Original Investigation

Association of Aspirin and NSAID Use With Risk of Colorectal Cancer According to Genetic Variants

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IMPORTANCE Use of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) is associated with lower risk of colorectal cancer.

OBJECTIVE To identify common genetic markers that may confer differential benefit from aspirin or NSAID chemoprevention, we tested gene × environment interactions between regular use of aspirin and/or NSAIDs and single-nucleotide polymorphisms (SNPs) in relation to risk of colorectal cancer.

DESIGN, SETTING, AND PARTICIPANTS Case-control study using data from 5 case-control and 5 cohort studies initiated between 1976 and 2003 across the United States, Canada, Australia, and Germany and including colorectal cancer cases (n=8634) and matched controls (n=8553) ascertained between 1976 and 2011. Participants were all of European descent.

EXPOSURES Genome-wide SNP data and information on regular use of aspirin and/or NSAIDs and other risk factors.

MAIN OUTCOMES AND MEASURES Colorectal cancer.

RESULTS Regular use of aspirin and/or NSAIDs was associated with lower risk of colorectal cancer (prevalence, 28% vs 38%; odds ratio [OR], 0.69 [95% CI, 0.64-0.74]; P = 6.2 x 10^{-28}) compared with nonregular use. In the conventional logistic regression analysis, the SNP rs2965667 at chromosome 12p12.3 near the MGST1 gene showed a genome-wide significant interaction with aspirin and/or NSAID use (P = 4.6 x 10^{-9} for interaction). Aspirin and/or NSAID use was associated with a lower risk of colorectal cancer among individuals with rs2965667-TT genotype (prevalence, 28% vs 38%; OR, 0.66 [95% CI, 0.61-0.70]; P = 7.7 x 10^{-13}) but with a higher risk among those with rare (4%) TA or AA genotypes (prevalence, 35% vs 29%; OR, 1.89 [95% CI, 1.27-2.81]; P = .002). In case-only interaction analysis, the SNP rs16973225 at chromosome 15q25.2 near the IL16 gene showed a genome-wide significant interaction with use of aspirin and/or NSAIDs (P = 8.2 x 10^{-9} for interaction). Regular use was associated with a lower risk of colorectal cancer among individuals with rs16973225-AA genotype (prevalence, 28% vs 38%; OR, 0.66 [95% CI, 0.62-0.71]; P = 1.9 x 10^{-30}) but was not associated with risk of colorectal cancer among those with less common (9%) AC or CC genotypes (prevalence, 36% vs 39%; OR, 0.97 [95% CI, 0.78-1.20]; P = .76).

CONCLUSIONS AND RELEVANCE In this genome-wide investigation of gene × environment interactions, use of aspirin and/or NSAIDs was associated with lower risk of colorectal cancer, and this association differed according to genetic variation at 2 SNPs at chromosomes 12 and 15. Validation of these findings in additional populations may facilitate targeted colorectal cancer prevention strategies.

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Considerable evidence demonstrates that use of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) is associated with lower risk of colorectal neoplasms. However, the mechanisms behind this association are not well understood. Routine use of aspirin, NSAIDs, or both for chemoprevention of cancer is not currently recommended because of uncertainty about risk-benefit profile. Hence, understanding the interrelationship between genetic markers and use of aspirin and NSAIDs, also known as gene × environment interactions, can help to identify population subgroups defined by genetic background that may preferentially benefit from chemopreventive use of these agents and offer novel insights into underlying mechanisms of carcinogenesis.

Previous genetic studies have examined the association of aspirin, NSAIDs, or both with colorectal cancer according to a limited number of candidate genes or pathways. Thus, to comprehensively identify common genetic markers that characterize individuals who may obtain differential benefit from aspirin and NSAIDs, we conducted a discovery-based, genome-wide analysis of gene × environment interactions, as defined by single-nucleotide polymorphisms (SNPs) in relation to risk of colorectal cancer.

Methods

Study Population and Harmonization of Environmental Data

We included individual-level data pooled from a case-control study from the Colon Cancer Family Registry (CCFR) and 9 studies from the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO) that were initiated between 1976 and 2003 and that enrolled cases of colorectal cancer diagnosed between 1976 and 2011 and matched controls across the United States, Canada, Australia, and Germany (Table 1). The cohorts are described in the eAppendix in the Supplement. All cases were defined as invasive colorectal adenocarcinoma and confirmed by medical record, pathology report, or death certificate. For prospective cohorts, nested case-control sets were constructed by fixing the cohort at a point at which risk set sampling was used to select cases and controls. For other case-control studies, population-based controls were used. For all studies, controls were matched on age, sex, and race/ethnicity; for some studies, controls were also matched on additional factors, such as enrollment date and trial group.

Study-specific eligibility and our multistep data harmonization procedure are described in the eAppendix in the Supplement. Briefly, within each study, all exposure information, including use of aspirin, NSAIDs, or both, was collected by in-person interviews, structured questionnaires, or both with the reference time for cohort studies as the time of enrollment (Women’s Health Initiative [WHI]; prostate, Lung, Colorectal and Ovarian Cancer Screening Trial [PLCO]; and Vitamins and Lifestyle [VITAL]) or blood draw (Health Professionals Follow-up Study [HPFS] and Nurses’ Health Study [NHS]). Individuals with missing data on use of aspirin and NSAIDs were excluded. The precise definition of regular use of aspirin, NSAIDs, or both, which was determined individually by each study cohort, is provided in Table 1.

All participants provided written or oral informed consent, and studies were reviewed and approved by their respective institutional review boards or ethics committees.

Statistical Methods

A detailed description for genotyping, quality assurance and quality control, and imputation is provided in the Supplement. Mean sample and SNP call rates, and concordance rates for blinded duplicates, are listed in eTable 1 in the Supplement. In brief, genotyped SNPs were excluded based on call rate (<98%), lack of Hardy-Weinberg equilibrium (HWE) in controls (P < 1 × 10−8), and minor allele frequency (MAF) (MAF <5% for WHI Set 1, Diet, Activity and Lifestyle Study [DALS] Set 1, and Northern Ontario Familial Colorectal Cancer Registry [OFCCR]; MAF <5/No. of samples for each other study). Because imputation of genotypes is standard practice in genetic association analysis, all autosomal SNPs of each study were imputed to the CEPH collection (CEU) population in the HapMap II using IMPUTE (CCFR), BEAGLE (OFCCR), and MACH (all other studies).

After imputation and quality-control analyses, a total of approximately 2.7 million SNPs were used in the analysis. To reduce heterogeneity, all analyses were restricted to samples self-reported as of European descent and clustering with Utah residents with Northern/Western European ancestry from the CEU population in principal component analysis, including the HapMap II populations as reference.

Statistical analyses were conducted centrally on individual-level data. We adjusted for age at reference time, sex, center, and racial composition using the first 3 principal components from EIGENSTRAT to account for population substructure. Each directly genotyped SNP was coded as 0, 1, or 2 copies of the variant allele. For imputed SNPs, we used the expected number of copies of the variant allele, which provides unbiased test statistics. Both genotyped and imputed SNPs were examined as continuous variables (ie, assuming log-additive effects).

We analyzed each study separately using logistic regression models and combined study-specific results using fixed effect to obtain summary odds ratios (ORs) and 95% CIs. We calculated P values for heterogeneity using the Cochran Q test. Fixed-effect meta-analysis is routinely used in genome-wide association studies (GWAS) because it is the most powerful approach for identifying disease-associated variants. Furthermore, in our study fixed effect was more appropriate than
random effects, since the Q-Q plots and the P value distributions indicated minimal heterogeneity across studies. Moreover, the effects may not fit a Gaussian distribution as required by the random-effects model, and the limited number of included studies may lead to an imprecise estimate of heterogeneity.\(^5\)

To test for gene × environment interactions between SNPs and the regular use of aspirin, NSAIDs, or both (including use of aspirin only, NSAIDs only, or both aspirin and NSAIDs) or the regular use of aspirin only, we used conventional case-control logistic regression and case-only interaction analyses. Equations for the models used in the interaction analyses are provided in the eAppendix in the Supplement. We examined genome-wide correlations between SNPs and use of aspirin, NSAIDs, or both using linear regression analysis and did not observe deviation from independence. For all genome-wide gene × environment interaction analyses, \(P < 5.0 \times 10^{-8}\) (2-sided), which yields a genome-wide significance level of .05, was considered statistically significant.

As described in the eAppendix in the Supplement, for each SNP showing gene × environment interaction with use of aspirin, NSAIDs, or both, we estimated the association of such use with colorectal cancer risk stratified by SNP genotypes, as well as associations in strata defined by SNP and use of aspirin, NSAIDs, or both with 1 common reference group. We also estimated absolute risks associated with use of aspirin, NSAIDs, or both among individuals defined by specific genotypes based on Surveillance, Epidemiology, and End Results age-adjusted colorectal cancer incidence rates (eAppendix in the Supplement).

All analyses were conducted using R 3.1.2 (R Foundation for Statistical Computing [http://www-r-project.org]).

### Results

The characteristics of the 8634 colorectal cancer cases and 8553 controls of European descent within each cohort from the CCFR and GECCO are provided in Table 1. As shown in the Figure, compared with nonregular use, regular use of aspirin, NSAIDs, or both (prevalence, 28% vs 38%; OR, 0.69 [95% CI, 0.64-0.74]; \(P = 6.2 \times 10^{-28}\), \(P = .02\) for heterogeneity) or aspirin only...
Prevalence, 24% vs 31%; OR, 0.71 [95% CI, 0.66-0.77]; \( P = \text{5.0} \times 10^{-19} \); \( P = .01 \) for heterogeneity) was associated with lower risk of colorectal cancer.

For the conventional logistic regression interaction analysis between each SNP and aspirin and/or NSAID use, the \( P \) values are shown in the Manhattan plot and Q-Q plot (eFigure 1 in the Supplement). At chromosome 12p12.3, we observed SNP rs2965667 (MAF = 1.7%) showing a genome-wide significant interaction with regular use of aspirin, NSAIDs, or both (\( P = 4.6 \times 10^{-9} \) for interaction). The SNP rs10505806 (MAF = 3.8%), which had the second-lowest \( P \) value, was also found in the same locus, but it did not reach genome-wide significant interaction (\( P = 5.5 \times 10^{-8} \) for interaction). These 2 top SNPs (rs2965667 and rs10505806) were highly correlated (\( D' = 1.0 \) and \( r^2 = 0.74 \) in HapMap CEU).

In stratified analysis, compared with nonregular use, regular use of aspirin, NSAIDs, or both was statistically significantly associated with lower risk of colorectal cancer among individuals with rs2965667-TT genotype (prevalence, \( 28\% \) vs \( 38\% \); OR, 0.66 [95% CI, 0.61-0.70]; \( P = 7.7 \times 10^{-33} \)), which comprised 96% (\( n = 16 \, 465 \)) of the population. In contrast, a higher risk was observed among the 4% (\( n = 722 \)) of the population with TA or AA genotypes (prevalence, \( 35\% \) vs \( 29\% \); OR, 1.89 [95% CI, 1.27-2.81]; \( P = .002 \)).

As expected, stratified results for the highly correlated rs10505806 were similar to those for rs2965667. Compared with nonregular use, regular use of aspirin, NSAIDs, or both was statistically significantly associated with lower risk of colorectal cancer among individuals with rs10505806-AA genotype (prevalence, \( 28\% \) vs \( 38\% \); OR, 0.66 [95% CI, 0.61-0.70]; \( P = 8.7 \times 10^{-33} \)), which comprised 95% (\( n = 16 \, 328 \)) of the population. In contrast, a higher risk was observed among the 5% (\( n = 859 \)) of the population with AT or TT genotypes (prevalence, \( 35\% \) vs \( 31\% \); OR, 1.56 [95% CI, 1.12-2.16]; \( P = .008 \)) (Table 2 and eFigure 2 in the Supplement).

Both of these 2 highly correlated SNPs (rs2965667 and rs10505806) were imputed across all studies (100% of study population).
samples), with a mean imputation $R^2$ of 0.7 for rs2965667 and 0.8 for rs10505806 (eTable 3 in the Supplement). To further validate accuracy of imputation, we conducted direct genotyping of rs10505806 in participants enrolled in the NHS (553 cases and 955 controls) and the HPFS (403 cases and 401 controls).

The overall concordance of the SNP rs10505806 between imputed vs genotyped data was high (Pearson correlation coefficient $r$ of 0.89). Among the total 956 cases and 1356 controls within NHS and HPFS whom we also directly genotyped rs10505806, we compared the gene × environment interaction statistical effect using direct genotype data with the imputed data. We confirmed no material difference in interaction estimates ($P = .50$ for heterogeneity) between imputed data (OR, 2.57 [95% CI, 1.02-6.43]; $P = .045$ for interaction) and directly genotyped data (OR, 2.19 [95% CI, 1.04-4.59]; $P = .04$ for interaction).

In case-only interaction analysis, SNP rs16973225 at chromosome 15q25.2 showed a genome-wide significant interaction with regular use of aspirin, NSAIDs, or both ($P = 8.2 \times 10^{-9}$ for interaction). In the stratified analysis, compared with nonregular use, regular use of aspirin, NSAIDs, or both was statistically significantly associated with lower risk of colorectal cancer.

Table 2. Risk for Colorectal Cancer According to Regular Use of Aspirin and/or NSAIDs, Stratified by the Genotypes of rs2965667, rs10505806, and rs16973225a

<table>
<thead>
<tr>
<th>SNP/Genotype</th>
<th>Use of Aspirin and/or NSAIDs</th>
<th>Nonregular</th>
<th>Regularb</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2965667TT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participants, No.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>5933</td>
<td>2325</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>5088</td>
<td>3119</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>Base modeld</td>
<td>1 [Reference]</td>
<td>0.66 (0.61-0.70)</td>
<td>$7.7 \times 10^{-33}$</td>
</tr>
<tr>
<td></td>
<td>Multivariable-adjusted model</td>
<td>1 [Reference]</td>
<td>0.63 (0.59-0.68)</td>
<td>$2.3 \times 10^{-35}$</td>
</tr>
<tr>
<td>$P$ value for interactionf</td>
<td></td>
<td></td>
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<tr>
<td>rs10505806AA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participants, No.</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
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<td>2301</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>5039</td>
<td>3092</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>Base modeld</td>
<td>1 [Reference]</td>
<td>0.66 (0.61-0.70)</td>
<td>$8.7 \times 10^{-33}$</td>
</tr>
<tr>
<td></td>
<td>Multivariable-adjusted model</td>
<td>1 [Reference]</td>
<td>0.63 (0.59-0.68)</td>
<td>$4.2 \times 10^{-35}$</td>
</tr>
<tr>
<td>$P$ value for interactionf</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>rs16973225AT or TT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participants, No.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>283</td>
<td>154</td>
<td></td>
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<tr>
<td>Controls</td>
<td>293</td>
<td>129</td>
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<tr>
<td>OR (95% CI)</td>
<td>Base modeld</td>
<td>1 [Reference]</td>
<td>1.56 (1.12-2.16)</td>
<td>$0.008$</td>
</tr>
<tr>
<td></td>
<td>Multivariable-adjusted model</td>
<td>1 [Reference]</td>
<td>1.42 (1.01-2.00)</td>
<td>$0.045$</td>
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<tr>
<td>$P$ value for interactionf</td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(continued)
cancer among individuals with rs16973225-AA genotype (prevalence, 28% vs 38%; OR, 0.66 [95% CI, 0.62-0.71]; \( P = 1.9 \times 10^{-30} \)), which comprised 91% (n = 15,616) of the population, but was not associated with risk of colorectal cancer among those with AC or CC genotypes (prevalence, 36% vs 39%; OR, 0.97 [95% CI, 0.78-1.20]; \( P = .76 \)) (Table 2 and Figure 2 in the Supplement), which comprised 9% (n = 1,568) of the population.

The SNP rs16973225 was directly genotyped in 9 of 15 study sets and was imputed with high quality (\( R^2 = 0.9 \)) in the remaining 6 study sets (38% of study samples) (eTable 3 in the Supplement). To validate imputation of rs16973225, we compared the gene \( \times \) environment interaction statistical effect with colorectal cancer between imputed vs genotyped study sets in case-only interaction analysis. We found that the interaction statistical effect size was not different (\( P = .73 \)) for heterogeneity within cohorts based on imputed data (OR, 1.68 [95% CI, 1.30-2.17]; \( P = 4.7 \times 10^{-5} \)) for interaction) compared with cohorts based on directly genotyped data (OR, 1.59 [95% CI, 1.28-1.97]; \( P = 4.2 \times 10^{-5} \)) for interaction). In the case-only analysis of aspirin only, we did not observe genome-wide significant interactions.

Table 2. Risk for Colorectal Cancer According to Regular Use of Aspirin and/or NSAIDs, Stratified by the Genotypes of rs2965667, rs10505806, and rs16973225* (continued)

| SNP/Genotype | Use of Aspirin and/or NSAIDs | | |
|--------------|-----------------------------|-----------------------------|
|              | Nonregular | Regular \(^b\) | \( P \) Value |
| rs16973225\(^a\) | | | |
| AA | | | |
| Cases | 5686 | 2181 | 0.66 (0.62-0.71) | 1.9 \( \times 10^{-30} \) |
| Controls | 4840 | 2909 | | |
| OR (95% CI) | Base model\(^d\) | 1 [Reference] | 0.66 (0.62-0.71) | 1.9 \( \times 10^{-30} \) |
| | Multivariable-adjusted model\(^e\) | 1 [Reference] | 0.63 (0.59-0.68) | 3.5 \( \times 10^{-33} \) |
| AC or CC | | | |
| Participants, No. | | | |
| Cases | 491 | 274 | | |
| Controls | 492 | 311 | | |
| OR (95% CI) | Base model\(^d\) | 1 [Reference] | 0.97 (0.78-1.20) | .76 |
| | Multivariable-adjusted model\(^e\) | 1 [Reference] | 0.93 (0.75-1.17) | .55 |
| \( P \) value for interaction\(^f\) | | | | 8.2 \( \times 10^{-9} \) |

Abbreviations: NSAID, nonsteroidal anti-inflammatory drug; OR, odds ratio; SNP, single-nucleotide polymorphism.

* The numbers of cases and controls were from the base model. For the SNP rs16973225, the total sample size is slightly smaller than in Table 1 because of missing genotype (n = 3).

\(^b\) Regular use of aspirin only, NSAIDs only, or both aspirin and NSAIDs.

\(^c\) SNPs rs2965667 and rs10505806 were identified from conventional logistic regression analysis.

\(^d\) Odds ratios in base models are adjusted for age at the reference time, sex, center, and the first 3 principal components from EIGENSTRAT.

\(^e\) Odds ratios in multivariable-adjusted models are adjusted for age at the reference time, sex, center, and the first 3 principal components from EIGENSTRAT, smoking status (never, former, or current smoker), body mass index, alcohol consumption, and red meat consumption.

\(^f\) \( P \) values for interaction were calculated after adjusting for age at the reference time, sex, center, and the first 3 principal components from EIGENSTRAT.

The SNP rs2965667 showing a genome-wide significant interaction with use of aspirin, NSAIDs, or both in conventional logistic regression case-control analysis also appeared as a notable variant in case-only interaction analysis, although it did not achieve a genome-wide significance level (\( P = 7.5 \times 10^{-8} \) for interaction). Similarly, the SNP rs16973225 reaching a genome-wide significant interaction with use of aspirin, NSAIDs, or both in case-only interaction analysis also showed evidence for gene \( \times \) environment interaction in conventional logistic regression analysis (\( P = 2.2 \times 10^{-4} \) for interaction).

The results for the 3 SNPs showing gene \( \times \) environment interaction (rs2965667, rs10505806, and rs16973225) did not materially change after adjusting for additional colorectal cancer risk factors, including smoking status, body mass index, alcohol consumption, and red meat consumption (Table 2 and eTable 4 in the Supplement). For these 3 SNPs, we report the ORs for use of aspirin, NSAIDs, or both across genotypes corresponding to 0, 1, or 2 copies of the variant allele (eTable 5 in the Supplement) and the ORs for each SNP by strata of use of aspirin, NSAIDs, or both with 1 common reference group (eTable 6 in the Supplement), to fully describe the interaction.
We estimated absolute risks associated with use of aspirin, NSAIDs, or both among individuals with specific genotypes defined by each of these 3 SNPs. Compared with non-use of aspirin, NSAIDs, or both, regular use was associated with 16.6 fewer colorectal cancer cases per 100,000 individuals with the rs2965667-TT genotype per year; 16.7 fewer colorectal cancer cases per 100,000 individuals with the rs10505806-AA genotype per year; and 16.8 fewer colorectal cancer cases per 100,000 individuals with the rs16973225-AA genotype per year. In contrast, regular use of aspirin, NSAIDs, or both was associated with 34.7 additional colorectal cancer cases per 100,000 individuals with rs2965667-TA or -AA genotypes per year; 21.1 additional colorectal cancer cases per 100,000 individuals with rs10505806-AT or -TT genotypes per year; and only 1.5 fewer colorectal cancer cases per 100,000 with rs16973225-AC or -CC genotypes per year.

Discussion

Consistent with the preponderance of experimental, epidemiologic, and clinical trial evidence, we found that use of aspirin, NSAIDs, or both was associated with overall lower risk of colorectal cancer in this large genome-wide investigation of gene × environment interaction, which included 86,340 colorectal cancer cases and 8,553 controls. However, we identified that use of aspirin, NSAIDs, or both was differentially associated with colorectal cancer risk according to genetic variation at 2 highly correlated SNPs at chromosome 12p12.3 (rs2965667 and rs10505806) using a conventional logistic regression analysis.

These SNPs are 927 kb to 971 kb downstream from microsomal glutathione S-transferase 1 (MGST1 [NCBI Entrez Gene 4257]) (eFigure 3 in the Supplement), a member of the superfamily of membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG). MGST1 has high sequence homology to prostaglandin E synthase (MGST1L1 [NCBI Entrez Gene 9536]), another homologue of the MAPEG family that shares 38% of its DNA sequences with MGST7.16 MGST1 and MGST1L1 are up-regulated in several cancers, including colorectal cancer.17,18 MGST1L1 is coexpressed and functionally coupled to prostaglandin-endoperoxide synthase 2 (PTGS2; also known as cyclooxygenase 2 [COX-2]), and the combined activity of MGST1L1 and COX-2 increases production of proinflammatory prostaglandin E2 (PGE2), which promotes carcinogenesis through several mechanisms, including stimulation of WNT signaling, an essential oncogenic pathway of colorectal cancer.19,20 An in vitro experiment has demonstrated that NSAIDs can inhibit expression of MGST1L1 and COX-2, thereby blocking COX-2-mediated synthesis of PGE2 in human colon carcinoma cells.21

Taken together, both MGST1L1 and the closely related gene MGST1 may influence NSAID-mediated inhibition of colorectal carcinogenesis partially through involvement in the PGE2-induced WNT signaling pathway. This finding is consistent with strong biological evidence linking genes in WNT signaling; use of aspirin, NSAIDs, or both; and colorectal cancer.22,23

Another candidate gene in this region is LIM domain only 3 (LMO3 [NCBI Entrez Gene 55885]), a known oncogene located about 686 kb upstream from rs2965667 (eFigure 3 in the Supplement). Altered expression of LMO3 may contribute to the development of several cancers, such as neuroblastoma and lung cancer.24,25

The SNP rs2965667 is also located about 970 kb upstream from phosphatidylinositol-4-phosphate 3-kinase, catalytic subunit type 2 gamma (PIK3CG [NCBI Entrez Gene 5288]) (eFigure 3 in the Supplement). The protein encoded by the PIK3CG gene belongs to the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) family, which plays a critical role in cancer.26 Experimental evidence suggests that activation of PI3K signaling enhances production of COX-2 and PGE2, which results in inhibition of apoptosis in colon cancer cell lines that can be restored with NSAID-mediated blockade of PI3K.27

Moreover, our previous study found that regular use of aspirin after diagnosis was associated with longer survival among the 15% to 30% of patients with colorectal cancer and with a mutation in phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA [NCBI Entrez Gene 5290]), one of the PI3K family genes.28 Markedly improved survival associated with aspirin according to PIK3CA status was also found in an analysis within a separate clinical trial cohort.29 Further investigations for the joint effect of these genes would be helpful to better understand the underlying molecular mechanisms of aspirin, NSAIDs, and colorectal cancer.

In the case-only interaction analysis, another SNP, rs16973225 at chromosome 15q25.2, was identified with genome-wide significant association. This SNP is about 625 kb upstream of interleukin 16 (IL16 [NCBI Entrez Gene 3603]) (eFigure 4 in the Supplement). As a multifunctional cytokine, IL16 plays a critical role in proinflammatory processes, including inflammatory bowel disease, Clostridium difficile-associated colitis, and many cancers, including colorectal.30,31-33 Moreover, IL16 may stimulate monocye induction of proinflammatory cytokines associated with tumorigenesis, including IL6 and tumor necrosis factor alpha,34,35 induction of COX-2 expression, and activation of WNT signaling.36 This evidence suggests the possibility that polymorphisms in or near the IL16 gene may regulate the production of inflammatory cytokines that modify the chemopreventive effect of aspirin or NSAIDs on colorectal cancer. It is plausible that those GWAS-identified promising loci outside of known coding regions affect more distant genes rather than the closest gene, since GWAS loci may be enhancers that can influence gene expression over a span of several hundred kilobases.37

Our study has several strengths. First, our large sample size facilitated detection of genome-wide gene × environment interactions, even using a conventional logistic regression or case-only interaction analysis and accounting for the stringent threshold for statistical significance. Second, we identified variants near genes possessing high functional plausibility given their critical roles in inflammation and prostaglandin synthesis, which have been mechanistically
linked to use of aspirin or NSAIDs and colorectal carcinogenesis.

We acknowledge some limitations. First, heterogeneity exists in the definition of regular use of aspirin, NSAIDs, or both and the range of exposure periods encompassed by each study. However, we used a standardized harmonization process on a range of environmental variables, including use of aspirin, NSAIDs, or both across 10 cohort and case-control studies. The forest plots (Figure) show the consistency of the association between use of aspirin, NSAIDs, or both and colorectal cancer on a per-study level, and the pooled risk estimate (ie, OR) is remarkably similar to those from prior studies.28-30 Thus, bias attributable to heterogeneity in the definition and period of exposure is likely to be minimal.

Second, we acknowledge that SNP rs2965667 and the highly correlated rs10505806 are relatively rare and imputed in all studies. However, we directly genotyped rs10505806 in cases and controls within 2 cohorts included in our study population. The high overall concordance ($r = 0.89$) between imputed and directly genotyped data and the consistent gene × environment interaction statistical effect using either imputed or directly genotyped data support our assumption that our results are not greatly affected by the amount of imputed data.

Although prior GWAS-based studies have traditionally examined promising findings within a replication cohort, we did not split our data into discovery and replication sets because the most powerful analytical approach is a combined analysis across all studies.39 This approach is increasingly used as more individual-level GWAS data are becoming available.40 Moreover, the consistency of our findings and lack of heterogeneity across distinct study cohorts supports the validity of the results.

Conclusions

In this genome-wide investigation of gene × environment interactions, use of aspirin, NSAIDs, or both was associated with lower risk of colorectal cancer, and the association of these medications with colorectal cancer risk differed according to genetic variation at 2 SNPs at chromosomes 12 and 15. Validation of these findings in additional populations may facilitate targeted colorectal cancer prevention strategies.
Baron reported holding a use patent for aspirin as a colorectal chemopreventive agent. Dr. Zanke reported holding a patent licensed to Arcturus. Dr. Chan reported receiving personal fees from Bayer Healthcare, Pozen, and Pfizer. No other authors reported disclosures.

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**REFERENCES**


