A Study of Prostaglandin Pathway Genes and Interactions with Current Nonsteroidal Anti-inflammatory Drug Use in Colorectal Adenoma

Todd L. Edwards1,2, Martha J. Shrubsole1,3,5, Qiuyin Cai1,3, Guoliang Li1, Qi Dai1,5, Douglas K. Rex7, Thomas M. Ulbright8, Zhenming Fu1, Harvey J. Murff1,3,5, Walter Smalley4,5,6, Reid Ness4, and Wei Zheng1,3,5

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. In addition, 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 (PG) 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 carried out 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 PGE receptor 3 (PTGER3) and 15-hydroxyprostaglandin dehydrogenase (HPGD), both strong biologic candidates for influence on polyp risk. We did not observe the previously reported effects and effect modification in PG–endoperoxide synthase 2 (PTGS2), PGE receptor 2 (PTGER2), or PGE 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. *Cancer Prev Res; 5(6): 855–63. ©2012 AACR.*

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 COX enzymes are usually expressed in response to inflammation and by cancerous and precancerous tissues; nonsteroidal anti-inflammatory drugs (NSAID) inhibit the formation of prostanooids 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, in which 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 adenomas (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 interindividual 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 biologic 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 (SNP) with apparent effects on adenoma risk for interactions with current NSAID use.

Materials and Methods

Study population and data collection

The TCPS 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 multiethnic population.

Excluded from the study were participants who had genetic CRC 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 designed for the southern United States (24). Participants provided DNA either before or after colonoscopy. Participants recruited before colonoscopy were asked to donate a 15 mL of blood sample. A total of 5,504 participants provided a blood sample. Buccal cell or Oragene kit samples were collected from 1,079 participants who chose not to provide a blood sample, or if they were recruited after colonoscopy.

In both study populations, colonoscopic procedures were carried out 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. 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 2 adenomas were further classified as having multiple adenomas. An advanced adenoma was defined as meeting one of the following criteria: (i) size ≥1 cm, (ii) tubulovillous or villous, or (iii) high-grade dysplasia.

Two independent samples of participants from TCPS and TIARS were evaluated for associations between genetic variation in PG pathway genes and adenoma risk in a 2-stage design. In the discovery phase, genotypes from a genome-wide association study (GWAS) 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. PGE synthase (PTGES), 15-hydroxyprostaglandin dehydrogenase (HPGD), PG–endo-peroxide synthase 2 (PTGS2), hydroxysteroid (11-beta) dehydrogenase 2 (HSD11B2), PGE receptor 4 (PTGER4), PGE receptor 3 (PTGER3), PGE receptor 2 (PTGER2), and PGE receptor 1 (PTGER1) were assayed for association with adenoma risk. This subset of all possible genes that are involved in PG-mediated inflammation, NSAID metabolism, PG 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 done using the Affymetrix Genome-Wide Human SNP Array 5.0 (Affymetrix, Inc.) to agnostically detect associations with adenoma risk throughout the genome. Imputation was done using IMPUTEv2.2 (25) with reference panels of densely genotyped SNPs from Table 1.

Table 1. Characteristics of study participants by phase, the TCPS (2003–2010) and TIARS (1996–2006)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>Cases (N = 958)</th>
<th>Controls (N = 909)</th>
<th>( P^a,b )</th>
<th>Total</th>
<th>Cases (N = 1,593)</th>
<th>Controls (N = 2,376)</th>
<th>( P^a,b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study population (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCPS</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>NA</td>
<td>87.5</td>
<td>68.8</td>
<td>100</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TIARS</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td>12.5</td>
<td>31.2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Study site (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vanderbilt University</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>NA</td>
<td>94.0</td>
<td>85</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Indiana University</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td>6.0</td>
<td>15</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Age [y, mean (SD)]</td>
<td>58.5 (7.4)</td>
<td>59.0 (7.3)</td>
<td>58.1 (7.5)</td>
<td>&lt;0.001</td>
<td>57.1</td>
<td>58.2 (7.1)</td>
<td>56.6 (7.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex (female, %)</td>
<td>26.5</td>
<td>26.3</td>
<td>26.6</td>
<td>0.885</td>
<td>40.6</td>
<td>27.9</td>
<td>49.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Indications for colonoscopy (%)c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Screening</td>
<td>56.7</td>
<td>56.6</td>
<td>60.3</td>
<td></td>
<td>56.7</td>
<td>52.8</td>
<td>61.0</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>43.3</td>
<td>43.5</td>
<td>36.8</td>
<td></td>
<td>43.3</td>
<td>47.2</td>
<td>39.0</td>
<td></td>
</tr>
<tr>
<td>Educational attainment (%)c</td>
<td></td>
<td></td>
<td></td>
<td>0.021</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High school or less</td>
<td>25.9</td>
<td>31.7</td>
<td>25.6</td>
<td></td>
<td>24.3</td>
<td>27.0</td>
<td>19.8</td>
<td></td>
</tr>
<tr>
<td>Some college</td>
<td>25.1</td>
<td>25.7</td>
<td>26.6</td>
<td></td>
<td>26.5</td>
<td>28.4</td>
<td>23.7</td>
<td></td>
</tr>
<tr>
<td>College graduate</td>
<td>20.0</td>
<td>18.8</td>
<td>20.8</td>
<td></td>
<td>19.3</td>
<td>18.6</td>
<td>18.9</td>
<td></td>
</tr>
<tr>
<td>Graduate or professional education</td>
<td>22.8</td>
<td>20.9</td>
<td>24.1</td>
<td></td>
<td>23.7</td>
<td>15.7</td>
<td>27.2</td>
<td></td>
</tr>
<tr>
<td>Race (white, %)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>NA</td>
<td>100</td>
<td>100</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>CRC family history (%)c</td>
<td>8.4</td>
<td>9.6</td>
<td>7.7</td>
<td>&lt;0.001</td>
<td>8.0</td>
<td>8.0</td>
<td>7.9</td>
<td>0.790</td>
</tr>
<tr>
<td>Regular cigarette smoking (%)c</td>
<td>58.4</td>
<td>64.9</td>
<td>54.1</td>
<td>&lt;0.001</td>
<td>57.7</td>
<td>70.3</td>
<td>52.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Regular alcohol consumption (%)c</td>
<td>50.2</td>
<td>54.7</td>
<td>48.4</td>
<td>&lt;0.001</td>
<td>49.3</td>
<td>53.7</td>
<td>48.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m², mean)d</td>
<td>28.3</td>
<td>28.5</td>
<td>28.1</td>
<td>0.073</td>
<td>28.0</td>
<td>28.9</td>
<td>27.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Regularly exercised (%)c</td>
<td>51.4</td>
<td>51.6</td>
<td>52.3</td>
<td>0.83</td>
<td>52.9</td>
<td>45.8</td>
<td>54.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NSAID use (%)c</td>
<td></td>
<td></td>
<td></td>
<td>0.007</td>
<td></td>
<td></td>
<td></td>
<td>0.343</td>
</tr>
<tr>
<td>Current</td>
<td>39.1</td>
<td>40.0</td>
<td>38.7</td>
<td></td>
<td>45.1</td>
<td>34.3</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>10.9</td>
<td>5.0</td>
<td>13.6</td>
<td></td>
<td>6.2</td>
<td>5.7</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>50.0</td>
<td>55.0</td>
<td>47.7</td>
<td></td>
<td>48.7</td>
<td>60.0</td>
<td>43.6</td>
<td></td>
</tr>
<tr>
<td>Total energy intake (kcal/d, mean)c</td>
<td>2,330</td>
<td>2,333.7</td>
<td>2,292.4</td>
<td>0.89</td>
<td>2,301</td>
<td>2,376.9</td>
<td>2,190.3</td>
<td>0.064</td>
</tr>
</tbody>
</table>

aDerived from ANOVA for continuous variables and \( \chi^2 \) test for categorical variables.

b\( P \) value for case–control comparison.

cStandardized by age (40–49, 50–59, 60–64, and ≥65 years old) and sex distribution of all study participants.

dStandardized by age distribution (40–49, 50–59, 60–64, and ≥65 years old) of all study participants.
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.) 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 S1. Follow-up genotyping of candidate SNPs in which association signals were observed was carried out using Sequenom iPLEX Gold genotyping.

**Quality control**

Quality control (QC) procedures were conducted 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 BRMM-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 whose X-chromosome heterozygosity was inconsistent with their reported sex. Sixteen participants who were first- or second-degree relatives with other study participants were removed from further analysis. A total of 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 22 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) \( P < 1 \times 10^{-6} \). 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, 8 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 less than 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 by more than 5\%, HWE \( P < 0.001 \), and MAF agreement with phase 1. All 8 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 in which 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 S2A to S4B.

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/m\(^2\)), and sex. We also evaluated risk for multiple adenoma and advanced adenoma in the SNPs that were genotyped for the replication phase. In addition, models were fit to evaluate interactions between the 8 candidate SNPs and NSAID exposure, encoded as current versus former and never users in both phases of the analysis. Use was defined as taking NSAIDs at least 3 times a week for at least 1 year. Former users had stopped NSAID use for 1 year or more but did not significantly differ from never users with regard to adenoma risk in either phase, and so these 2 classes of participants were merged in the analysis (data not shown).

A meta-analysis was done by 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). In addition, stratified analysis of the genotypes at SNP rs12647154 were conducted to estimate ORs 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 CRC, 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 were consistent between phases. The direction for NSAID use is in the opposite direction, and this is mostly because of 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, as 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 3 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 OR, 0.84 (95% CI, 0.70–0.96), and P value = 0.023. The SNP rs6846029 in the HPGD was associated with adenoma risk with OR, 0.85 (95% CI, 1.04–1.75; P value = 0.026) and at rs12647154 OR, 0.93 (95% CI, 0.79–1.0; P value = 0.414). In PTGER3, the unadjusted estimates for rs14485048 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 (PTG2, PTGER1, HSD11B2, PTGER4, and 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, 0.89 (95% CI, 0.80–0.98; P value = 0.03), and rs12647154 was weakly associated in the replication study with OR, 0.90 (95% CI, 0.79–1.02; P value = 0.116). We also tested these 8 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,

### Table 2. Meta-analysis results with unadjusted P values for 8 selected SNPs from COX pathway genes the TCPS (2003–2010) and TIARS (1996–2006)

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Referent Allele</th>
<th>Discovery OR (95% CI)</th>
<th>P</th>
<th>Replication OR (95% CI)</th>
<th>P</th>
<th>Meta OR (95% CI)</th>
<th>P</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTGER2</td>
<td>rs17125318</td>
<td>G</td>
<td>0.65 (0.48–0.88)</td>
<td>0.005</td>
<td>1.01 (0.87–1.18)</td>
<td>0.856</td>
<td>0.85 (0.75–0.96)</td>
<td>0.008</td>
<td>--</td>
</tr>
<tr>
<td>PTGER2</td>
<td>rs1254600b</td>
<td>G</td>
<td>0.69 (0.49–0.99)</td>
<td>0.037</td>
<td>0.97 (0.79–1.18)</td>
<td>0.762</td>
<td>0.84 (0.73–0.99)</td>
<td>0.028</td>
<td>--</td>
</tr>
<tr>
<td>PTGER3</td>
<td>rs3765414</td>
<td>T</td>
<td>1.32 (1.04–1.68)</td>
<td>0.021</td>
<td>1.06 (0.89–1.26)</td>
<td>0.492</td>
<td>1.12 (0.96–1.31)</td>
<td>0.158</td>
<td>--</td>
</tr>
<tr>
<td>PTGER3</td>
<td>rs14485048</td>
<td>T</td>
<td>1.21 (0.99–1.46)</td>
<td>0.056</td>
<td>1.05 (0.91–1.20)</td>
<td>0.489</td>
<td>1.09 (0.97–1.24)</td>
<td>0.153</td>
<td>--</td>
</tr>
<tr>
<td>PTGER3</td>
<td>rs7541936</td>
<td>C</td>
<td>1.19 (0.99–1.45)</td>
<td>0.062</td>
<td>1.05 (0.91–1.20)</td>
<td>0.499</td>
<td>1.09 (0.96–1.23)</td>
<td>0.176</td>
<td>--</td>
</tr>
<tr>
<td>HPGD</td>
<td>rs6846029</td>
<td>T</td>
<td>0.82 (0.70–0.96)</td>
<td>0.011</td>
<td>1.07 (0.96–1.19)</td>
<td>0.241</td>
<td>1.05 (0.97–1.21)</td>
<td>0.223</td>
<td>--</td>
</tr>
<tr>
<td>HPGD</td>
<td>rs3797013</td>
<td>A</td>
<td>1.28 (0.97–1.68)</td>
<td>0.079</td>
<td>0.83 (0.70–0.98)</td>
<td>0.032</td>
<td>0.88 (0.77–1.00)</td>
<td>0.054</td>
<td>--</td>
</tr>
<tr>
<td>HPGD</td>
<td>rs12647154</td>
<td>A</td>
<td>0.87 (0.73–1.04)</td>
<td>0.120</td>
<td>0.90 (0.79–1.02)</td>
<td>0.116</td>
<td>0.89 (0.80–1.01)</td>
<td>0.073</td>
<td>--</td>
</tr>
</tbody>
</table>

*Indicates whether the nonreferent allele increases risk (+) or decreases risk (−) in the discovery and replication stages, respectively.

*Analyses conditioned upon PTGER2 SNP rs17125318.

*Analyses conditioned upon PTGER3 SNP rs3765414.

*Analyses conditioned upon HPGD SNP rs6846029.
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 8 SNPs identified for replication. For single SNPs, 5 of 8 SNPs had effects in the same direction in both phases, and 2 SNPs had a P value less than 0.05 in the combined analysis (Table 2). For interactions with current NSAID use, 6 of 8 tests were in the same direction in both phases, and HPGD SNP rs12647154 had a nominally significant interaction in the meta-analysis ORmeta, 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, ORmeta, 0.88 (95% CI, 0.77–1.00; P value = 0.054) and at rs12647154, ORmeta, 0.89 (95% CI, 0.73–1.01; P value = 0.073). ORs for adenomas were estimated for individual genotypes at rs12647154, stratified by NSAID status (Supplementary Table S8). Similar trends of effect sizes were observed in both the discovery and replication samples, in which current NSAID users carrying the C allele at rs12647154 had approximately 30% reduced risk of adenoma, whereas 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 8 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 adenomas. 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 biologic and epidemiologic data. Many other phenotypes have been ascribed to HPGD and PTGER2 in human and animal studies, showing that these genes are important in multiple biologic pathways, from crucial steps of development and tumor suppression to inflammation homeostasis.

Previous studies of PG 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 PG molecule and greatly reduces inflammatory activity in vivo, is the primary enzyme for PG degradation (40). Two recent genetic studies of CRC identified SNPs in HPGD as marginally associated with CRC (41, 42). HPGD is highly

<table>
<thead>
<tr>
<th>Gene</th>
<th>Test</th>
<th>Discovery</th>
<th>Replication</th>
<th>Meta</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTGER2</td>
<td>rs17125318×NSAID</td>
<td>0.69 (0.38–1.25)</td>
<td>0.220</td>
<td>0.79 (0.55–1.14)</td>
</tr>
<tr>
<td>PTGER2</td>
<td>rs12546000×NSAID</td>
<td>0.78 (0.52–1.19)</td>
<td>0.255</td>
<td>0.99 (0.74–1.33)</td>
</tr>
<tr>
<td>PTGER2</td>
<td>rs3765414×NSAID</td>
<td>0.90 (0.56–1.46)</td>
<td>0.675</td>
<td>0.87 (0.58–1.28)</td>
</tr>
<tr>
<td>PTGER2</td>
<td>rs41485048×NSAID</td>
<td>1.02 (0.69–1.49)</td>
<td>0.921</td>
<td>0.92 (0.67–1.26)</td>
</tr>
<tr>
<td>PTGER2</td>
<td>rs7541936×NSAID</td>
<td>0.99 (0.68–1.45)</td>
<td>0.963</td>
<td>0.93 (0.68–1.27)</td>
</tr>
<tr>
<td>HPGD</td>
<td>rs6846029×NSAID</td>
<td>1.02 (0.75–1.39)</td>
<td>0.894</td>
<td>0.87 (0.68–1.11)</td>
</tr>
<tr>
<td>HPGD</td>
<td>rs3797013×NSAID</td>
<td>1.62 (0.94–2.77)</td>
<td>0.081</td>
<td>1.03 (0.71–1.49)</td>
</tr>
<tr>
<td>HPGD</td>
<td>rs12647154×NSAID</td>
<td>0.79 (0.56–1.10)</td>
<td>0.164</td>
<td>0.69 (0.52–0.92)</td>
</tr>
</tbody>
</table>

Indicates whether the nonreferent allele increases risk (+) or decreases risk (−) in the discovery and replication stages, respectively.

Analyses conditioned upon PTGER2 SNP rs17125318.

Analyses conditioned upon PTGER3 SNP rs3765414.

Analyses conditioned upon HPGD SNP rs6846029.
expressed in normal colon mucosa, but expression is lost in human colon cancer cells (43, 44). A recent study showed 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, in which 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 overexpression in CRC has been associated with microsatellite instability, independent of the CpG island methylator phenotype (46). Polymorphisms in PTGER2 have also been associated with CRC 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 PG 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).

Although there is a definite role for PG 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 CRC GWAS did not identify PG pathway genes with genome-wide levels of significance, which may have occurred because of the subtle influence of these genes, or effect modification that was not modeled in those studies (52). However, even modest effects of genetic variants can provide some insight into biologic 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 carried out. 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.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: M.J. Shrubsole, R. Ness, W. Zheng
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M.J. Shrubsole, Q. Dai, D. Rex, T.M. Ulbright, R. Ness, W. Zheng
Writing, review, and/or revision of the manuscript: T.L. Edwards, M.J. Shrubsole, Q. Dai, D. Rex, T.M. Ulbright, Z. Fu, H.J. Murff, R. Ness, W. Zheng
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M.J. Shrubsole, Q. Dai, W. Zheng
Study supervision: M.J. Shrubsole, W. Zheng

Grant Support
This study was supported through the National Cancer Institute grants P50CA95103, R01CA121060, and R01AT004660. T.L. Edwards is supported by a Vanderbilt Clinical and Translational Research Scholar Award S5KL2RR024975 (to T.L. Edwards, in part). The TCPS was conducted by the Survey and Biospecimen Shared Resource supported in part by the Vanderbilt-Ingram Cancer Center (P30 CA 68485).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received October 5, 2011; revised March 26, 2012; accepted March 28, 2012; published OnlineFirst May 2, 2012.

References
Edwards et al.


Cancer Prevention Research

A Study of Prostaglandin Pathway Genes and Interactions with Current Nonsteroidal Anti-inflammatory Drug Use in Colorectal Adenoma

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


Updated version Access the most recent version of this article at: doi:10.1158/1940-6207.CAPR-11-0459

Supplementary Material Access the most recent supplemental material at: http://cancerpreventionresearch.aacrjournals.org/content/suppl/2012/05/02/1940-6207.CAPR-11-0459.DC1

Cited articles This article cites 49 articles, 18 of which you can access for free at: http://cancerpreventionresearch.aacrjournals.org/content/5/6/855.full.html#ref-list-1

Citing articles This article has been cited by 2 HighWire-hosted articles. Access the articles at: /content/5/6/855.full.html#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.