Gallinger**[14,15], **Stephen B Gruber**[16], **Robert W Haile**[17,18], **Tabitha A Harrison**[1], **Michael Hoffmeister**[7], **Eric J Jacobs**[10], **Mark A Jenkins**[19], **Amit D. Joshi**[5,20], **Li Li**[21], **Yi Lin**[1], **Noralane M Lindor**[22], **Loïc Le Marchand**[23], **Vicente Martin**[24,25], **Roger Milne**[26], **Robert MacInnis**[26], **Victor Moreno**[24,27,28], **Hongmei Nan**[29,30], **Polly A Newcomb**[1,2], **John D Potter**[1,2,31], **Gad Rennert**[32,33,34], **Hedy Rennert**[32,33,34], **Martha L Slattery**[35], **Steve N Thibodeau**[36], **Stephanie J Weinstein**[6], **Michael O Woods**[37], **Andrew T Chan**[4,5,38], **Emily White**[1,2], **Li Hsu**[1], **Ulrike Peters**[1,2]

[1]Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA [2]Department of Epidemiology, University of Washington School of Public Health, Seattle, WA 98195, USA [3]Department of Cancer Epidemiology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL 33612, USA [4]Division of Gastroenterology, Massachusetts General Hospital, Boston, MA 02114, USA [5]Clinical and Translational Epidemiology Unit, Massachusetts General Hospital, Boston, MA 02108, USA [6]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA [7]Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ), Heidelberg, 69120, Germany [8]Division of Preventive Oncology, German Cancer Research Center (DKFZ) and National Center for Tumor Diseases (NCT), Heidelberg, 69120, Germany [9]German Cancer Consortium (DKTK), Heidelberg, 69120, Germany [10]Behavioral and Epidemiology Research Group, American Cancer Society, Atlanta, GA 30303, USA [11]Public Health Sciences, University of Virginia, Charlottesville, VA 22903, USA [12]Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, 69009, Germany [13]University Cancer Center Hamburg, University Hospital Hamburg-Eppendorf, Hamburg, 20251, Germany [14]Department of Pathology and Laboratory Medicine, Lunenfeld-Tanenbaum Research Institute, Toronto, Ontario M5G 1X5, Canada [15]Division of General Surgery, Toronto General Hospital, Toronto, Ontario, M5G 2C4, Canada [16]Department of Preventive Medicine, USC Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033, USA [17]Department of Health Research and Policy (Epidemiology), Stanford University School of Medicine, Palo Alto, CA 94305, USA [18]Department of Medicine (Oncology), Stanford Cancer Institute, Palo Alto, CA 94305, USA [19]Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Parkville, VIC 3010, Australia [20]Department of Epidemiology, Harvard School of Public Health, Boston, MA 02115,

[*]Correspondence: xwang23@fredhutch.org; 1100 Fairview Ave N, M4-B402, Seattle WA 98109 USA.

USA [21]Department of Family Medicine, University of Virginia, Charlottesville, VA 22908, USA [22]Department of Health Sciences Research, Mayo Clinic, Scottsdale, AZ 85259 USA [23]Epidemiology Program, University of Hawaii Cancer Center, Honolulu, HI 96813, USA [24]CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, 28029, Spain [25]Biomedicine Institute (IBIOMED), University of León, León, 24071, Spain [26]Cancer Epidemiology Division, Cancer Council Victoria, Melbourne, Victoria, 3004 Australia [27]Cancer Prevention and Control Program, Catalan Institute of Oncology-IDIBELL, L'Hospitalet de Llobregat, Barcelona, 08908, Spain [28]Department of Clinical Sciences, Faculty of Medicine, University of Barcelona, Barcelona, 08007, Spain [29]Department of Epidemiology, Richard M. Fairbanks School of Public Health, Indiana University, Indianapolis, IN 46202, USA [30]Melvin and Bren Simon Cancer Center, Indiana University, Indianapolis, IN 46202, USA [31]Centre for Public Health Research, Massey University, Wellington 6021, New Zealand [32]Department of Community Medicine and Epidemiology, Lady Davis Carmel Medical Center, Haifa 3436212, Israel [33]Ruth and Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa 3436212, Israel [34]Clalit National Cancer Control Center, Haifa 3436212, Israel [35]Department of Internal Medicine, University of Utah Health Sciences Center, Salt Lake City, UT 84132, USA [36]Department of Laboratory Medicine & Pathology, Mayo Clinic, Rochester, MN 55905, USA [37]Discipline of Genetics, Memorial University of Newfoundland, St. John's, NL A1B 3V6, Canada. [38]Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA

Abstract

Background—Regular use of non-steroidal anti-inflammatory drugs (NSAIDs) is associated with lower risk of colorectal cancer (CRC). Genome-wide interaction analysis on single variants (G×E) has identified several SNPs that may interact with NSAIDs to confer CRC 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 CRC risk. Methods: Genetically predicted gene expressions were tested for interaction with NSAID use on CRC risk among 19,258 CRC cases and 18,597 controls from 21 observational studies. A Mixed Score Test for Interactions (MiSTi) approach was used to jointly assess G×E effects which are modeled via fixed interaction effects of the weighted burden within each gene sets (burden) and residual G×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 CRC risk, including *DPP10* ($P_{G\times E}=1.96\times10^{-4}$), *KRT16* ($P_{G\times E}=2.3\times10^{-4}$), *CD14* ($P_{G\times E}=9.38\times10^{-4}$), and *CYP27A1* ($P_{G\times E}=1.44\times10^{-3}$). There was a significant interaction between expression level of *RP11–89N17* and regular use of aspirin only on CRC risk ($P_{G\times E}=3.23\times10^{-5}$). No interactions were observed between predicted gene expression and non-aspirin NSAID use at FDR<0.2.

Conclusion—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 CRC.

Keywords

Gene-environment interaction; set-based testing; colorectal cancer; non-steroidal anti-inflammatory drugs; aspirin

Introduction

Long-term use of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) has been consistently found to significantly reduce the incidence and mortality of colorectal cancer (CRC) in both randomized clinical trials and observational studies(1–3). Despite its promising chemopreventive effects, aspirin is recommended for CRC 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.

In order to provide more precise guidelines for population stratification, the chemopreventive mechanisms of NSAIDs on CRC 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 cyclooxygenase [COX]-2) activity and subsequent formation of prostaglandin E2 (PGE_2)(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/β-catenin signaling pathway(11) and NFκB-signaling pathway(12,13) might modify the effect of NSAIDs on CRC risk. An in *vitro* study also showed that NSAIDs inhibited the extracellular-signal-regulated kinase (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 has 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 CRC risk by incorporating functional information from a publicly available database.

Materials and Methods

Study participants

We used epidemiological and genetic data from 19,258 CRC cases and 18,597 controls from 21 participating studies in three international CRC 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 1.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. Further, 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.

Ethic 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 (FHCRC) 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(+/− 1Mb 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/predictdb/). A total of 4,840 genes (predictive R^2>1%, with at least 3 SNPs in our data) were included in subsequent CRC 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 (Supplemental Figure 1). 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 non-aspirin NSAIDs only), measures of both frequency and duration of regular intake were defined using study-specific definitions of regular use of aspirin and/or non-aspirin NSAIDs, instead of one uniform definition due to variability in questions across studies (Supplemental Table 1). Use of aspirin included both low-dose aspirin (81 mg), and regular or extra-strength aspirin (≥325 mg). Use of non-aspirin 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 non-aspirin NSAIDs. All participating studies, except PHS, collected use of aspirin and non-aspirin NSAIDs separately. PHS only collected aspirin use, and was not included in the analysis of non-aspirin NSAID use.

Statistical Analysis

We used individual level genotype and environmental data for this interaction analysis. We used MiSTi(26), a mixed effects score test, for gene-based interaction test of regular use of any NSAID on CRC risk. The MiSTi approach considers a hierarchal 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 CRC 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 Supplemental Table 1. Regular use of any NSAID was lower among CRC cases (31.3%) than controls (39.2%), and NSAIDs was associated with 26% lower risk of CRC (OR=0.74; 95% CI: 0.69, 0.80; Figure 1A). A slightly smaller effect was observed for aspirin only, where regular use of only aspirin was associated with a 23% lower risk of CRC (OR=0.77; 95% CI: 0.71, 0.83; Figure 1B).

Among 4,840 genes tested, genetically predicted gene expressions of four genes had an interaction with regular use of any NSAIDs on CRC risk at FDR<0.2 (Table 1). All four of these interactions were detected by the burden component test (Figure 2). The most significant gene, *DPP10,* is located on chromosome 2q14.1 ($P_{G\times E}=1.96\times10^{-4}$), 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 ($P_{G\times E}=2.32\times10^{-4}$), 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 ($P_{G\times E}=9.38\times10^{-4}$). 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 CRC risk ($P_{G\times E}=1.44\times10^{-3}$).

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 CRC among never or non-regular users of NSAIDs, but with lower risk of CRC among regular users of NSAIDs (Table 2). Higher expression of *CD14* was associated with lower risk of CRC among regular users of NSAIDs only. Higher estimated expressions of *CYP27A1* was associated with lower risk of CRC among never or non-regular users of NSAIDs, but with higher risk of CRC 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 CRC risk, we did not find that the observed interactions were driven by specific individual SNPs (Supplemental Table 2). Interaction analysis between these genes and different types of NSAID use (aspirin only, non-aspirin NSAIDs only) did not suggest the observed significant interactions were driven by either aspirin or non-aspirin NSAIDs (Supplemental Table 3). In sensitivity analysis that only included participants from cohort studies, the estimated associations between predicted gene expression levels and CRC risk stratified by NSAID use remain in the same directions for all four genes.

Lastly, using the random effects test, we observed the predicted expression of a long non-coding RNA gene *RP11–89N17* interacted with regular use of aspirin only use on CRC risk at FRD<0.2 (Variance component $P_{G\times E}=3.23\times10^{-5}$; Figure 3). This gene is located on

chromosome 7, with 60 SNPs explaining 3.93% of the expression (Table 1). 6 of these SNPs were not available in our data. No genes significantly interacted with regular use of non-aspirin NSAIDs on CRC 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 CRC risk (FDR<0.2). In addition, we found that a gene encoding altered expression of a long non-coding RNA gene, as predicted beyond genetic variants, modified the effects of regular use of aspirin on CRC risk (FDR<0.2).

The *DPP10* gene, encoding dipeptidyl-peptidase 10 (DPP10), was the most strongly associated gene with regular use of NSAIDs on CRC 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 CRC among never or non-regular users of NSAIDs, while DPP10 was associated with lower CRC 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).

The second gene that was shown to interact with NSAID use is *KRT16*, encoding cytokeratin 16, which is associated with activation and hyper-proliferation 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 CRC risk among never or non-regular users of NSAIDs; however, our study further suggested that this association is no longer observed among regular users of NSADIs, 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 NFκB and PTGS-2 activation(36). The binding between lipopolysaccharide and CD14 protein was found to induce cytokines such as tumor necrosis factor α (TNF-α) 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 advanced stage CRC patients compared to earlier stage

CRC patients and healthy controls, and is highly correlated with level of plasma carcinoembryonic antigen, suggesting that *CD14* expression levels may be associated with CRC 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 CRC, compared to adjacent non-malignant tissues(39,40). Survival analysis has also shown that low expression of CYP27A1 was associated with improved survival among CRC patients(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* was only associated with lower risk of CRC among never or non-regular users of NSAIDs, where the risk of CRC 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 CRC risk. Evidence has shown that long non-coding RNAs (lncRNAs) 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 CRC 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 CRC risk. One study found that RP11–89N17 was down-regulated in lung tumor tissues, compared to normal tissues, using 272 human serial analysis of gene expression (SAGE) libraries (47). It is possible that aspirin might share similar chemopreventive mechanisms through the expression of RP11–89N17 in CRC.

Our study has several strengths as the largest study to-date that investigates interaction between genes and NSAID use on CRC 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 estimated 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. Lastly, 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-CRC 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, since gene expression levels changes depending 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 CRC 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. Since expression levels of different genes may differ across populations, our results may not be necessarily generalizable to other ancestry groups. Lastly, we acknowledge that we used a liberal FDR threshold instead of Bonferroni correction for multiple testing, since 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 CRC risk. Follow-up studies are needed to confirm the function of these genes in relation to NSAID use and CRC development. Independent replication is also warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

CPS-II: The authors thank the CPS-II participants and Study Management Group for their invaluable contributions to this research. The authors would also like to acknowledge the contribution to this study from central cancer registries supported through the Centers for Disease Control and Prevention National Program of Cancer Registries, and cancer registries supported by the National Cancer Institute Surveillance Epidemiology and End Results program.

DACHS: We thank all participants and cooperating clinicians, and Ute Handte-Daub, Utz Benscheid, Muhabbet Celik and Ursula Eilber for excellent technical assistance.

Harvard cohorts (HPFS, NHS, PHS): The study protocol was approved by the institutional review boards of the Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health, and those of participating registries as requiredWe would like to thank the participants and staff of the HPFS, NHS and PHS for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. The authors assume full responsibility for analyses and interpretation of these data. Kentucky: We would like to acknowledge the staff at the Kentucky Cancer Registry.

GECCO: The authors thank all those at the GECCO Coordinating Center for helping bring together the data and people that made this project possible.

NFCCR: The authors wish to acknowledge the contribution of Alexandre Belisle and the genotyping team of the McGill University and Génome Québec Innovation Centre, Montréal, Canada, for genotyping the Sequenom panel in the NFCCR samples. Funding was provided to Michael O. Woods by the Canadian Cancer Society Research Institute.

OFCCR: Additional funding toward genetic analyses of OFCCR includes the Ontario Research Fund, the Canadian Institutes of Health Research, and the Ontario Institute for Cancer Research, through generous support from the Ontario Ministry of Research and Innovation.

PLCO: This study is supported by Intramural Research Program of the Division of Cancer Epidemiology and Genetics and supported by contracts from the Division of Cancer Prevention, National Cancer Institute, NIH. The authors thank the PLCO Cancer Screening Trial screening center investigators and the staff from Information Management Services Inc and Westat Inc. Most importantly, we thank the study participants for their contributions that made this study possible.

PMH: The authors would like to thank the study participants and staff of the Hormones and Colon Cancer study.

WHI: The authors thank the WHI investigators and staff for their dedication, and the study participants for making the program possible. A full listing of WHI investigators can be found at: http://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Short%20List.pdf

Funding and Grant Support

Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO): National Cancer Institute, National Institutes of Health, U.S. Department of Health and Human Services (U01 CA137088, R01 CA059045, R01201407). This research was funded in part through the NIH/NCI Cancer Center Support Grant P30 CA015704. Genotyping/Sequencing services were provided by the Center for Inherited Disease Research (CIDR). CIDR is fully funded through a federal contract from the National Institutes of Health to The Johns Hopkins University, contract number HHSN268201200008I.

The ATBC Study is supported by the Intramural Research Program of the U.S. National Cancer Institute, National Institutes of Health, and by U.S. Public Health Service contract HHSN261201500005C from the National Cancer Institute, Department of Health and Human Services.

COLO2&3: National Institutes of Health (R01 CA60987).

The Colon Cancer Family Registry (CFR) Illumina GWAS was supported by funding from the National Cancer Institute, National Institutes of Health (grant numbers U01 CA122839, R01 CA143247). The Colon CFR/CORECT Affymetrix Axiom GWAS and OncoArray GWAS were supported by funding from National Cancer Institute, National Institutes of Health (grant number U19 CA148107 to S Gruber). The Colon CFR participant recruitment and collection of data and biospecimens used in this study were supported by the National Cancer Institute, National Institutes of Health (grant number U01 CA167551) and through cooperative agreements with the following Colon CFR centers: Australasian Colorectal Cancer Family Registry (NCI/NIH grant numbers U01 CA074778 and U01/U24 CA097735 to M. A. Jenkins), USC Consortium Colorectal Cancer Family Registry (NCI/NIH grant numbers U01/U24 CA074799 to R.W. Haile), Mayo Clinic Cooperative Family Registry for Colon Cancer Studies (NCI/NIH grant number U01/U24 CA074800 to N.M Lindor), Ontario Familial Colorectal Cancer Registry (NCI/NIH grant number U01/U24 CA074783 to S. Gallinger), Seattle Colorectal Cancer Family Registry (NCI/NIH grant number U01/U24 CA074794 to P.A. Newcomb), and University of Hawaii Colorectal Cancer Family Registry (NCI/NIH grant number U01/U24 CA074806 to L. Le Marchand), Additional support for case ascertainment was provided from the Surveillance, Epidemiology and End Results (SEER) Program of the National Cancer Institute to Fred Hutchinson Cancer Research Center (Control Nos. N01-CN-67009 and N01-PC-35142, and Contract No. HHSN2612013000121), the Hawai'i Department of Health (Control Nos. N01-PC-67001 and N01-PC-35137, and Contract No. HHSN26120100037C, and the California Department of Public Health (contracts HHSN261201000035C awarded to the University of Southern California, and the following state cancer registries: AZ, CO, MN, NC, NH, and by the Victoria Cancer Registry and Ontario Cancer Registry.

Colorectal Cancer Transdisciplinary (CORECT) Study: The CORECT Study was supported by the National Cancer Institute, National Institutes of Health (NCI/NIH), U.S. Department of Health and Human Services (grant numbers U19 CA148107, R01 CA81488, P30 CA014089, R01 CA197350,; P01 CA196569; R01 CA201407) and National Institutes of Environmental Health Sciences, National Institutes of Health (grant number T32 ES013678).

CPS-II: The American Cancer Society funds the creation, maintenance, and updating of the Cancer Prevention Study-II (CPS-II) cohort. This study was conducted with Institutional Review Board approval.

CRCGEN: Colorectal Cancer Genetics & Genomics, Spanish study was supported by Instituto de Salud Carlos III, co-funded by FEDER funds –a way to build Europe– (grants PI14-613 and PI09-1286), Agency for Management of University and Research Grants (AGAUR) of the Catalan Government (grant 2017SGR723), and Junta de Castilla y León (grant LE22A10-2). Sample collection of this work was supported by the Xarxa de Bancs de Tumors de Catalunya sponsored by Pla Director d'Oncología de Catalunya (XBTC), Plataforma Biobancos PT13/0010/0013 and ICOBIOBANC, sponsored by the Catalan Institute of Oncology.

DACHS: This work was supported by the German Research Council (BR 1704/6-1, BR 1704/6-3, BR 1704/6-4, CH 117/1-1, HO 5117/2-1, HE 5998/2-1, KL 2354/3-1, RO 2270/8-1 and BR 1704/17-1), the Interdisciplinary Research Program of the National Center for Tumor Diseases (NCT), Germany, and the German Federal Ministry of Education and Research (01KH0404, 01ER0814, 01ER0815, 01ER1505A and 01ER1505B).

DALS: National Institutes of Health (R01 CA48998 to M. L. Slattery).

Harvard cohorts (HPFS, NHS, PHS): HPFS is supported by the National Institutes of Health (P01 CA055075, UM1 CA167552, U01 CA167552, R01 CA137178, R01 CA151993, R35 CA197735, K07 CA190673, and P50 CA127003), NHS by the National Institutes of Health (R01 CA137178, P01 CA087969, UM1 CA186107, R01 CA151993, R35 CA197735, K07 CA190673, and P50 CA127003) and PHS by the National Institutes of Health (R01 CA042182).

Kentucky: This work was supported by the following grant support: Clinical Investigator Award from Damon Runyon Cancer Research Foundation (CI-8); NCI R01CA136726.

MCCS cohort recruitment was funded by VicHealth and Cancer Council Victoria. The MCCS was further supported by Australian NHMRC grants 509348, 209057, 251553 and 504711 and by infrastructure provided by Cancer Council Victoria. Cases and their vital status were ascertained through the Victorian Cancer Registry (VCR) and the Australian Institute of Health and Welfare (AIHW), including the National Death Index and the Australian Cancer Database.

MEC: National Institutes of Health (R37 CA54281, P01 CA033619, and R01 CA063464).

MECC: This work was supported by the National Institutes of Health, U.S. Department of Health and Human Services (R01 CA81488 to SBG and GR).

NFCCR: This work was supported by an Interdisciplinary Health Research Team award from the Canadian Institutes of Health Research (CRT 43821); the National Institutes of Health, U.S. Department of Health and Human Serivces (U01 CA74783); and National Cancer Institute of Canada grants (18223 and 18226). The authors wish to acknowledge the contribution of Alexandre Belisle and the genotyping team of the McGill University and Génome Québec Innovation Centre, Montréal, Canada, for genotyping the Sequenom panel in the NFCCR samples. Funding was provided to Michael O. Woods by the Canadian Cancer Society Research Institute.

OFCCR: National Institutes of Health, through funding allocated to the Ontario Registry for Studies of Familial Colorectal Cancer (U01 CA074783); see CCFR section above. Additional funding toward genetic analyses of OFCCR includes the Ontario Research Fund, the Canadian Institutes of Health Research, and the Ontario Institute for Cancer Research, through generous support from the Ontario Ministry of Research and Innovation.

PLCO: Intramural Research Program of the Division of Cancer Epidemiology and Genetics and supported by contracts from the Division of Cancer Prevention, National Cancer Institute, NIH,

PMH: National Institutes of Health (R01 CA076366 to P.A. Newcomb).

VITAL: National Institutes of Health (K05 CA154337).

WHI: The WHI program is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, U.S. Department of Health and Human Services through contracts HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HHSN268201100003C, HHSN268201100004C, and HHSN271201100004C.

References

1. Flossmann E, Rothwell PM, British Doctors Aspirin T, the UKTIAAT. Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. Lancet 2007;369(9573):1603–13 doi 10.1016/S0140-6736(07)60747-8. [PubMed: 17499602]
2. Rothwell PM, Wilson M, Elwin CE, Norrving B, Algra A, Warlow CP, et al. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. Lancet 2010;376(9754):1741–50 doi 10.1016/S0140-6736(10)61543-7. [PubMed: 20970847]
3. Chan AT, Giovannucci EL, Meyerhardt JA, Schernhammer ES, Curhan GC, Fuchs CS. Long-term use of aspirin and nonsteroidal anti-inflammatory drugs and risk of colorectal cancer. JAMA 2005;294(8):914–23 doi 10.1001/jama.294.8.914. [PubMed: 16118381]
4. Chubak J, Whitlock EP, Williams SB, Kamineni A, Burda BU, Buist DS, et al. Aspirin for the Prevention of Cancer Incidence and Mortality: Systematic Evidence Reviews for the U.S. Preventive Services Task Force. Ann Intern Med 2016;164(12):814–25 doi 10.7326/M15-2117. [PubMed: 27064482]
5. Zheng SL, Roddick AJ. Association of Aspirin Use for Primary Prevention With Cardiovascular Events and Bleeding Events: A Systematic Review and Meta-analysis. JAMA 2019;321(3):277–87 doi 10.1001/jama.2018.20578. [PubMed: 30667501]
6. Ulrich CM, Bigler J, Potter JD. Non-steroidal anti-inflammatory drugs for cancer prevention: promise, perils and pharmacogenetics. Nat Rev Cancer 2006;6(2):130–40 doi 10.1038/nrc1801. [PubMed: 16491072]
7. Drew DA, Cao Y, Chan AT. Aspirin and colorectal cancer: the promise of precision chemoprevention. Nat Rev Cancer 2016;16(3):173–86 doi 10.1038/nrc.2016.4. [PubMed: 26868177]
8. Thun MJ, Jacobs EJ, Patrono C. The role of aspirin in cancer prevention. Nat Rev Clin Oncol 2012;9(5):259–67 doi 10.1038/nrclinonc.2011.199. [PubMed: 22473097]
9. Liao X, Lochhead P, Nishihara R, Morikawa T, Kuchiba A, Yamauchi M, et al. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. N Engl J Med 2012;367(17):1596–606 doi 10.1056/NEJMoa1207756. [PubMed: 23094721]
10. Nan H, Hutter CM, Lin Y, Jacobs EJ, Ulrich CM, White E, et al. Association of aspirin and NSAID use with risk of colorectal cancer according to genetic variants. JAMA 2015;313(11):1133–42 doi 10.1001/jama.2015.1815. [PubMed: 25781442]
11. Nan H, Morikawa T, Suuriniemi M, Imamura Y, Werner L, Kuchiba A, et al. Aspirin use, 8q24 single nucleotide polymorphism rs6983267, and colorectal cancer according to CTNNB1 alterations. J Natl Cancer Inst 2013;105(24):1852–61 doi 10.1093/jnci/djt331. [PubMed: 24317174]
12. Andersen V, Christensen J, Overvad K, Tjonneland A, Vogel U. Polymorphisms in NFkB, PXR, LXR and risk of colorectal cancer in a prospective study of Danes. BMC Cancer 2010;10:484 doi 10.1186/1471-2407-10-484. [PubMed: 20836841]
13. Seufert BL, Poole EM, Whitton J, Xiao L, Makar KW, Campbell PT, et al. IkappaBKbeta and NFkappaB1, NSAID use and risk of colorectal cancer in the Colon Cancer Family Registry. Carcinogenesis 2013;34(1):79–85 doi 10.1093/carcin/bgs296. [PubMed: 23002237]
14. Pan MR, Chang HC, Hung WC. Non-steroidal anti-inflammatory drugs suppress the ERK signaling pathway via block of Ras/c-Raf interaction and activation of MAP kinase phosphatases. Cell Signal 2008;20(6):1134–41 doi 10.1016/j.cellsig.2008.02.004. [PubMed: 18374541]
15. Hutter CM, Chang-Claude J, Slattery ML, Pflugeisen BM, Lin Y, Duggan D, et al. Characterization of gene-environment interactions for colorectal cancer susceptibility loci. Cancer Res 2012;72(8):2036–44 doi 10.1158/0008-5472.CAN-11-4067. [PubMed: 22367214]
16. Newcomb PA, Baron J, Cotterchio M, Gallinger S, Grove J, Haile R, et al. Colon Cancer Family Registry: an international resource for studies of the genetic epidemiology of colon cancer. Cancer Epidemiol Biomarkers Prev 2007;16(11):2331–43 doi 10.1158/1055-9965.EPI-07-0648. [PubMed: 17982118]

17. Schmit SL, Edlund CK, Schumacher FR, Gong J, Harrison TA, Huyghe JR, et al. Novel Common Genetic Susceptibility Loci for Colorectal Cancer. J Natl Cancer Inst 2019;111(2):146–57 doi 10.1093/jnci/djy099. [PubMed: 29917119]
18. Schumacher FR, Schmit SL, Jiao S, Edlund CK, Wang H, Zhang B, et al. Genome-wide association study of colorectal cancer identifies six new susceptibility loci. Nat Commun 2015;6:7138 doi 10.1038/ncomms8138. [PubMed: 26151821]
19. Peters U, Jiao S, Schumacher FR, Hutter CM, Aragaki AK, Baron JA, et al. Identification of Genetic Susceptibility Loci for Colorectal Tumors in a Genome-Wide Meta-analysis. Gastroenterology 2013;144(4):799–807.e24 doi 10.1053/j.gastro.2012.12.020. [PubMed: 23266556]
20. Peters U, Hutter CM, Hsu L, Schumacher FR, Conti DV, Carlson CS, et al. Meta-analysis of new genome-wide association studies of colorectal cancer risk. Hum Genet 2012;131(2):217–34 doi 10.1007/s00439-011-1055-0. [PubMed: 21761138]
21. Zanke BW, Greenwood CM, Rangrej J, Kustra R, Tenesa A, Farrington SM, et al. Genome-wide association scan identifies a colorectal cancer susceptibility locus on chromosome 8q24. Nat Genet 2007;39(8):989–94 doi 10.1038/ng2089. [PubMed: 17618283]
22. McCarthy S, Das S, Kretzschmar W, Delaneau O, Wood AR, Teumer A, et al. A reference panel of 64,976 haplotypes for genotype imputation. Nat Genet 2016;48(10):1279–83 doi 10.1038/ng.3643. [PubMed: 27548312]
23. Gamazon ER, Wheeler HE, Shah KP, Mozaffari SV, Aquino-Michaels K, Carroll RJ, et al. A gene-based association method for mapping traits using reference transcriptome data. Nat Genet 2015;47(9):1091–8 doi 10.1038/ng.3367. [PubMed: 26258848]
24. Torres JM, Gamazon ER, Parra EJ, Below JE, Valladares-Salgado A, Wacher N, et al. Cross-tissue and tissue-specific eQTLs: partitioning the heritability of a complex trait. Am J Hum Genet 2014;95(5):521–34 doi 10.1016/j.ajhg.2014.10.001. [PubMed: 25439722]
25. Jeon J, Du M, Schoen RE, Hoffmeister M, Newcomb PA, Berndt SI, et al. Determining Risk of Colorectal Cancer and Starting Age of Screening Based on Lifestyle, Environmental, and Genetic Factors. Gastroenterology 2018;154(8):2152–64 e19 doi 10.1053/j.gastro.2018.02.021. [PubMed: 29458155]
26. Su YR, Di CZ, Hsu L, Genetics, Epidemiology of Colorectal Cancer C. A unified powerful set-based test for sequencing data analysis of GxE interactions. Biostatistics 2017;18(1):119–31 doi 10.1093/biostatistics/kxw034. [PubMed: 27474101]
27. Yu DM, Yao TW, Chowdhury S, Nadvi NA, Osborne B, Church WB, et al. The dipeptidyl peptidase IV family in cancer and cell biology. FEBS J 2010;277(5):1126–44 doi 10.1111/j.1742-4658.2009.07526.x. [PubMed: 20074209]
28. Kim SH, Choi H, Yoon MG, Ye YM, Park HS. Dipeptidyl-peptidase 10 as a genetic biomarker for the aspirin-exacerbated respiratory disease phenotype. Ann Allergy Asthma Immunol 2015;114(3):208–13 doi 10.1016/j.anai.2014.12.003. [PubMed: 25592153]
29. Hamberg M, Hedqvist P, Radegran K. Identification of 15-hydroxy-5,8,11,13-eicosatetraenoic acid (15-HETE) as a major metabolite of arachidonic acid in human lung. Acta Physiol Scand 1980;110(2):219–21 doi 10.1111/j.1748-1716.1980.tb06656.x. [PubMed: 7211407]
30. Rauzi F, Kirkby NS, Edin ML, Whiteford J, Zeldin DC, Mitchell JA, et al. Aspirin inhibits the production of proangiogenic 15(S)-HETE by platelet cyclooxygenase-1. FASEB J 2016;30(12):4256–66 doi 10.1096/fj.201600530R. [PubMed: 27633788]
31. Machesney M, Tidman N, Waseem A, Kirby L, Leigh I. Activated keratinocytes in the epidermis of hypertrophic scars. Am J Pathol 1998;152(5):1133–41. [PubMed: 9588880]
32. Wang YN, Chen YJ, Chang WC. Activation of extracellular signal-regulated kinase signaling by epidermal growth factor mediates c-Jun activation and p300 recruitment in keratin 16 gene expression. Mol Pharmacol 2006;69(1):85–98 doi 10.1124/mol.105.016220. [PubMed: 16214953]
33. Endo H, Sugioka Y, Nakagi Y, Saijo Y, Yoshida T. A novel role of the NRF2 transcription factor in the regulation of arsenite-mediated keratin 16 gene expression in human keratinocytes. Environ Health Perspect 2008;116(7):873–9 doi 10.1289/ehp.10696. [PubMed: 18629308]

34. Evstafieva AG, Kovaleva IE, Shoshinova MS, Budanov AV, Chumakov PM. Implication of KRT16, FAM129A and HKDC1 genes as ATF4 regulated components of the integrated stress response. PLoS One 2018;13(2):e0191107 doi 10.1371/journal.pone.0191107. [PubMed: 29420561]
35. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science 1990;249(4975):1431–3. [PubMed: 1698311]
36. Kojima M, Morisaki T, Izuhara K, Uchiyama A, Matsunari Y, Katano M, et al. Lipopolysaccharide increases cyclo-oxygenase-2 expression in a colon carcinoma cell line through nuclear factor-kappa B activation. Oncogene 2000;19(9):1225–31 doi 10.1038/sj.onc.1203427. [PubMed: 10713711]
37. Li C, Luo X, Lin Y, Tang X, Ling L, Wang L, et al. A Higher Frequency of CD14+ CD169+ Monocytes/Macrophages in Patients with Colorectal Cancer. PLoS One 2015;10(10):e0141817 doi 10.1371/journal.pone.0141817. [PubMed: 26509874]
38. Cali JJ, Russell DW. Characterization of human sterol 27-hydroxylase. A mitochondrial cytochrome P-450 that catalyzes multiple oxidation reaction in bile acid biosynthesis. J Biol Chem 1991;266(12):7774–8. [PubMed: 1708392]
39. Anderson MG, Nakane M, Ruan X, Kroeger PE, Wu-Wong JR. Expression of VDR and CYP24A1 mRNA in human tumors. Cancer Chemother Pharmacol 2006;57(2):234–40 doi 10.1007/s00280-005-0059-7. [PubMed: 16180015]
40. Matusiak D, Benya RV. CYP27A1 and CYP24 expression as a function of malignant transformation in the colon. J Histochem Cytochem 2007;55(12):1257–64 doi 10.1369/jhc.7A7286.2007. [PubMed: 17875655]
41. Swan R, Alnabulsi A, Cash B, Alnabulsi A, Murray GI. Characterisation of the oxysterol metabolising enzyme pathway in mismatch repair proficient and deficient colorectal cancer. Oncotarget 2016;7(29):46509–27 doi 10.18632/oncotarget.10224. [PubMed: 27341022]
42. Hendrikx T, Jeurissen ML, Bieghs V, Walenbergh SM, van Gorp PJ, Verheyen F, et al. Hematopoietic overexpression of Cyp27a1 reduces hepatic inflammation independently of 27-hydroxycholesterol levels in Ldlr(−/−) mice. J Hepatol 2015;62(2):430–6 doi 10.1016/j.jhep.2014.09.027. [PubMed: 25281859]
43. Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. Cell 2009;136(4):629–41 doi 10.1016/j.cell.2009.02.006. [PubMed: 19239885]
44. Liu J, Wan L, Lu K, Sun M, Pan X, Zhang P, et al. The Long Noncoding RNA MEG3 Contributes to Cisplatin Resistance of Human Lung Adenocarcinoma. PLoS One 2015;10(5):e0114586 doi 10.1371/journal.pone.0114586. [PubMed: 25992654]
45. Malek E, Jagannathan S, Driscoll JJ. Correlation of long non-coding RNA expression with metastasis, drug resistance and clinical outcome in cancer. Oncotarget 2014;5(18):8027–38 doi 10.18632/oncotarget.2469. [PubMed: 25275300]
46. Guo H, Liu J, Ben Q, Qu Y, Li M, Wang Y, et al. The aspirin-induced long non-coding RNA OLA1P2 blocks phosphorylated STAT3 homodimer formation. Genome Biol 2016;17:24 doi 10.1186/s13059-016-0892-5. [PubMed: 26898989]
47. Gibb EA, Vucic EA, Enfield KS, Stewart GL, Lonergan KM, Kennett JY, et al. Human cancer long non-coding RNA transcriptomes. PLoS One 2011;6(10):e25915 doi 10.1371/journal.pone.0025915. [PubMed: 21991387]
48. Glebov OK, Rodriguez LM, Soballe P, DeNobile J, Cliatt J, Nakahara K, et al. Gene expression patterns distinguish colonoscopically isolated human aberrant crypt foci from normal colonic mucosa. Cancer Epidemiol Biomarkers Prev 2006;15(11):2253–62 doi 10.1158/1055-9965.EPI-05-0694. [PubMed: 17119054]
49. LaPointe LC, Dunne R, Brown GS, Worthley DL, Molloy PL, Wattchow D, et al. Map of differential transcript expression in the normal human large intestine. Physiol Genomics 2008;33(1):50–64 doi 10.1152/physiolgenomics.00185.2006. [PubMed: 18056783]
50. Noble CL, Abbas AR, Cornelius J, Lees CW, Ho GT, Toy K, et al. Regional variation in gene expression in the healthy colon is dysregulated in ulcerative colitis. Gut 2008;57(10):1398–405 doi 10.1136/gut.2008.148395. [PubMed: 18523026]

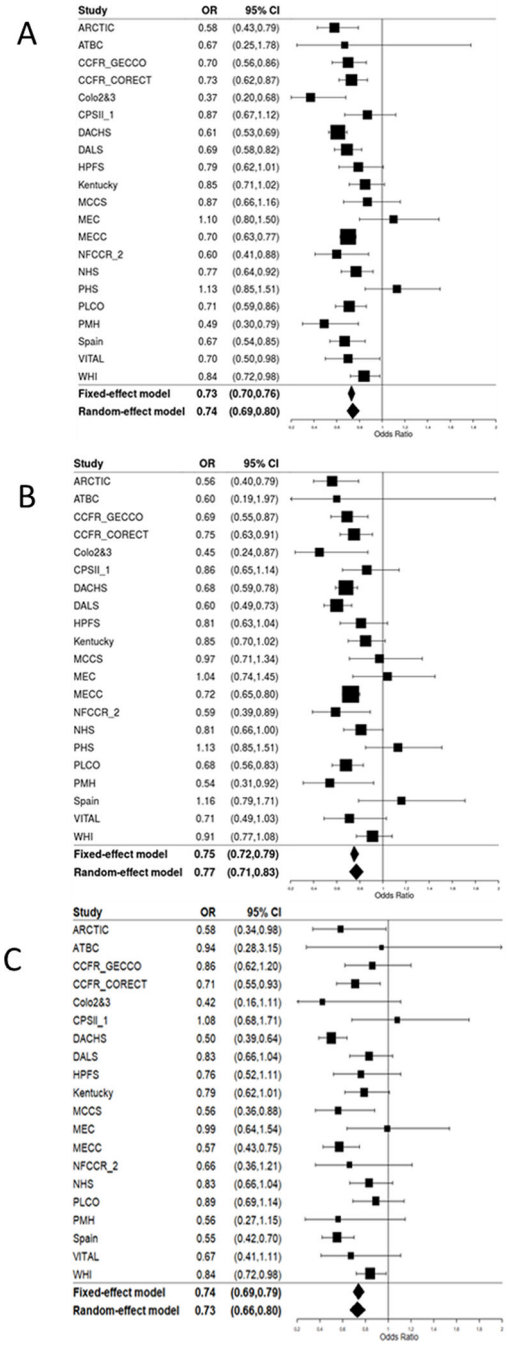

Figure 1. Main effect of regular use of any NSAID (A), aspirin (B) or non-aspirin NSAIDs (C) on colorectal cancer risk

(A) Regular use of any NSAID (N = 39,290)

* I^2 = 45.7%; p-heterogeneity = 0.001

(B) Regular use of aspirin only (N = 38,784)

* I^2 = 44.4%; p-heterogeneity = 0.001

(C) Regular use of non-aspirin NSAIDs only (N = 37,645)

* I^2 = 2.2%; p-heterogeneity = 0.012

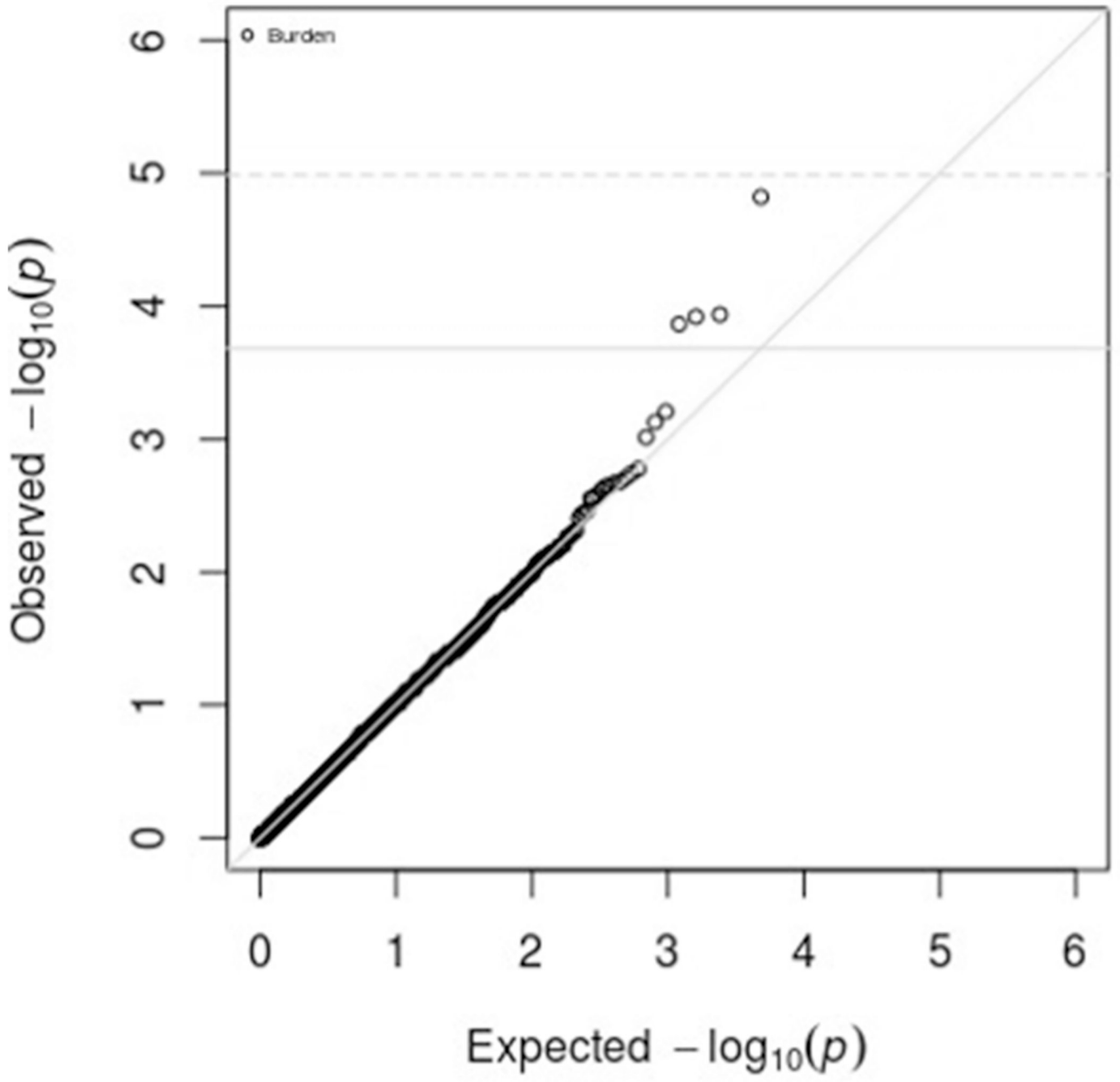

Figure 2. Quantile-quantile plot of p-values of the fixed effects in gene-any NSAID use interaction.

* N genes = 4,840

** Dotted line (- - -): p-value threshold of Bonferroni correction (1×10^{-5}); Solid line (——): p-value threshold for FDR at 0.2.

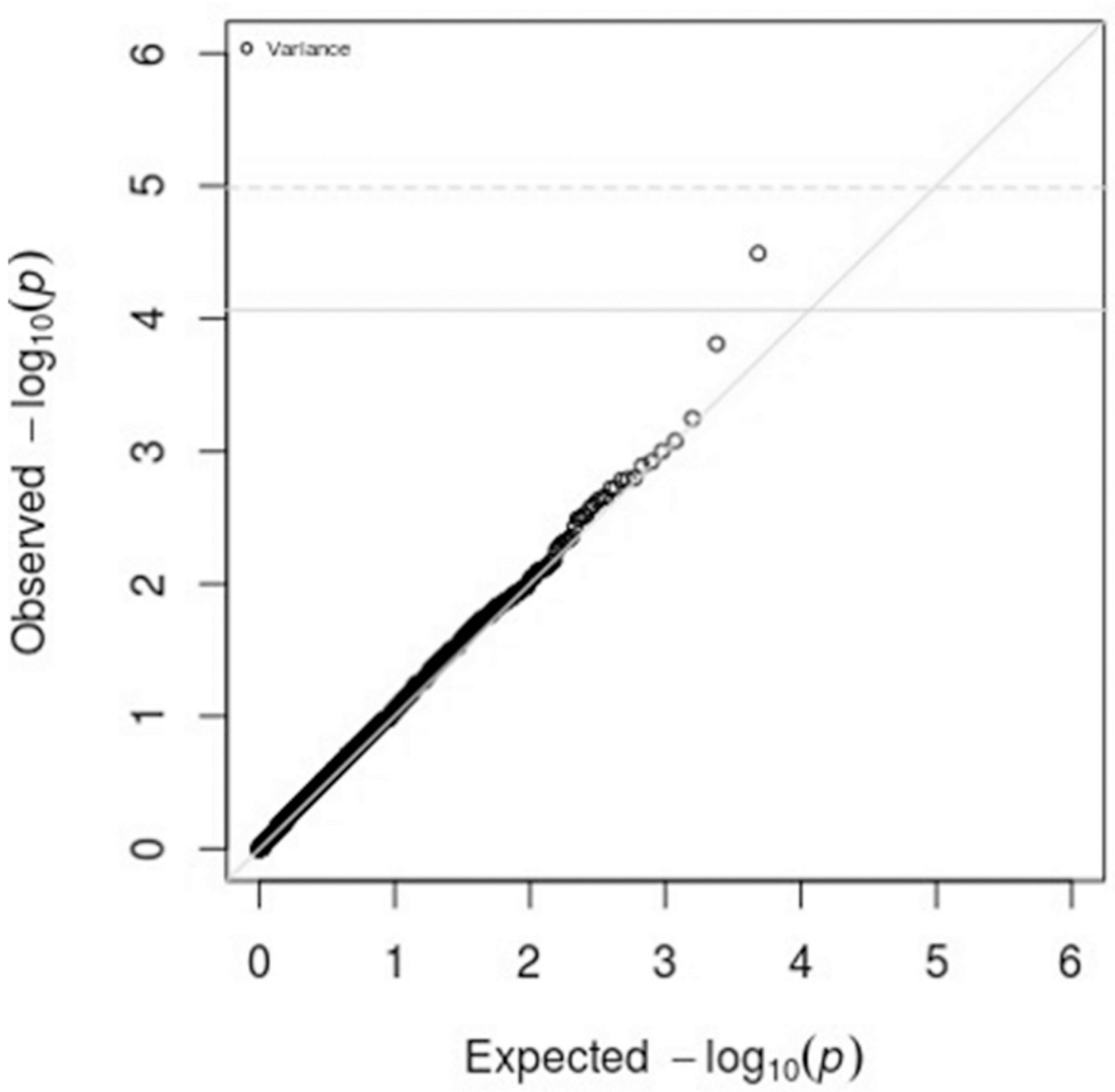

Figure 3. Quantile-quantile plot of p-values of the random effects in gene-aspirin use interaction.

* N genes = 4,840

** Dotted line (- - -): p-value threshold of Bonferroni correction (1×10^{-5}); Solid line (——): p-value threshold for FDR at 0.2.

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^2	$P_{G\times E}$
Any NSAID	*DPP10*	2q14.1	8	4.46%	1.96×10^{-4}
	KRT16	17q21.2	10	2.80%	2.32×10^{-4}
	CD14	5q31.3	14	2.90%	9.38×10^{-4}
	CYP27A1	2q35	6	11.45%	1.44×10^{-3}
Aspirin	*RP11–89N17*	7p14.3	60	3.93%	3.23×10^{-5}

*R^2: The variance of the gene expressions explained by the SNPs in each gene set, downloaded from the publicly available PredictDB Repository (http://hakyimlab.org/predictdb/)

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 non-regular NSAID users			Regular NSAID users		
		OR[a]	95% CI	p-value	OR[a]	95% CI	p-value
DPP10	−0.18 (0.13)	1.04	(1.01, 1.06)	0.005	0.94	(0.91, 0.97)	<0.001
KRT16	−0.22 (0.07)	1.04	(1.01, 1.06)	0.004	0.96	(0.93, 1.00)	0.031
CD14	0.16 (0.16)	1.01	(0.99, 1.04)	0.421	0.96	(0.92, 0.99)	0.014
CYP27A1	0.24 (0.18)	0.96	(0.94, 0.99)	0.002	1.05	(1.01, 1.08)	0.009

[a]Odds ratio per standard deviation increase in estimated gene expression level