A study of prostaglandin pathway genes and interactions with current non-steroidal anti-inflammatory drug use in colorectal adenoma

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ABSTRACT

Colorectal cancer (CRC) is the second leading cause of cancer-related death, and usually arises from colorectal polyps. Screening and removal of polyps reduce mortality from CRC. Colorectal polyps are known to aggregate in families; however the genetic determinants for risk of polyps are unknown. Additionally, it has been shown that nonsteroidal anti-inflammatory drug (NSAID) use decreases the risk of CRC and the incidence and size of polyps. In this study, we used data from the Tennessee Colorectal Polyp Study and the Tennessee-Indiana Adenoma Recurrence Study to evaluate selected genes from the prostaglandin metabolism and signaling pathways for association with risk of polyps and for interactions with NSAIDs. Our design consisted of discovery and replication phases for a total of 2,551 Caucasian polyp cases and 3,285 Caucasian controls. We performed multivariable logistic regression to test for association in both the discovery and replication phase and further examined the results with meta-analysis. We detected association signals in the genes prostaglandin E receptor 3 (PTGER3) and 15-hydroxyprostaglandin dehydrogenase (HPGD), both strong biological candidates for influence on polyp risk. We did not observe the previously reported effects and effect modification in prostaglandin-endoperoxide synthase 2 (PTGS2), prostaglandin E receptor 2 (PTGER2), or prostaglandin E receptor 4 (PTGER4), although we did observe a single nucleotide polymorphism in PTGER2 associated with risk of multiple adenomas. We also observed effect modification of the HPGD signal by NSAID exposure.
INTRODUCTION

Colorectal cancer (CRC) is the second most common cause of cancer death in North America and the fourth most diagnosed cancer (1). The vast majority of CRCs are derived from neoplastic colorectal polyps (2, 3), and colorectal adenomas are commonly recognized precursors to CRC(2). CRC risk has been shown to be modulated by environmental and genetic factors, in addition to epigenetic phenomena that associate with tumors. In CRC pathways, normal colonic epithelium is transformed as the result of the progressive accumulation of genetic and epigenetic alterations such as somatic mutations through gain-of-function, loss-of-function, and subsequent genomic instability.

The cyclooxygenase (COX) enzymes are usually expressed in response to inflammation and by cancerous and pre-cancerous tissues; non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the formation of prostanoids by antagonizing the activity of COX enzymes (4). It has long been recognized that COX upregulation increases the metastatic potential of cancer cells (5). Overexpression of COX-2 occurs in 50% of colon adenomas and 85% of colon cancers and is considered a key and early oncogenic event in colorectal carcinogenesis (6). In animal studies knockouts of prostaglandin (PG) receptors have lowered rates of adenomatous polyps and cancer (7).

Inhibition of the PG pathways also have been shown to reduce tumor cell proliferation, increase apoptosis, and reduce angiogenesis. Previous studies have shown that people and animals taking NSAIDs experience lower rates of precancerous growths, cancers, and cancer-related deaths (8, 9). Clinical trials evaluating the use of NSAIDs and selective COX-2 inhibitors for colorectal adenoma prevention yielded some promising results, where persons taking the drugs had as much as 45% fewer adenomas than the placebo group (10-15). This reduction in risk of adenoma has been consistently observed for regular users of NSAIDs (16-19), and is more pronounced for larger adenoma (16, 20). Interactions between NSAIDs and genetic
variation in COX genes have also been associated with protection from cancers and adenomas (21-23)

In this study, we investigated whether inter-individual genetic variation within candidate genes in the PG pathway is a determinant of risk for adenoma formation in participants from the Tennessee Colorectal Polyp Study (TCPS) and the Tennessee-Indiana Adenoma Recurrence Study (TIARS). Given the evidence that PG genes have a biological role in adenoma incidence, it stands to reason that these genes may harbor alleles that influence the fate of colorectal epithelial cells through a mechanism related to the activity of NSAIDs. We also evaluated single nucleotide polymorphisms (SNPs) with apparent effects on adenoma risk for interactions with current NSAID use.

MATERIALS AND METHODS

Study population and data collection:

The Tennessee Colorectal Polyp Study was a colonoscopy-based case-control study conducted in Nashville, TN from 2003 to 2010. Eligible participants, aged between 45 and 70 years old, were identified from patients scheduled for colonoscopy at the Vanderbilt Gastroenterology Clinic and the Veteran’s Affairs Tennessee Valley Health System Nashville Campus. Demographic properties of all participants are described in Table 1. For the purposes of the association analyses we only included participants of Caucasian race, although original recruitment for TCPS was from a multi-ethnic population. Excluded from the study were participants who had genetic colorectal cancer syndromes, a prior history of inflammatory bowel disease, prevalent adenomatous polyps, or any cancer other than nonmelanoma skin cancer. Among eligible participants, 65% provided informed consent, and subsequently 84% completed telephone interviews and 75% completed a food frequency questionnaire (FFQ) designed for the southern United States (US)(24). Participants provided DNA either prior to or after colonoscopy. Participants recruited prior to colonoscopy were asked
to donate a 15 mL blood sample. 5504 participants provided a blood sample. Buccal cell or Oragene kit samples were collected from 1079 participants who chose not to provide a blood sample, or if they were recruited after colonoscopy. DNA was obtained from blood for 82.9% of participants, and mouthwash buccal samples or Oragene samples for 16.3% of participants. The study was approved by the Vanderbilt University Institutional Review Board, the Veterans’ Affairs Tennessee Valley Health System Institutional Review Board, and the Veterans’ Affairs Tennessee Valley Health System Research and Development Committee,

Participants were also included as adenoma cases from the Tennessee-Indiana Adenoma Recurrence Study, a retrospective cohort study conducted in Nashville, Tennessee, and Indianapolis, Indiana, United States. Eligible participants, aged between 40 and 75 years old, were identified from patients diagnosed during colonoscopy with an advanced or multiple adenomas between January, 1996 and December, 2002 at the Vanderbilt Gastroenterology Clinic, Veterans’ Affairs Tennessee Valley Health System Nashville campus, Indiana University Hospital, the Richard L. Roudebush Veterans Administration Medical Center, and Wishard Memorial Hospital. Excluded from TIARS were patients who could not speak or understand English, had genetic colorectal cancer syndromes (e.g. hereditary non-polyposis colorectal cancer or familial adenomatous polyposis), were participating in an intervention trial to prevent adenoma recurrence, had a prior history of colon resection, inflammatory bowel disease, adenomas, or any cancer other than non-melanoma skin cancers or were a current resident in a correctional facility. Overall, 1,643 eligible individuals were identified. Potential participants who were not known to be deceased were contacted first by letter and then by telephone. 670 participants provided written informed consent. Deceased individuals (351) were also included in the study. The overall participation rate was 62.1%. A standardized telephone interview was conducted by trained interviewers to obtain information on follow-up examinations, medication use since baseline, demographics, medical history, family history, reproductive history, anthropometry, and lifestyle. Among participants, 706 (63.7%) completed the telephone
interview. Beginning in May 2004, buccal cell samples were collected from participants or a saliva sample was collected using an Oragene™ kit. 532 participants (48.0%) provided a buccal and/or Oragene sample. The study was approved by the Vanderbilt University Institutional Review Board, the Veterans’ Affairs Tennessee Valley Health System Institutional Review Board, the Veterans’ Affairs Tennessee Valley Health System Research and Development Committee, and the Indiana University Institutional Review Board Development Committee.

In both study populations, colonoscopic procedures were performed and reported using standard clinical protocols by the patient’s gastroenterologist. Any identified polyps were removed using biopsy forceps or snare techniques. All pathology diagnoses were determined by hospital pathologists and reported as part of routine care. Data were abstracted from these reports to classify study participants into the following groups: adenomas only, hyperplastic polyps only, presence of both adenomas and hyperplastic polyps, and polyp-free controls. In order to be classified as polyp free, the participant had to have a complete colonoscopy reaching the cecum without the observation of polyps. Participants with at least two adenomas were further classified as having multiple adenomas. An advanced adenoma was defined as meeting one of the following criteria: 1) size ≥ 1 cm, 2) tubulovillous or villous, or 3) high-grade dysplasia.

Two independent samples of participants from TCPS and TIARS were evaluated for associations between genetic variation in prostaglandin pathway genes and adenoma risk in a 2-stage design. In the discovery phase, genotypes from a genome-wide association study were supplemented with additional genotyping assays to complete genomic coverage of those genes, and then imputed to the 1000 Genomes and HapMap reference panels. In the replication phase, selected SNPs were genotyped in an independent sample of participants, and results from both phases were combined using meta-analysis.

Genotyping
Genes were selected from the PG signaling and metabolism pathways for analysis. Prostaglandin E synthase (PTGES), 15-hydroxyprostaglandin dehydrogenase (HPGD), prostaglandin-endoperoxide synthase 2 (PTGS2), hydroxysteroid (11-beta) dehydrogenase 2 (HSD11B2), prostaglandin E receptor 4 (PTGER4), prostaglandin E receptor 3 (PTGER3), prostaglandin E receptor 2 (PTGER2) and prostaglandin E receptor 1 (PTGER1) were assayed for association with adenoma risk. This subset of all possible genes that are involved in prostaglandin-mediated inflammation, NSAID metabolism, prostaglandin synthesis and catabolism, and other relevant pathways were selected to refine the scope of this study to genes most proximal to the phenomenon of adenoma chemoprevention by NSAIDs.

Initial genotyping was performed using the Affymetrix Genome-Wide Human SNP Array 5.0 (Affymetrix, Inc., Santa Clara, CA) to agnostically detect associations with adenoma risk throughout the genome. Imputation was performed using IMPUTEv2.2 (25) with reference panels of densely genotyped SNPs from the International HapMap Project Phase 3 data (26) and data from the 1000 Genomes project (27). Genomic coverage of regions of interest in the discovery sample were augmented with further genotyping using Sequenom iPLEX Gold genotyping (Sequenom, Inc., San Diego, CA) in the PTGES, HPGD, and PTGER1 to ensure at least 80% coverage of known common variants in the Caucasian population. The proportion of common SNPs from the International HapMap Project phase 2 data that were tagged with at least an \( r^2 \) of 0.8 are given in Supplementary Table 1. Follow-up genotyping of candidate SNPs where association signals were observed was performed using Sequenom iPLEX Gold genotyping.

**Quality Control**

Quality control (QC) procedures were performed on CEL files using the Dynamic Model (DM) algorithm in the Affymetrix Power Tools software package. Genotypes were called in the remaining samples using the BRLMM-P algorithm (28). The average concordance of genotypes assessed using the PLINK software package within duplicate QC participants was 99.9% (29).
The PLINK --sex-check option did not discover any participants who’s X-chromosome heterozygosity was inconsistent with their reported sex. Sixteen participants who were 1st or 2nd degree relatives with other study participants were removed from further analysis. 165 Participants who were missing greater than 5% of their autosomal genotypes were removed from further analysis. Population stratification was assessed by comparing the study participants to reference panels from the HapMap Phase 3 participants using EIGENSTRAT (30), resulting in the removal of twenty-two participants with apparent ancestral differences from the rest of the sample.

For SNP QC, SNPs were removed if they were missing in greater than 5% of participants, or if the minor allele frequency (MAF) in the samples that passed sample QC was less than 1%. After related and admixed participants were removed, SNPs were removed for major deviations from Hardy-Weinberg equilibrium (HWE) p<1x10⁻⁶. After sample and SNP QC procedures, 402,326 SNPs remained in 958 adenoma cases and 909 adenoma controls. In the COX genes, a total of 1,145 genotyped and high-quality imputed SNPs remained after QC.

In the replication phase of the study, eight SNPs were selected from COX pathway genes based on the statistical significance of tests of association, imputation quality and allele frequency. SNPs were required to be nominally significant for tests of main effect association in primary analyses or be associated with p-value < 0.15 in conditional analyses adjusted for the index SNP in each gene, have an imputation quality information score from SNPTEST of at least 0.8, and an allele frequency of at least 10%. These SNPs were genotyped using the Sequenom genotyping system in 2,028 cases and 3,087 controls. Samples were checked for duplications, and 41 pairs were removed. Also participants who did not self-report as Caucasian were removed from this analysis (394 cases, 618 controls). These SNPs were evaluated for concordance among replicate QC participants (99%), missing data > 5%, HWE p<0.001, and minor allele frequency agreement with Phase 1. All eight SNPs passed QC checks. The final data for association analysis consisted of 1,593 cases and 2,376 controls.
Statistical Analysis

In data from the GWAS, we assessed the relationship between genetic variation in candidate genes and the risk of colorectal adenoma using the software package SNPTESTv2.2.0 with the “–method score” option, using logistic regression with frequentist tests, and assuming an additive effect of SNP alleles on risk, adjusted for age and sex (31). In genes where there were multiple nominally significant SNPs we conducted conditional tests of association for the remaining SNPs, adjusting for the most significant SNP, age, and sex. This procedure mitigates the effect of LD-induced significance and helps identify associations at SNPs that are potentially due to LD with independent mutations on distinct haplotypic backgrounds. Summaries of the LD among the index and conditional SNPs are provided in Supplementary Tables 2a-4b.

The SNPs genotyped for replications were evaluated for association with adenoma risk using PLINK with logistic regression, adjusting for age, body mass index (BMI: kg/m²) and sex. We also evaluated risk for multiple adenoma and advanced adenoma in the SNPs that were genotyped for the replication phase. Additionally, models were fit to evaluate interactions between the eight candidate SNPs and NSAID exposure, encoded as current vs. former and never users in both phases of the analysis. Use was defined as taking NSAIDs at least three times a week for at least one year. Former users had stopped NSAID use for one year or more, but did not significantly differ from never users with regard to adenoma risk in either phase, and so these two classes of participants were merged in the analysis (data not shown).

A meta-analysis was performed combining the results from both phases of the investigation for both SNPs and SNP x NSAID interactions using the software METAL for the combined sample size of 2,551 cases and 3,285 controls (32). Additionally, stratified analysis of the genotypes at SNP rs12647154 were conducted to estimate odds ratios of adenoma for each genotype versus the referent allele homozygotes, stratified by NSAID status. All reported p-values are 2-sided.
RESULTS

Demographic Data

Analysis of demographic variables for this study identified several associations with risk of adenoma (Table 1). In the discovery phase, cases were significantly older than controls, less educated, were more likely to have a family history of colorectal cancer, were more likely to drink and smoke, and more likely to be current NSAID users. In the replication phase, cases were older, less likely to be female, less educated, were more likely to drink and smoke, had significantly higher BMI, and were less likely to exercise. Differences in these associations between phases are mostly attributable to statistical significance, as the direction of effects for family history, exercise, and BMI are consistent between phases. The direction for NSAID use is in the opposite direction, and this is mostly due to a higher proportion of current NSAID users in the control group of the replication phase.

Genetic Main Effects

Referent alleles were assigned at random for analysis of SNP data, since there are not strong a priori reasons for specifying a particular allele at a SNP as referent in GWAS. As a result, effect sizes may be presented as protective, but we only know the magnitude of the association, and not the true direction with regard to population prevalence without making risk estimates from prospective data. SNPs in three genes from the GWAS data were nominally significantly associated with risk of adenoma in the discovery participants (Table 2). No SNPs in the other 5 genes had a p-value for association with risk of adenoma of less than 0.05. The SNP rs3765414 in PTGER3 was associated with adenoma risk with an odds ratio (OR) = 1.31 (95% CI = 1.04-1.66), and p-value=0.023. The SNP rs6846029 in the HPGD was associated with adenoma risk with OR = 0.84 (95% CI = 0.71-0.96), and p-value=0.013. The SNP rs17125318 in the PTGER2 was associated with adenoma risk with OR = 0.84 (95% CI = 0.71-0.96), and p-value=0.004.
These SNPs were genotyped for replication. All other SNPs in these genes with p-values smaller than 0.05 are presented in Supplementary Tables 5-7.

**Conditional Analyses**

Analyses adjusting for the most significant index SNP were conducted in each gene in order to find associations that were conditionally independent of the index signal. We identified and genotyped three index SNPs and five additional SNPs of interest using this approach. There were two SNPs in the gene **HPGD** after adjusting for rs6846029, two SNPs in the gene **PTGER3** with adjustment for rs3765414, and one SNP in **PTGER2** after adjusting for rs17125318. The two additional SNPs in **HPGD** were rs3797013 with OR = 1.28 (95% CI = 0.97-1.68) and rs12647154 with OR = 0.87 (95% CI = 0.73-1.04). Estimates for **HPGD** SNPs without adjustment for rs6846029 at rs3797013 were OR = 1.34 (95% CI = 1.04-1.75; p-value = 0.026), and at rs12647154 OR = 0.93 (95% CI = 0.79-1.10; p-value = 0.414). In **PTGER3**, the unadjusted estimates for rs41485048 were OR = 1.17 (95% CI = 0.97-1.42; p-value = 0.101), and for rs7541963 they were OR = 1.16 (95% CI = 0.96-1.41; p-value = 0.110). At the **PTGER2** SNP rs1254600, the unadjusted OR = 1.1 (95% CI = 0.90-1.35; p-value = 0.339).

No SNPs in the remaining genes of interest in this study (**PTGS2**, **PTGER1**, **HSD11B2**, **PTGER4**, **PTGES**) were nominally significant (data not shown). Additional adjustment for use of NSAIDs did not substantively change estimates of effects, significance, or seem to be a confounder for the association of SNPs in the candidate genes.

**Replication of associated SNPs**

The genotyped SNPs were evaluated for association with adenoma risk in an independent set of self-reported European-ancestry participants (**Table 2**). The same conditional models were fit for the replication study as were used to identify the SNPs in the discovery phase. Two SNPs were nominally associated with adenoma risk; rs3797013 with OR = 0.81 (95% CI = 0.70-0.98; p-value = 0.03), and rs12647154 was weakly association in the replication study with OR = 0.90 (95% CI = 0.79-1.02; p-value = 0.116). We also tested these eight SNPs for association with
risk of multiple and advanced adenomas. The PTGER2 SNP rs1254600 was associated with risk of multiple adenomas in the discovery phase with OR = 0.73 (95% CI = 0.53-0.99, p-value 0.046), but not in the replication phase with OR = 0.65 (95% CI = 0.29-1.40, p-value = 0.263). The meta-analysis of this SNP was also significant, with OR = 0.71 (95% CI = 0.57-0.88, p-value = 0.0007). No other SNP was significantly associated with risk of either multiple or advanced adenomas in either phase (data not shown).

**Meta-analyses**

Meta-analyses of the combined discovery and replication samples for SNPs and interactions were conducted for the eight SNPs identified for replication. For single SNPs, five of eight SNPs had effects in the same direction in both phases, and two SNPs had a p-value less than 0.05 in the combined analysis (Table 2). For interactions with current NSAID use, six of eight tests were in the same direction in both phases, and HPGD SNP rs12647154 had a nominally significant interaction in the meta-analysis $OR_{meta} = 0.76$ (95% CI = 0.62-0.85; p-value = 0.00005), with a p-value = 0.42 for a 1-degree-of-freedom test of heterogeneity (Table 3). For rs12647154, the C allele decreased risk of adenoma, and this protective phenomenon was synergistic with current NSAID use. The nominally significant SNPs from the meta-analysis were the PTGER2 SNPs rs17125318 and rs1254600. Two HPGD SNPs trended toward an association at rs3797013, $OR_{meta} = 0.88$ (95% CI = 0.77-1.00; p-value = 0.054), and at rs12647154, $OR_{meta} = 0.89$ (95% CI = 0.73-1.01; p-value = 0.073). Odds ratios for adenomas were estimated for individual genotypes at rs12647154, stratified by NSAID status (Supplemental Table 8). Similar trends of effect sizes were observed in both the discovery and replication samples, where current NSAID users carrying the C allele at rs12647154 had approximately 30% reduced risk of adenoma, while participants with those genotypes who were not current NSAID users were not protected.

**DISCUSSION**
In this study we have conducted a detailed 2-stage analysis of eight candidate genes from the PG synthesis pathway for association with risk of colorectal adenoma. We also evaluated interactions with NSAID exposure on adenoma risk for the most statistically implicated SNPs and found evidence for effect modification by NSAIDs. We detected nominally significant associations in *PTGER2*, and an interaction in *HPGD* in the combined meta-analysis of both stages. We also detected an association between *PTGER2* and the risk of multiple adenoma. No statistical signal in this analysis survives a formal multiple-testing correction for all tests, although the interaction result in *HPGD* withstands a Bonferroni correction within tests of interaction. The *HPGD* SNP rs12647154 had consistent direction of effect in both phases for both main effect and interaction analyses. The hypotheses relating the genes studied here to colorectal adenoma are well-supported by biological and epidemiological data. Many other phenotypes have been ascribed to *HPGD* and *PTGER2* in human and animal studies, demonstrating that these genes are important in multiple biological pathways, from crucial steps of development and tumor suppression to inflammation homeostasis.

Previous studies of prostaglandin pathway genetic variants on risk of adenoma have produced generally subtle and inconsistent results. Some subtle effect modification was recently observed for *PTGER2* and *PTGER4* SNPs and NSAID exposure (33). We did not observe the previously reported effects and effect modification in *PTGS2*, although we did not test for interactions unless we observed some marginal effect of a SNP on risk of adenoma (34-39).

The gene product of *HPGD*, which oxidizes the prostaglandin molecule and greatly reduces inflammatory activity *in vivo*, is the primary enzyme for prostaglandin degradation (40). Two recent genetic studies of CRC identified SNPs in *HPGD* as marginally associated with CRC (41, 42). *HPGD* is highly expressed in normal colon mucosa, but expression is lost in human colon cancer cells (43, 44). A recent study demonstrated that the adenoma-preventive activity of the NSAID celecoxib is abrogated in *HPGD* knockout animals, and that participants who develop adenoma in clinical trials investigating chemoprevention by celecoxib have lower
average colonic HPGD expression levels than participants who do not develop adenoma (45). Whether the interaction observed here is a result of linkage disequilibrium with a mutation affecting gene expression or secondary to a change in HPGD catalytic activity is unknown and requires further study, where the primary hypothesis of interest is that the high-risk A allele at rs12647154 is associated with lower levels of HPGD expression. We do not know of any study in the literature that specifically evaluates this mechanism.

Similarly, PTGER2 has been implicated in several cancer traits, and cancer-related phenomena. PTGER2 over expression in colorectal cancer has been associated with microsatellite instability, independent of the CpG island methylator phenotype (46). Polymorphisms in PTGER2 have also been associated with colorectal cancer risk in humans (42). We found in this study PTGER2 variants associated with the risk of multiple adenomas only.

The biology of PTGER3 is also complex and related to cancer risk and progression. The gene PTGER3 encodes one of several prostaglandin receptors. PTGER3 has been shown to regulate the ability of breast cancer tumors to undergo angiogenesis and tumor growth (47-49). Mitogenic activity in CRC cells has also been linked to PTGER3 (50). Studies of tumor cell migrations have suggested that PTGER3 contributes to metastasis by upregulation of VEGF-receptor signaling (51).

While there is a definite role for prostaglandin biology in the pathophysiology of colorectal adenoma, the effects of genetic polymorphisms in PG pathway genes on risk of adenoma are modest. The effects we describe here are subtle and do not explain a large proportion of risk. A recent well-powered meta-analysis of colorectal cancer GWAS did not identify PG pathway genes with genome-wide levels of significance, which may have occurred due to the subtle influence of these genes, or effect modification which was not modeled in those studies (52). However, even modest effects of genetic variants can provide some insight into biological mechanisms.
The TCPS and TIARS together are one of the largest colonoscopy-based case-control studies of colorectal polyps, providing adequate power to detect subtle effects on risk. Only controls with complete colonoscopies were included in the analysis, protecting against misclassification of cases and controls. Additional SNPs were also assayed in addition to the GWAS SNPs in the discovery phase of the study to provide adequate coverage of the candidate genes before imputation was performed. The final analysis consisted of 2,551 cases and 3,285 controls, a sample size sufficient to detect a modest effect of OR between 1.1 and 1.5 with a MAF of 0.1 or larger at multiple-comparisons-corrected levels of significance. For interactions, the final analysis had 80% power to detect an interaction OR of 1.5 to 1.8 with MAF of 0.1 or more.

In summary, modest direct effects on risk of adenoma were detected in the combined sample for HPGD and PTGER2 variants. Effect modification by NSAID exposure was observed, suggesting that the role of these genes in adenoma formation is modified by PG inhibition. Further studies may be required to completely identify the roles of these genes in adenoma formation.

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Table 1. Characteristics of study participants by phase, the Tennessee Colorectal Polyp Study (2003-2010) and Tennessee-Indiana Adenoma Recurrence Study (1996-2006).

<table>
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<tr>
<th>Characteristic</th>
<th>Discovery (N = 1,867)</th>
<th>Replication (N = 3,969)</th>
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<td>Total Cases (N=958)</td>
<td>Controls (N=909)</td>
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<td>Study Population (%)</td>
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<tr>
<td>NSAID use (%)</td>
<td></td>
<td></td>
<td>0.007</td>
</tr>
<tr>
<td>Current</td>
<td>39.1</td>
<td>40.0</td>
<td>38.7</td>
</tr>
<tr>
<td>Former</td>
<td>10.9</td>
<td>5.0</td>
<td>13.6</td>
</tr>
<tr>
<td>Never</td>
<td>50.0</td>
<td>55.0</td>
<td>47.7</td>
</tr>
<tr>
<td>Total energy intake (kcal/day, mean)</td>
<td>2330</td>
<td>2333.7</td>
<td>2292.4</td>
</tr>
</tbody>
</table>

1 Derived from ANOVA for continuous variables and \(\chi^2\) test for categorical variables.
2 Standardized by age (40-49, 50-59, 60-64, and \(\geq\)65 years old) and sex distribution of all study participants.
Standardized by age distribution (40-49, 50-59, 60-64, and ≥65 years old) of all study participants

P-value for case-control comparison
Table 2. Meta-analysis results with unadjusted p-values for 8 selected SNPs from COX pathway genes

the Tennessee Colorectal Polyp Study (2003-2010) and Tennessee-Indiana Adenoma Recurrence Study (1996-2006).

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Discovery</th>
<th>Replication</th>
<th>Meta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Referent</td>
<td>OR  95% CI</td>
<td>P-value  OR  95% CI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Allele</td>
<td>P-value</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G</td>
<td>0.65 0.48-0.88</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G</td>
<td>0.69 0.49-0.98</td>
<td>0.037</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T</td>
<td>1.32 1.04-1.68</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T</td>
<td>1.21 0.99-1.46</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T</td>
<td>1.19 0.99-1.45</td>
<td>0.062</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T</td>
<td>0.82 0.70-0.96</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>1.28 0.97-1.68</td>
<td>0.079</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>0.87 0.73-1.04</td>
<td>0.120</td>
</tr>
</tbody>
</table>

*Analyses conditioned upon HPGD SNP rs6846029
†Analyses conditioned upon PTCR3 SNP rs3765414
‡Analyses conditioned upon PTGER2 SNP rs17125318
ΔIndicates whether the non-referent allele increases risk (+), or decreases risk (-) in the discovery and replication stages, respectively.
Table 3. Results from tests of interaction with unadjusted p-values for selected SNPs and NSAID exposure, the Tennessee Colorectal Polyp Study (2003-2010) and Tennessee-Indiana Adenoma Recurrence Study (1996-2006).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Test</th>
<th>Discovery</th>
<th>Replication</th>
<th>Meta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OR 95% CI</td>
<td>P-value</td>
<td>OR 95% CI</td>
</tr>
<tr>
<td>PTGER2</td>
<td>rs17125318xNSAID</td>
<td>0.69</td>
<td>0.38-1.25</td>
<td>0.220</td>
</tr>
<tr>
<td>PTGER2</td>
<td>rs1254600xNSAID†</td>
<td>0.78</td>
<td>0.52-1.19</td>
<td>0.255</td>
</tr>
<tr>
<td>PTGER3</td>
<td>rs3765414xNSAID</td>
<td>0.90</td>
<td>0.56-1.46</td>
<td>0.675</td>
</tr>
<tr>
<td>PTGER3</td>
<td>rs41485048xNSAID‡</td>
<td>1.02</td>
<td>0.69-1.49</td>
<td>0.921</td>
</tr>
<tr>
<td>PTGER3</td>
<td>rs7541936xNSAID‡</td>
<td>0.99</td>
<td>0.68-1.45</td>
<td>0.963</td>
</tr>
<tr>
<td>HPGD</td>
<td>rs6846029xNSAID</td>
<td>1.02</td>
<td>0.75-1.39</td>
<td>0.894</td>
</tr>
<tr>
<td>HPGD</td>
<td>rs3797013xNSAID*</td>
<td>1.62</td>
<td>0.94-2.77</td>
<td>0.081</td>
</tr>
<tr>
<td>HPGD</td>
<td>rs12647154xNSAID*</td>
<td>0.79</td>
<td>0.56-1.10</td>
<td>0.164</td>
</tr>
</tbody>
</table>

*Analyses conditioned upon HPGD SNP rs6846029
†Analyses conditioned upon PTGER3 SNP rs3765414
‡Analyses conditioned upon PTGER2 SNP rs17125318

ΔIndicates whether the non-referent allele increases risk (+), or decreases risk (-) in the discovery and replication stages, respectively.
A study of prostaglandin pathway genes and interactions with current non-steroidal anti-inflammatory drug use in colorectal adenoma

Todd Edwards, Martha J. Shrubsole, Qiuyin Cai, et al.

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