Aspirin Use, 8q24 Single Nucleotide Polymorphism rs6983267, and Colorectal Cancer According to CTNNB1 Alterations

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Background

Regular aspirin use reduces the risk for colorectal cancer (CRC), possibly through inhibition of WNT/cadherin-associated protein β1 (CTNNB1 or β-catenin) signaling. The single nucleotide polymorphism (SNP) rs6983267 on chromosome 8q24 is a CRC susceptibility locus that affects binding activity of transcription factor 7 like-2 (TCF7L2) to CTNNB1, thereby altering expression of target oncogenes, including MYC.

Methods

We evaluated regular aspirin use and CRC risk according to genotypes of SNP rs6983267 and CTNNB1 expression status in two prospective case-control studies (840 CRC case patients and 1686 age- and race-matched control subjects) nested within the Nurses’ Health Study and the Health Professionals Follow-up Study. We estimated odds ratios (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression models. All statistical tests were two-sided.

Results

A lower risk of CRC was associated with regular aspirin use and the T allele of rs6983267. The effect of aspirin was confined to individuals with protective T allele of rs6983267 (additive matching factors–adjusted OR for T allele = 0.83; 95% CI = 0.74 to 0.94; \(P_{\text{trend}} = .002; P_{\text{interaction}} = .01\)). Additionally, the T allele of rs6983267 was associated with a reduced expression of MYC oncogene (\(P_{\text{trend}} = .03\)). Moreover, among individuals with protective T allele, the effect of regular aspirin use was limited to those with positive nuclear CTNNB1 expression. In a functional analysis, in vitro treatment of LS174T cells (a cell line heterozygous for rs6983267) with aspirin was statistically significantly associated with higher G/T allelic ratio of TCF7L2 immunoprecipitated DNA (\(P = .03\)).

Conclusions

Our results support an influence of aspirin on WNT/CTNNB1 signaling and suggest that aspirin chemoprevention may be tailored according to rs6983267 genotype.


Considerable evidence demonstrates that regular aspirin use reduces the risk of colorectal neoplasms (1–5). However, routine use of aspirin for chemoprevention of colorectal cancer is not currently recommended because of uncertainty about its risk–benefit profile. It remains unclear whether there are specific markers that can identify individuals who may obtain differential benefit from aspirin chemoprevention. Substantial data show that the anticancer benefit of aspirin is mediated by inhibition of prostaglandin synthase (PTGS or cyclooxygenase [COX]) enzymes responsible for the conversion of arachidonic acid to prostaglandins (6–9). We have previously shown that regular aspirin use is associated with a lower risk of colorectal cancers that overexpress PTGS2 (COX-2) but not risk of cancers that do not overexpress PTGS2 (COX-2) (10). Emerging evidence suggests that aspirin may also modulate WNT/cadherin-associated protein β1 (CTNNB1 or β-catenin) signaling, one of the most essential oncogenic pathways in colorectal cancer (11–13). Aspirin inhibits COX-mediated synthesis of prostaglandin E2 (PGE2) that stimulates the CTNNB1 pathway (14,15). Through COX-independent pathways, aspirin also induces phosphorylation, ubiquination, and degradation of CTNNB1 (11). Thus, the net result of aspirin is inhibition of CTNNB1 signaling (Figure 1, A and B).

Previous genome-wide association studies have consistently identified a single nucleotide polymorphism (SNP) rs6983267 on chromosome 8q24 as a susceptibility locus for colorectal cancer, with the T allele associated with a 15% to 18% lower risk of colorectal cancer (16–18). The most proximate gene in this region is MYC, residing 335 kb downstream from rs6983267 (19). Previous in vivo and in vitro experiments have demonstrated that the T allele of the SNP rs6983267 impairs binding of WNT/CTNNB1 pathway-related transcription factor 7 like-2 (TCF7L2), inhibiting the MYC promoter (Figure 1, C and D) (20–22). Most recently, SNP rs6983267 has been shown to functionally reduce MYC expression, inducing resistance to intestinal tumorigenesis (23).
Based on these results, we examined whether the effect of aspirin use on colorectal cancer risk varied according to rs6983267 genotype. We hypothesized that if aspirin influences colorectal carcinogenesis through modulation of WNT signaling, individuals may derive differential benefit with regular aspirin use according to rs6983267 genotype. If confirmed, such associations would be a novel example of a gene–environment interaction in which the effect of a genome-wide association study susceptibility locus for colorectal cancer was modified by the presence or absence of a known environmental factor (eg, medication use) associated with disease. Moreover, to further validate such a gene–environment interaction, we confirmed its biological plausibility by determining whether rs6983267 is associated with differential MYC expression in tumors and whether the joint effect of aspirin and the rs6983267 T allele may be particularly pronounced for tumors that appear more dependent on CTNNB1 activation for their growth (24).

**Methods**

**Study Population**
Details on the study population for each case–control study nested within the Nurses’ Health Study (NHS) and Health Professionals Follow-up Study (HPFS) are described in the Supplementary Methods (available online). Briefly, in each nested case–control study, we randomly selected between one and three control subjects within the same cohort from participants who were free of colorectal cancer at the same time the colorectal cancer was diagnosed in the case patients. Control subjects were matched to each case patient on...
ethnicity, year of birth, and month/year of blood sampling (25). In total, 840 (n = 472 in the NHS diagnosed up to June 2010; n = 368 in the HPFS diagnosed up to January 2008) case patients and 1686 (n = 1013 in the NHS; n = 673 in the HPFS) control subjects were included in our analysis. Among the 840 case patients with blood samples in this study, we were able to successfully obtain tissue specimens suitable for assessment of CTNNB1 status in 275 case patients and for assessment of MYC status in 189 case patients. The institutional review boards at Brigham and Women’s Hospital and the Harvard School of Public Health approved this study.

Assessment of Aspirin Use
Assessment of aspirin use in both the NHS and HPFS has been described in detail previously (1,26,27). Briefly, in 1980 we asked NHS participants whether they used aspirin, the number of pills taken each week, and the number of years of use. We updated this information every 2 years, except for 1986, with specific questions on the number of aspirin tablets taken per week (in categories of number taken). In the 1986 HPFS questionnaire and in questionnaires every 2 years thereafter, we inquired whether the men in the study used aspirin two or more times per week. Beginning in 1992, we also asked these men the average number of tablets taken per week (in categories of number taken). In both cohorts, we specifically inquired about standard-dose (325 mg) aspirin tablets. However, to reflect secular trends in consumption of low-dose (81 mg) aspirin (baby aspirin), the questionnaires after 1992 asked participants to convert four baby aspirin tablets to one adult standard-dose tablet when responding.

Laboratory Assays
Details on laboratory assays, including genotyping and assessment of nuclear CTNNB1 and MYC expression, are described in the Supplementary Methods (available online). Briefly, the SNP rs6983267 was genotyped by the 5’ nuclease assay (TaqMan), using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). We confirmed that the SNP rs6983267 genotype was in Hardy–Weinberg equilibrium among the control subjects using the \( \chi^2 \) test (\( P = .29 \) for NHS; \( P = .24 \) for HPFS). We used immunohistochemistry to assess the expression of CTNNB1 and MYC based on immunostaining intensity (24). A pathologist who was unaware of any data concerning the participants interpreted CTNNB1 and MYC expression in the nuclei of tumor cells using a standardized grading system indicating progressively increasing degrees of overexpression (absent, weak, moderate, or strong). Consistent with our prior studies, if the intensity of immunostaining was moderate or strong, tumors were classified as positive; otherwise they were classified as negative for CTNNB1 or MYC overexpression. Our method for validation of our classification of CTNNB1 expression has been described previously (24). For the agreement study, a second pathologist blinded to any data examined 292 randomly selected case patients for CTNNB1 expression. The concordance between the 2 observers was 0.90 (\( k = 0.80; P < .0001 \) (28)), indicating substantial agreement.

Statistical Analysis
We used unconditional logistic regression models adjusting for matching factors and other known or suspected colorectal cancer risk factors to estimate odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for the association of aspirin use and the SNP rs6983267 with colorectal cancer risk in each cohort. Logistic regression was also used to assess the association between the SNP rs6983267 and MYC expression among cases. For all analyses, we used the cumulative average intake of aspirin before diagnosis...
(June 2010 for NHS and January 2008 for HPFS) to reduce within-person variation and to better estimate long-term intake (29). In NHS, women who reported taking two or more standard aspirin tablets (325 mg) per week were classified as regular users and those who reported taking less than two tablets per week were classified as nonregular users (26). In the HPFS, we defined men who reported taking aspirin at least two times per week as regular users and those who reported taking aspirin less than two times per week as nonregular users (27). We also examined duration of regular aspirin use by calculating the number of years of use according to response to all biennial questionnaires before each 2-year follow-up interval (1,26). We conducted analyses stratified by genotype and assessed statistical significance of interaction by using the Wald test for cross-product terms of variant genotype and aspirin use in the logistic regression adjusted for matching factors. All statistical analyses were two-sided and carried out using SAS version 9.2 (SAS Institute, Cary, NC).

**Chromatin Immunoprecipitation Sequenom**

To confirm the biological plausibility of the relationship between aspirin and the SNP rs6983267, we further conducted functional analysis using allele-specific chromatin immunoprecipitation sequenom (ChIP-Seq) to compare the effect of treatment with different concentrations of aspirin on the binding of TCF7L2 to the G and T alleles in LS174T, a cell line heterozygous for rs6983267 (Figure 2). Details on the method are described in the Supplementary Methods (available online). Briefly, the LS174T cell line was cultured in Dulbecco’s modified Eagle medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. A stock solution of 1 M aspirin (Sigma-Aldrich, St Louis, MO) was freshly made in ethanol before the drug treatment. After growing to approximately 80% confluence, the cells were treated with 0.1 mM aspirin, 1 mM aspirin, or ethanol vehicle for 48 hours. Samples from six independent ChIP experiments were analyzed, and each biological replicate was performed in triplicate. For each condition, the ratio of allelic peak heights (G/T allelic ratio) as reported by the Sequenom software was retrieved. The G/T ratio of the input sample was used to normalize the G/T ratio of the TCF7L2 immunoprecipitated sample. An analysis of variance model was used to assess whether the normalized G/T ratio differed when treated with 1 mM, 0.1 mM, or vehicle. A Tukey’s test was used for further evaluation of pairwise comparisons.

**Results**

The baseline characteristics of this study population of 840 case patients of colorectal cancer and 1686 control subjects are presented in Table 1. The mean age at diagnosis of colorectal cancer case patients was 65.9 years for women and 70.5 years for men. Compared with control subject, both male and female colorectal cancer case patients were less likely to have used aspirin or nonsteroidal anti-inflammatory drugs (NSAIDs), were more likely to have a family history of colorectal cancer, were more likely to smoke, consumed higher amounts of alcohol, and had lower intakes of total calcium. Table 1. Basic characteristics of colorectal cancer case patients and control subjects in the nested case–control study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>NHS*</th>
<th>HPFS*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case patients</td>
<td>Control subjects</td>
<td>Case patients</td>
</tr>
<tr>
<td>Mean age at diagnosis, y</td>
<td>65.9</td>
<td>—</td>
<td>70.5</td>
</tr>
<tr>
<td>Mean age at blood draw, y</td>
<td>59.5</td>
<td>59.6</td>
<td>65.9</td>
</tr>
<tr>
<td>Nonwhite, %</td>
<td>1.5</td>
<td>0.4</td>
<td>6.5</td>
</tr>
<tr>
<td>Regular aspirin use, %†</td>
<td>37.5</td>
<td>46.8</td>
<td>45.4</td>
</tr>
<tr>
<td>Regular nonsteroidal anti-inflammatory drug use, %‡</td>
<td>33.1</td>
<td>41.6</td>
<td>22.6</td>
</tr>
<tr>
<td>Mean body mass index, kg/m²</td>
<td>25.9</td>
<td>25.9</td>
<td>26.3</td>
</tr>
<tr>
<td>Mean physical activity, METs hours/wk§</td>
<td>15.8</td>
<td>15.9</td>
<td>30.7</td>
</tr>
<tr>
<td>Colorectal cancer in a parent or sibling, %</td>
<td>22.3</td>
<td>16.3</td>
<td>20.7</td>
</tr>
<tr>
<td>Former or current smoker, %</td>
<td>57.1</td>
<td>55.3</td>
<td>60.9</td>
</tr>
<tr>
<td>Mean alcohol consumption, g/d‡</td>
<td>7.0</td>
<td>6.1</td>
<td>13.8</td>
</tr>
<tr>
<td>Former or current postmenopausal hormone use, %‡</td>
<td>61.7</td>
<td>64.8</td>
<td>—</td>
</tr>
<tr>
<td>Mean beef, pork, or lamb as a main dish, servings/wk</td>
<td>1.9</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>Mean total calcium intake, mg/d</td>
<td>959.7</td>
<td>1019.2</td>
<td>942.8</td>
</tr>
<tr>
<td>Mean total folate intake, µg/d</td>
<td>422.0</td>
<td>442.7</td>
<td>530.2</td>
</tr>
</tbody>
</table>

* HPFS = Health Professionals Follow-Up Study (men); NHS = Nurses’ Health Study (women).
† Regular aspirin user was defined as intake of at least two 325-mg tablets per week in the NHS (or at least two times per week in the HPFS).
‡ Regular nonsteroidal anti-inflammatory drug use was defined as intake at least two days per week.
§ MET denotes metabolic equivalent. Met hours = sum of the average time/week in each activity × MET value of each activity. One MET, the energy spent sitting quietly, is equal to 3.5 mL of oxygen uptake per kilograms of body weight per minute for a 70 kg adult.
¶ Nutrient values (calcium and folate) represent the mean of energy-adjusted intakes.
of calcium and folate. Among men, case patients had a higher body mass index and were less physically active. Among women, case patients were less likely to have used postmenopausal hormones.

We examined the association between SNP rs6983267 and risk of colorectal cancer (Table 2). Risk estimates were consistent within each independent cohort ($P_{\text{interaction}} = .93$). Among both women (NHS) and men (HPFS), we observed a lower risk of colorectal cancer associated with the T allele of rs6983267 (additive matching factors–adjusted OR for T allele = 0.83; 95% CI = 0.74 to 0.94; $P_{\text{trend}} = .002$). Compared with individuals with the GG genotype, the matching factors–adjusted odds ratios of colorectal cancer risk were 0.85 (95% CI = 0.69 to 1.04) for those with the GT genotype and 0.69 (95% CI = 0.54 to 0.87) for those with the TT genotype. These associations did not materially change after adjusting for additional colorectal cancer risk factors.

We examined whether the influence of regular aspirin use on colorectal cancer risk varied according to allelic variation at SNP rs6983267 (Table 3). Among individuals of any genotype, we observed a statistically significant inverse association between regular aspirin use and the risk of colorectal cancer (multivariable OR = 0.71; 95% CI = 0.60 to 0.85; $P_{\text{trend}} = .0001$). Regular aspirin use was also associated with a lower risk of both proximal (multivariable OR = 0.73; 95% CI = 0.56 to 0.95; $P_{\text{trend}} = .02$) and distal cancer (multivariable OR = 0.57; 95% CI = 0.41 to 0.80; $P_{\text{trend}} = .001$) (data not shown). Moreover, we considered the possibility that the association of regular aspirin use with colorectal cancer risk may be confined to the heaviest users. However, in the analyses in which we examined aspirin according to dose categories, we found that even relatively modest doses of aspirin were inversely associated with colorectal cancer risk (Table 4). The effect of aspirin was confined to individuals with at least one protective T allele ($P_{\text{interaction}} = .01$). Compared with nonuse, regular aspirin use was associated with multivariable odds ratio 0.61 (95% CI = 0.47 to 0.79) among those with GT genotypes and 0.52 (95% CI = 0.35 to 0.78) among those with the TT genotypes. In contrast, regular aspirin use was not associated with lower risk among individuals with GG genotypes (multivariable OR = 0.99; 95% CI = 0.70 to 1.40) (Table 3). Results were consistent in separate analyses of each cohort (data not shown).

Considering that aspirin and other nonaspirin NSAIDs may share some anticancer mechanisms, we conducted additional analyses that considered use of both aspirin and nonaspirin NSAIDs, use of either aspirin or nonaspirin NSAIDs, and use of nonaspirin NSAIDs only. The effect size for the risk of colorectal cancer was similar across each of these different exposure variables. Compared with nonregular users, regular users for each exposure variable were statistically significantly associated with a reduced risk of colorectal cancer (for use of both aspirin and NSAIDs: multivariable OR = 0.58, 95% CI = 0.45 to 0.75, $P < .0001$; for use of either aspirin or NSAIDs: multivariable OR = 0.73, 95% CI = 0.61 to 0.86, $P = .0003$; for use of NSAIDs only: multivariable OR = 0.79, 95% CI = 0.65 to 0.96, $P = .02$). Moreover, for each of these exposure variables, we did not observe any statistically significant interactions with rs6983267 genotypes on the risk of colorectal cancer ($P_{\text{interaction}} > .08$ for each variable; data not shown).

We further evaluated the associations between dose of regular aspirin use as well as duration of aspirin use and the risk of colorectal cancer (multivariable OR = 0.73; 95% CI = 0.56 to 0.95; $P_{\text{trend}} = .02$) and distal cancer (multivariable OR = 0.57; 95% CI = 0.41 to 0.80; $P_{\text{trend}} = .001$) (data not shown). Moreover, we considered the possibility that the association of regular aspirin use with colorectal cancer risk may be confined to the heaviest users. However, in the analyses in which we examined aspirin according to dose categories, we found that even relatively modest doses of aspirin were inversely associated with colorectal cancer risk (Table 4). The effect of aspirin was confined to individuals with at least one protective T allele ($P_{\text{interaction}} = .01$). Compared with nonuse, regular aspirin use was associated with multivariable odds ratio 0.61 (95% CI = 0.47 to 0.79) among those with GT genotypes and 0.52 (95% CI = 0.35 to 0.78) among those with the TT genotypes. In contrast, regular aspirin use was not associated with lower risk among individuals with GG genotypes (multivariable OR = 0.99; 95% CI = 0.70 to 1.40) (Table 3). Results were consistent in separate analyses of each cohort (data not shown).

### Table 2. Association between the single nucleotide polymorphism rs6983267 and colorectal cancer risk

<table>
<thead>
<tr>
<th>SNP</th>
<th>Cases/controls</th>
<th>Total OR (95% CI)†</th>
<th>Multivariable OR (95% CI)‡</th>
<th>Total OR (95% CI)†</th>
<th>Multivariable OR (95% CI)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs6983267</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

* Percentages may not sum to 100 due to rounding. CI = confidence interval; HPFS = Health Professionals Follow-up Study (men); NHS = Nurses’ Health Study (women); OR = odds ratio

† Multivariable logistic regression odds ratios are adjusted for age, race, and sex (pooled data only).
‡ Multivariable logistic regression odds ratios are adjusted for age, race, sex (pooled data only), regular aspirin use (yes or no), regular nonsteroidal anti-inflammatory drug use (yes or no), body mass index (in kg, 5–9.9, 10–14.9, 15–19.9, 20–24.9, 25–29.9, 30–34.9, 35–39.9, ≥ 40 kg/m²), alcohol consumption (in g per day), smoking (never, former, current), and consumption of beef, pork, or lamb (0–1, 2–3, 4–5, ≥ 6 times per week).

§ Pooled data are from NHS and HPFS. Values for heterogeneity against the additive odds ratio were calculated using Cochran’s Q (Phenotype = G). All statistical tests were two-sided.
Table 3. Risk for colorectal cancer according to regular aspirin use, stratified by rs6983267 genotype

<table>
<thead>
<tr>
<th>rs6983267</th>
<th>Nonregular aspirin users</th>
<th>Regular aspirin users</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>496/857</td>
<td>344/829</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Multivariable OR (95% CI)*</td>
<td>1.00 (referent)</td>
<td>0.71 (0.60 to 0.84)</td>
<td>.0001</td>
</tr>
<tr>
<td>GT</td>
<td>127/221</td>
<td>111/184</td>
<td>.96</td>
</tr>
<tr>
<td>Multivariable OR (95% CI)*</td>
<td>1.00 (referent)</td>
<td>0.99 (0.72 to 1.38)</td>
<td>.0001</td>
</tr>
<tr>
<td>TT</td>
<td>250/399</td>
<td>155/411</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Multivariable OR (95% CI)*</td>
<td>1.00 (referent)</td>
<td>0.60 (0.47 to 0.76)</td>
<td>.0001</td>
</tr>
<tr>
<td>Multivariable OR (95% CI)†</td>
<td>1.00 (referent)</td>
<td>0.61 (0.47 to 0.79)</td>
<td>.0001</td>
</tr>
</tbody>
</table>

* Multivariable logistic regression odds ratios (ORs) are adjusted for age, race, sex, regular nonsteroidal anti-inflammatory drug use (yes or no), body mass index (in tertiles), physical activity (in tertiles), history of colorectal cancer in a parent or sibling (yes or no), smoking (never, former, or current smoker), alcohol consumption (0–4.9 g, 5–9.9 g, 10–14.9 g, or ≥15.0 g per day), postmenopausal hormone use (premenopausal, never, former, or current user), consumption of beef, pork, or lamb as a main dish (0–3 times per month, once a week, 2–4 times per week, or ≥5 times per week), and energy-adjusted calcium and folate intake (in tertiles).

† Multivariable logistic regression odds ratios are adjusted for age, race, sex, regular nonsteroidal anti-inflammatory drug use (yes or no), body mass index (in tertiles), physical activity (in tertiles), history of colorectal cancer in a parent or sibling (yes or no), smoking (never, former, or current smoker), alcohol consumption (0–4.9 g, 5–9.9 g, 10–14.9 g, or ≥15.0 g per day), postmenopausal hormone use (premenopausal, never, former, or current user), consumption of beef, pork, or lamb as a main dish (0–3 times per month, once a week, 2–4 times per week, or ≥5 times per week), and energy-adjusted calcium and folate intake (in tertiles).

cancer (Table 4). A lower risk of colorectal cancer was associated with higher dose of aspirin (P_trend = .0008) and long-term regular aspirin use (P_trend < .0001). Although the influence of aspirin dose and duration also appeared to be more evident among individuals carrying at least one T allele, there did appear to be a modest inverse association of aspirin use in the highest categories of dose (aspirin dose > 7 tablets/week: multivariable OR = 0.77; 95% CI = 0.41 to 1.42) and duration (aspirin duration > 10 years: multivariable OR = 0.70; 95% CI = 0.44 to 1.14) among individuals with GG genotype.

Regular aspirin use and allelic variation at rs698327 have previously been shown to influence the Wnt/CTNNB1 signaling pathway. Based on our finding that aspirin appears to only modify risk of colorectal cancer in carriers of any T allele at rs698327, we tested the joint effect of these two factors (gene × drug) on odds of developing colorectal cancer in carriers of the GG genotype. We found that the benefit of regular aspirin use on colorectal cancer risk appears confined to individuals with the T allele of rs6983267 on 8q24, a colorectal cancer susceptibility SNP (16–18, 30) associated with impaired binding of CTNNB1/TCF7L2 (20–22). Furthermore, we found that increased CTNNB1/TCF7L2 immunoprecipitated G/T allelic ratio was statistically significantly higher when treated with 1.0 mM compared than when treated with vehicle (mean = 0.12; 95% CI = 0.005 to 0.23).

Discussion

We found that the benefit of regular aspirin use on colorectal cancer risk appears confined to individuals with the T allele of rs6983267 on 8q24, a colorectal cancer susceptibility SNP (16–18, 30) associated with impaired binding of CTNNB1/TCF7L2 (20–22). Furthermore, our in vitro functional analysis for the relationship between aspirin and TCF7L2 immunoprecipitated G/T allelic ratio of rs6983267 supported the findings observed in our human study. To our knowledge, our finding is the first to associate a genetic susceptibility locus identified through genome-wide association studies for colorectal cancer as a modifier of drug response.

The WNT family proteins bind to cell-surface receptors, transducing signals to a cytoplasmic protein that inhibits phosphorylation and destruction of CTNNB1. CTNNB1 subsequently accumulates in the cytoplasm, eventually translocating into the nucleus, complexing with the TCF/LEF family transcription factor TCF7L2 (also known as TCF4), and thereby altering expression genotypes, the multivariable odds ratio for MYC overexpression was 0.72 (95% CI = 0.31 to 1.68) for the GT genotypes and 0.32 (95% CI = 0.11 to 0.89) for the TT genotypes (P_trend = .03; data not shown), supporting the hypothesis that the rs6983267 protective T allele is associated with lower MYC expression among individuals with colorectal cancer. In the functional analysis to further validate our observations, we found that among six independent ChIP-Seq experiments, each performed with three technical replicates, the normalized G/T allelic ratio of TCF7L2 immunoprecipitated DNA was statistically significantly different comparing the three conditions (P = .03) (Figure 2, A and B). From pairwise comparisons, the normalized G/T allelic ratio was statistically significantly higher when treated with 1.0 mM compared than when treated with vehicle (mean = 0.12; 95% CI = 0.005 to 0.23).
of target oncogenes, including MYC, the gene most proximate to SNP rs6983267. In colorectal cancer cell lines, we, and others have previously shown that the T allele of SNP rs6983267 impairs affinity to CTNNB1/TCF7L2 transcription complex, inhibiting the activity of MYC protooncogene (21,31). In a murine model, SNP rs6983267 has been shown to influence MYC expression and subsequent intestinal tumorigenesis (23). Consistent with these data, we found, in human populations, that the T allele was associated with not only a lower risk of colorectal cancer but also lower expression of MYC.

Similar to our previous findings in the populations within which this study cohort is nested, we found that regular aspirin use was associated with not only a lower risk of colorectal cancer but also lower expression of MYC.

Table 4. Risk for colorectal cancer according to dose of aspirin intake and duration of regular aspirin use, stratified by rs6983267 genotype

<table>
<thead>
<tr>
<th>rs6983267</th>
<th>Tablets of aspirin per week</th>
<th>Multivariable OR (95% CI)†</th>
<th>Multivariable OR (95% CI)‡</th>
<th>P trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>All genotypes</td>
<td>175/267</td>
<td>559/1154</td>
<td>100/259</td>
</tr>
<tr>
<td></td>
<td>Multivariable OR (95% CI)*</td>
<td>1.00 (referent)</td>
<td>0.75 (0.60 to 0.93)</td>
<td>0.60 (0.44 to 0.80)</td>
</tr>
<tr>
<td></td>
<td>Multivariable OR (95% CI)†</td>
<td>1.00 (referent)</td>
<td>0.74 (0.60 to 0.93)</td>
<td>0.60 (0.44 to 0.81)</td>
</tr>
<tr>
<td>GT</td>
<td>Case patients/control subjects</td>
<td>49/86</td>
<td>161/259</td>
<td>28/60</td>
</tr>
<tr>
<td></td>
<td>Multivariable OR (95% CI)*</td>
<td>1.00 (referent)</td>
<td>1.12 (0.75 to 1.69)</td>
<td>0.80 (0.45 to 1.42)</td>
</tr>
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<td>Multivariable OR (95% CI)†</td>
<td>1.00 (referent)</td>
<td>1.09 (0.70 to 1.69)</td>
<td>0.77 (0.41 to 1.42)</td>
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<td>TT</td>
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<td>34/59</td>
<td>114/279</td>
<td>16/70</td>
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<td>Multivariable OR (95% CI)*</td>
<td>1.00 (referent)</td>
<td>0.73 (0.45 to 1.18)</td>
<td>0.40 (0.20 to 0.81)</td>
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<td>Multivariable OR (95% CI)†</td>
<td>1.00 (referent)</td>
<td>0.74 (0.44 to 1.24)</td>
<td>0.39 (0.18 to 0.83)</td>
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P interaction: .08

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<th>rs6983267</th>
<th>Years of regular aspirin use</th>
<th>Multivariable OR (95% CI)†</th>
<th>Multivariable OR (95% CI)‡</th>
<th>P trend</th>
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<td>370/768</td>
<td>196/524</td>
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<td>1.00 (referent)</td>
<td>0.68 (0.56 to 0.83)</td>
<td>0.54 (0.43 to 0.67)</td>
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<td>Multivariable OR (95% CI)‡</td>
<td>1.00 (referent)</td>
<td>0.68 (0.56 to 0.84)</td>
<td>0.55 (0.43 to 0.70)</td>
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<td>GT</td>
<td>Case patients/control subjects</td>
<td>75/118</td>
<td>110/168</td>
<td>53/119</td>
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<td>1.00 (referent)</td>
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<td>Case patients/control subjects</td>
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<td>179/374</td>
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<td>1.00 (referent)</td>
<td>0.62 (0.47 to 0.83)</td>
<td>0.47 (0.34 to 0.66)</td>
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<td>Multivariable OR (95% CI)‡</td>
<td>1.00 (referent)</td>
<td>0.66 (0.48 to 0.89)</td>
<td>0.50 (0.35 to 0.71)</td>
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<td>P interaction: .20</td>
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* Multivariable logistic regression odds ratios (ORs) are adjusted for age, race, and sex. CI = confidence interval.
† Multivariable logistic regression odds ratios are adjusted for age, race, body mass index (in tertiles), physical activity (in tertiles), history of colorectal cancer in a parent or sibling (yes or no), smoking (never, former, or current smoker), alcohol consumption (g per day), postmenopausal hormone use (premenopausal, never, former, or current user), consumption of beef, pork, or lamb as a main dish (0–3 times per month, once a week, 2–4 times per week, or ≥5 times per week), and energy-adjusted calcium and folate intake (in tertiles). All statistical tests were two-sided.

Aspirin dampens CTNNB1 signaling through inhibition of PTGS (COX)–mediated synthesis of PGE2 (14,15). PGE2 stimulates the CTNNB1 pathway through EP2 receptors, coupling the heterotrimeric G proteins of the Gαi family and inhibiting the CTNNB1 destruction complex. This leads to release of GSK-3β from the complex, thereby blocking phosphorylation and destruction of CTNNB1. Aspirin also inhibits the CTNNB1 signaling through COX-independent pathways by directly inducing phosphorylation and subsequent degradation of CTNNB1 and downregulating target oncogene expression, including MYC and cyclin D1 (11,12,32). Thus, aspirin may lower risk of cancer through modulation of WNT/CTNNB1 pathways through either COX-dependent or -independent pathways (Figure 1, A and B).

In support of this mechanism, we found that the benefit of regular aspirin use on colorectal cancer risk appears confined to
individuals with the T allele of rs6983267, which is associated with impaired binding of CTNNB1/TCF7L2 and lower expression of MYC (21,22,33). In contrast, among individuals without a T allele (with GG genotype), aspirin use did not appear to reduce risk of colorectal cancer. The G allele of rs6983267 leads to constitutively active binding of CTNNB1/TCF7L2 and expression of MYC, promoting carcinogenesis (21,22,33). This, among individuals with only G allele, binding of CTNNB1/TCF7L2 is less sensitive to nuclear CTNNB1 levels, resulting in relative resistance to the effect of aspirin on inhibition of CTNNB1 accumulation. Furthermore, our in vitro functional analysis demonstrated that aspirin alters the TCF7L2 immunoprecipitated G/T allelic ratio, corroborating the association between rs6983267 and aspirin use in our human study. These data together suggest that susceptibility to the effects of aspirin on the WNT/CTNNB1 pathway requires a genetic background by which CTNNB1/TCF7L2 binding is not constitutively active (Figure 1, C and D).

To further confirm the biological relevance of this gene–environment interaction, we examined the effect of rs6983267 and aspirin in relation to distinct molecular features associated with enhanced WNT/CTNNB1 activation (24,34). Among individuals carrying GT/TT genotypes of rs6983267, we found the strongest association of regular aspirin use with the risk of colorectal cancer with positive nuclear CTNNB1 expression, a marker for a tumor with WNT activation (35–37). In contrast, aspirin use was not associated with risk of colorectal cancer with negative nuclear CTNNB1 expression irrespective of genotype. These findings support an influence of aspirin on the WNT pathway because it appears to preferentially reduce the risk of tumors that are more dependent on WNT signaling for their growth. Similarly, NSAIDs (aspirin or ibuprofen) inhibit nuclear CTNNB1 expression in human colon adenomas and colon cancer cell lines (11,35). Several other studies have also shown that NSAIDs inhibit WNT activity in colon tissue or adenomas of patients with familial adenomatous polyposis or Lynch syndrome (38,39) and in colon cancer cells (38,40–44).

Our study has several strengths. First, we used prospectively collected, updated, detailed data on aspirin use and other risk factors over long-term follow-up. Second, our matched control subjects were selected from the same cohort in which the case patients developed, minimizing population stratification or selection bias (45). Third, our findings were remarkably consistent between two independent cohorts. Fourth, among a large number of participants, we used tumor tissue to examine intratumoral markers of WNT signaling with greater mechanistic specificity. Last, our in vitro functional analysis of rs6983267 and aspirin supported our findings in the human study.

We acknowledge several limitations. Our study is observational. However, associations between aspirin use and colorectal cancer in our cohort (1,10,46) have been validated by the findings of randomized controlled trials (47–51). Second, we did not have tumor specimens available for all case patients. However, the risk factors in case patients with available tumor tissue did not appreciably differ from those in case patients without tumor tissue (10). Third, previous studies failed to detect a statistically significant gene–environment interaction between rs6983267, NSAID use, and colorectal cancer (52,53). However, these prior analyses were limited by a single ascertainment of NSAID exposure; heterogeneity in the timing of assessment (baseline or after diagnosis); a lack of information about NSAID type (aspirin vs nonaspirin NSAIDs), dose, or duration of use; and the inability to examine gene–environment interactions in the context of CTNNB1 expression. Fourth, in the
ChIP-Seq experiment, we used ethanol vehicle as a control group. However, because the aspirin used in our experiments was made in ethanol before the drug treatment, any deviations due to the ethanol vehicle would be minimized. Also, ethanol is a commonly used vehicle in treatment-related experiments. Last, based on our limited sample size, we cannot exclude the possibility that a longer duration of regular aspirin use may be associated with benefit in individuals with GG genotypes (Table 4).

In summary, our study demonstrates that aspirin reduces risk of colorectal cancer, particularly tumors with activated CTNNB1, among individuals with rs6983267 GT/TT genotypes but not among individuals with GG genotypes. Our results support an influence of aspirin on WNT signaling as an underlying mechanism for aspirin’s effect on carcinogenesis and suggest the possibility that aspirin chemoprevention may be tailored according to rs6983267 genotype. Moreover, as a compelling example of a novel gene–environment interaction with molecular specificity, our study highlights the power of genome-wide association studies to illuminate underlying biological pathways. Last, our study may have substantial clinical implications given the considerable body of evidence that already supports the effectiveness of aspirin in the prevention of colorectal cancer (1–5). Our findings extend this data by suggesting that aspirin’s chemopreventive benefit may be more effective in a subgroup of the population defined by genetic susceptibility for colorectal cancer.

References

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Notes
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