Prostaglandin H Synthase 2 Variant (Val511Ala) in African Americans May Reduce the Risk for Colorectal Neoplasia


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Abstract

Prostaglandin H synthase 2 (also known as cyclooxygenase-2) is thought to play a role in the prevention of colon cancer by aspirin, an inhibitor of the enzyme. We used DNA heteroduplex analysis to screen the prostan glandin H synthase 2 gene, to search for naturally occurring enzyme variants that may simulate the effects of aspirin. We found among African-Americans a single-nucleotide polymorphism that changes valine to alanine at residue 511 (V511A; GTT \rightarrow GCT; g.5937T>G; allele frequency 0.045). The polymorphism was also seen among Asian-Indians (allele frequency, 0.03) but not among Chinese, Filipinos, Hispanics, Japanese, Koreans, Samoans, and Caucasians. The amino acid change is predicted to open a 53 cubic angstrom cavity near the active site of the enzyme, but no change in \( V_{\text{max}}, K_m \), or thermal stability was observed for the variant enzyme in COS-1 cell transfection assays. Case-control analysis of African-Americans from two different study populations showed a 0.56 odds ratio for colorectal adenomas among polymorphism carriers (95% confidence interval, 0.25–1.27; 161 cases and 219 controls). A similar analysis of African-Americans nested in the Multietnic Cohort Study showed a 0.67 odds ratio for colorectal cancer (95% confidence interval, 0.28–1.56; 138 cases and 258 controls). Consistency of the results across all three of the studies is potentially compatible with a protective effect of the polymorphism, mimicking aspirin.

Introduction

PTGS\(^3\) enzymes convert arachidonic acid to prostaglandin \( H_2 \), a precursor to all of the other prostanoids. Two forms of human PTGS are known, PTGS1 and PTGS2. They are similar in crystal structure and inhibited by NSAIDs (1, 2), but the enzymes are encoded by different genes (3, 4). PTGS1 was first prepared from sheep and bull seminal vesicles (5–7), and may be a housekeeping enzyme involved in cell signaling. PTGS2, on the other hand, was discovered in chicken embryo fibroblasts induced by Rous sarcoma virus (8), in Swiss 3T3 mouse cells treated with tetradecanoylphorbol acetate (9), and in C127 mouse fibroblasts stimulated by serum or glucocorticoids (10). Both PTGS1 and PTGS2 localize to the nuclear envelope and endoplasmic reticulum of cells (11), but PTGS2 is absent from many cell types unless induced by tumor promoters, growth factors, or cytokines.

PTGS2 occurs at high levels in colon cancer (12–16). Support for the hypothesis that PTGS2 is involved in development of colon cancer comes from evidence that fewer polyps occurred after disruption of the PTGS2 gene in mice prone to intestinal polyposis (17). Also, celecoxib, a selective PTGS2 inhibitor, blocked colon tumors in mice (18, 19) and in patients with familial adenomatous polyposis (20, 21). In a case-control study of colon cancer, Kune et al. (22) unexpectedly found a 0.53 relative risk for cancer with regular aspirin use among 715 cases and 727 controls. Some 15 other studies have found similar results (reviewed in Ref. 23).

We hypothesized that naturally occurring PTGS2 variants might mimic long-term NSAID use (24) and give additional insight into biochemical mechanisms of colon cancer prevention. Here we describe: (a) identification of a PTGS2 polymor-

\(1\) The abbreviations used are: PTGS, prostaglandin H synthase; NSAID, nonsteroidal anti-inflammatory drug; Å, angstrom; OR, odds ratio; CI, confidence interval; USC, University of Southern California; UNC, University of North Carolina.

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phenism among African-Americans; (b) cell transfection experiments to check function of the polymorphism; (c) modeling of the crystal structure of the variant enzyme; and (d) epidemiological studies of the polymorphism in relation to colorectal adenomas and cancer.

Materials and Methods

Identification of PTGS2 Variants

Blood Specimens from Healthy Individuals. We used blood specimens from the University of California Los Angeles Tissue Typing Laboratory to search for common PTGS2 variants. Specimens were from blood drives aimed at finding bone marrow donors from specific ethnic groups. Random batches of specimens in acid-citrate-dextrose tubes without identifiers were obtained between February 1992 and November 1993. Most blood specimens were from the Los Angeles area, but the laboratory also received specimens from around the world. Volunteers were generally unrelated, between the ages of 18 and 65, and without a history of chronic disease, including cancer. There was no gender preference and no duplicate sampling. Ethnic groups surveyed were: African-American, Chinese (Hong Kong), Filipino, Hispanic, Indian (Asian), Japanese, Korean, Samoan, and Caucasian.

PCR. We used 1 × 1 mm squares of dried blood on blotter paper (No. 903 paper; Schleicher and Schuell, Keene, NH) for PCR templates. Each PCR contained 40 μM of each dNTP, 1 μM of each primer, 20 μg ml⁻¹ BSA, buffers recommended by Taq polymerase suppliers, and a total volume of 10–40 μl. MgCl₂ was optimized for each pair of primers. Heating was done in a Perkin-Elmer 9600, Applied Biosystems 9700, or MJ Research PTC-100 thermal cycler (95°C for 15 min; then the temperature was lowered to 85°C for 10 min while 0.25 units of Taq polymerase was added; then 26–32 cycles of 94°C for 30 s, the annealing temperature for 40 s, and 72°C for 60 s; then 72°C for 5 min). PCR primers were selected by use of a computer program (Table 1), with theoretical annealing temperatures matched to within 3–5°C.

Heteroduplex Analysis. We used DNA heteroduplex analysis to screen for variants in exons and flanking intron regions in 47 African-American and 47 Caucasian subjects (25, 26). PCR conditions were as above, with total volumes of 10 μl and 0.2 μCi of [α³²P]dCTP or 1 μCi of [α³²P]dATP (3000 Ci mmol⁻¹; ICN Pharmaceuticals, Irvine, CA). Primer pairs, annealing temperatures, and sizes of PCR products were: EX1L1 and EX1R1 (54°C; 329 bp); EX23L2 and EX23R2 (50°C; 627 bp); EX4L3 and EX4R3 (50–51°C; 364 bp); EX5L4 and EX5R4 (54°C; 319 bp); EX6L5 and EX6R3 (50°C; 659 bp); EX8L6 and EX8R6 (48°C; 413 bp); EX9L7 and EX9R7 (50°C; 296 bp); and EX10L and UTR-R (48°C; 603 bp). After amplification, products were heated to 98°C for 5 min, incubated at 68°C for 1 h to form heteroduplexes, and electrophoresed on 10% polyacrylamide gels to detect variants (27).

DNA Sequencing. Variants were identified by Sanger deoxy DNA sequencing (United States Biochemical, Cleveland, OH) through either direct sequencing of PCR products or sequencing of PCR products that were cloned into plasmids. For direct sequencing, PCR products were treated with exonuclease I and shrimp alkaline phosphatase (United States Biochemical) or purified from agarose gels (GENECLEAN; Bio101, Vista, CA). For plasmid sequencing, PCR products were cloned into a plasmid vector (TA cloning vector; Invitrogen Corp., San Diego, CA). Forty to 60 clones containing inserts were grown individually, pooled, and used for preparing plasmid DNA.

We also sequenced an AU-rich region 3’ of the PTGS2 stop codon in six subjects with the V511A polymorphism to look for DNA variation that might be associated with V511A. The AU-rich region is thought to play a role in degradation of mRNA (see “Results”). Specifically, we amplified exon 10 and the adjoining 3’ untranslated region (primers EX10L and UTR-R; 48°C; 603 bp) and used UTR-L and UTR-R as sequencing primers.

Transfection Experiments with the V511A Polymorphism

PTGS2 Expression Vectors and Site-directed Mutagenesis. A plasmid expression vector containing the human PTGS2 coding sequence was provided by W. Smith and D. DeWitt. We subcloned into plasmid pUC19 a SauI restriction fragment containing wild-type coding and flanking regions (1.9-kb total). The 5’ flanking region was: GTC GAC(SalI site)-CGA ATT(C/eoRl/ApoI site)-GCG GCC GCG TGA GAA CCG TTT ACC(24 bp junction piece)-ATG(start of PTGS2 coding sequence). The 3’ flanking region was: TAG(stop codon)-AAG TCG TAT GAT CAT (PTGS2 flanking sequence)-ACC CTT CCC TCG G6C GAA CAT AAG GCT TGG AAC GGT CGA C (40 bp junction piece)-GTC GAC(SalI site). We prepared a coding unit that produced the same amino acid sequence as described by Kosaka et al. (4).

We introduced the V511A variant into the coding sequence by site-directed mutagenesis [Quantum Biotechnologies, Inc., Laval, Quebec, Canada; mutagenic oligonucleotide: 5’-pGA AAC CAT GTG AAG AGC TGC ACC ATT

Table 1. Oligonucleotide primers used for PCR and/or DNA sequencing

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<th>Name</th>
<th>Sequence</th>
<th>Position</th>
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<td>EX5L4</td>
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<tr>
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<td>3’ primers</td>
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in a volume of 50

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USC/Kaiser study, which consisted of roughly 1700 subjects

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English, gave informed consent, and lived in the metropolitan
Los Angeles area. There was no history of invasive cancer,
inflammatory bowel disease, familial polyposis, previous
bowel surgery, symptoms suggestive of gastrointestinal dis-
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time diagnosis of an adenoma, confirmed by histology. Con-
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sigmoidoscopy date, and center. Indications for sigmoidoscopy
were “routine” for 44% of the control subjects and 45% of the
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a 45-min in-person interview, subjects provided information on
smoking, therapeutic drug use, physical activity, height, weight,
family history of cancer, and other factors. The interviewer was
unaware of case or control status for 70% of case subjects and
87% of controls. Overall participation rates in the study were
84% for cases and 82% for controls (number interviewed/number
eligible; Ref. 35).

Blood specimens from 140 African-Americans (42 cases
and 98 controls) came from the UNC study, which consisted of
roughly 800 subjects examined by colonoscopy at the UNC
Hospitals during the period from August 1998 through March
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fomed consent. They agreed to have biopsy specimens taken
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Dried blood spots on blotter paper were prepared from
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papers were labeled with a code number, but no other identifier.
One-mm squares of dried blood were tested in duplicate for the
V511A polymorphism by use of allele-specific PCR, without
knowledge of case or control status (see Fig. 1d). To double-
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polymorphism were amplified again and tested for the poly-
morphism by use of restriction enzyme AluI (site present in
variant allele; see Fig. 1c).

Epidemiological Studies of the V511A Polymorphism

Case-Control Analyses of Colorectal Adenomas. Subjects
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Modeling of the V511A Polymorphism
The crystal structures of ovine PTGS1 (3.1 Å resolution and
Rfactor = 18.5%; PDB entry 1CQE) and murine PTGS2 (3.1 Å
resolution and Rfactor = 28%; PDB entry 3PGH), both com-
pleted with the NSAID flurbiprofen, were superimposed by
least squares superposition of equivalent Co atoms (root square
mean = 0.51Å) and were used to generate images of enzyme
structure. For clarity in comparing crystallographic structures of
PTGS isozymes, we use a consensus numbering system that
starts with methionine 1 of the ovine PTGS1 enzyme. The
homologous amino acids corresponding to ovine PTGS1 and
human PTGS2 that are mentioned in the text are (PTGS1:
PTGS2): Met525:Val511; Leu384:Leu370; and Tyr385:
Tyr371. Thus, position 511 in PTGS2 is called position 525
whenever crystal structures are discussed below.

As the coordinates of human PTGS2 were not yet publicly
available, the L525V (leucine to valine) and L525A (leucine to
alanine) substitutions were modeled in the mouse PTGS2 struc-
ture, using Setor 4.14.7 (31), to evaluate the V525A substitution
in human PTGS2. This procedure was straightforward as the
sequences of PTGS2 from humans and mice are highly homol-
ogous (>95% identity) in this region, and because human
PTGS2, mouse PTGS2, and ovine PTGS1 are all structurally
homologous to each other (32, 33). Cavity volume measure-
ments were made using the program Grasp (34).

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Case-Control Analysis of Colorectal Cancer. DNA speci-
mens came from 396 African-Americans (138 cases and 258
controls) from the Multiethnic Cohort Study, a cohort assem-
bled to investigate diet and cancer in Hawaii and Los An-
geles (36). African-American participants (16.3% of a total

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results were also stratified by NSAID use, because such drugs inhibit PG2S2 and prevent some cases of colorectal cancer. Adjustment for factors besides age and gender was not possible, because other variables were not available in all three of the data sets. Conceivably, other factors may be confounders in such analyses, but there have been no reports of strong confounders of NSAID effects.

Results

Identification of PTGS2 Variants

Variants identified by heteroduplex analysis and DNA sequencing are shown in Table 2. Only the V511A polymorphism changed the amino acid sequence (Fig. 1). The V511A heterozygote frequency among African-American blood specimens from the University of California Los Angeles Tissue Typing Laboratory was 8 of 107 (allele frequency, 0.04). The result was consistent with the frequency in our epidemiological studies (0.045; see below). Among Indians, the V511A heterozygote frequency was 5 of 75 (allele frequency, 0.03). V511A was not detected among Chinese, Hispanic, or Caucasian individuals screened by heteroduplex analysis, or among Filipino, Japanese, Korean, or Samoan individuals screened by allele-specific PCR.

Our screening for variants in exon 1 also covered 193-bp directly 5' of the ATG start codon, which contains a cyclic AMP response element (TTCCGTCA; -193 to -187 relative to the ATG codon), the TATA box (TATAAAA; -165 to -159), and the mRNA cap site (at -134; Ref. 4). No variation in this promotor region was detected.

At the other end of the gene, the 2.9-kb region 3' of the stop codon is AU-rich and contains at least 21 copies of an AUUUA element and has been shown to inhibit protein translation (38). No variation in this promoter region was detected.

For DNA variants that might be associated with V511A. No
species and PTGS isozymes: mouse PTGS2 has a leucine at residue 525, in accordance with the consensus PTGS1/PTGS2 numbering used for crystal structure comparisons; see “Materials and Methods”). Val 525 in human PTGS2 abuts against Leu 384, which forms part of the active site wall and is a close neighbor of the radical donor/acceptor, Tyr 385. The residues that form the pocket are highly conserved among different species and PTGS isozymes: mouse PTGS2 has a leucine at position 525 instead of a valine, and ovine PTGS1 contains Met 525 and two other conservative substitutions. These differences cause little or no change in the packing of the pocket (Fig. 2; B-D). However, the replacement of Val 525 with Ala is predicted to open a 53 Å² (moderate-sized) cavity within the pocket and a smaller, adjacent 20 Å² hole (Fig. 2E).

The presence of a large cavity next to Leu 384 will alter its conformation and could directly perturb the structure of the active site. For example, movement of Leu 384 may allow the Tyr 385 catalytic residue to assume different conformations. Movement of Leu 384 into the cavity may also increase the space within the active site and allow nonproductive conformations of arachidonate (40, 41). Finally, the segment 384–388 forms an unusually stretched helix that contains the two key catalytic residues, Tyr 385 and His 388 (the proximal heme ligand). Repacking around Leu 384 could relax the stretched helix, allowing Tyr 385 to move away from its ideal position. These alterations would be expected to affect cyclooxygenase function.

### Epidemiological Studies of the V511A Polymorphism

#### V511A Allele Frequency
The V511A heterozygote frequency among African-American controls from the USC/Kaiser (12 of 121), UNC (10 of 98), and Multiethnic Cohort (21 of 258) studies was 43 of 477, corresponding to an allele frequency of 0.045 (95% CI, 0.033–0.060). No homozygote was found among all 776 of the cases and controls (1 in 492 expected with an allele frequency of 0.045).

#### V511A Effects
In the USC/Kaiser and UNC studies, the OR for colorectal adenomas with the V511A polymorphism was in the direction of a protective effect but not statistically significant, possibly because of small sample sizes (OR, 0.56; 95% CI, 0.25–1.27; Table 4B). Among African-Americans in the Multiethnic Cohort Study, the OR for colorectal cancer with the V511A polymorphism was 0.67 (95% CI, 0.28–1.56; Table 4C).

### Table 2
PTGS2 variants identified by screening blood samples from healthy individuals

<table>
<thead>
<tr>
<th>Variant</th>
<th>Exon or intron</th>
<th>Heterozygote frequency</th>
<th>Allele frequency (95% CI)</th>
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<td>Caucasians</td>
<td>African-Americans</td>
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<td>Exon 1</td>
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<td>2/47</td>
<td>0/47</td>
</tr>
<tr>
<td>g.&quot;5939T&gt;C (V511A)</td>
<td>Exon 10</td>
<td>8/107</td>
<td>0/100</td>
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### Table 3
Kinetic parameters and thermal stability for PTGS2 enzymes after transfection of wild-type and variant expression vectors in COS-1 cells

<table>
<thead>
<tr>
<th>PTGS2 enzyme</th>
<th>Vmax (nmol of O₂ uptake/min/mg of microsomal protein)</th>
<th>Km (µM)</th>
<th>Half-life at 37°C</th>
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</thead>
<tbody>
<tr>
<td>Val 511 (wild-type)</td>
<td>560 ± 97</td>
<td>1.1 µM</td>
<td>19 h</td>
</tr>
<tr>
<td>Ala 511 (variant)</td>
<td>630 ± 45</td>
<td>2.0 µM</td>
<td>18 h</td>
</tr>
</tbody>
</table>

variation was found (specimen numbers 3014, 3040, 3049, 3061, 3062, and 3065).

### Transfection Experiments with the V511A Polymorphism

The coding sequence with V511A was built into a plasmid vector by use of site-directed mutagenesis and expressed in COS-1 monkey kidney cells. Wild-type and variant PTGS2 were similar in Vmax, Km, and thermal stability at 37°C (Table 3).

### Modeling of the V511A Polymorphism

In the native PTGS2 enzyme, residue 511 (referred to below as residue 525, in accordance with the consensus PTGS1/PTGS2 numbering used for crystal structure comparisons; see “Materials and Methods”), lies inside a tightly packed hydrophobic pocket adjacent to the cyclooxygenase active site (Fig. 2A; Refs. 32, 33, 39). Val 525 in human PTGS2 abuts against Leu 384, which forms part of the active site wall and is a close neighbor of the radical donor/acceptor, Tyr 385. The residues that form the pocket are highly conserved among different species and PTGS isozymes: mouse PTGS2 has a leucine at position 525 instead of a valine, and ovine PTGS1 contains Met 525 and two other conservative substitutions. These differences cause little or no change in the packing of the pocket (Fig. 2; B–D). However, the replacement of Val 525 with Ala is predicted to open a 53 Å² (moderate-sized) cavity within the pocket and a smaller, adjacent 20 Å² hole (Fig. 2E).

The presence of a large cavity next to Leu 384 will alter its conformation and could directly perturb the structure of the active site. For example, movement of Leu 384 may allow the Tyr 385 catalytic residue to assume different conformations. Movement of Leu 384 into the cavity may also increase the space within the active site and allow nonproductive conformations of arachidonate (40, 41). Finally, the segment 384–388 forms an unusually stretched helix that contains the two key catalytic residues, Tyr 385 and His 388 (the proximal heme ligand). Repacking around Leu 384 could relax the stretched helix, allowing Tyr 385 to move away from its ideal position. These alterations would be expected to affect cyclooxygenase function.
Fig. 2. Changes in PTGS2 structure with the V511A polymorphism. Figures show the consensus PTGS1 and PTGS2 numbering, obtained by adding 14 to the human PTGS2 residue number (e.g., human Val 511 is numbered 525 in these figures). A, schematic of the PTGS monomer structure. Overall tertiary structures of PTGS1 and PTGS2 are similar. Tyr 385 (an essential catalytic residue) and the binding site of the NSAID drug flurbiprofen (flu) define the location of the cyclooxygenase active site. Adjacent to the cyclooxygenase active site is a hydrophobic pocket that surrounds residue 525 (yellow). Residue 525 is Met in PTGS1 (ovine), and Leu or Val in PTGS2 (mouse or human, respectively). B, close-up view of the hydrophobic pocket in ovine PTGS1. The tightly packed pocket consists of eight hydrophobic residues: Leu 384, Tyr 466, Phe 470, Phe 503, Leu 507, Met 522, Met 525, and Phe 529. Two polar residues, Ser 521 (light blue residue behind Met 522) and Glu 380 (red stick-bond residue) also form part of the pocket, but the polar side chains point away. Ser 530, the aspirin acetylation site, is shown for reference. C, close-up view of the hydrophobic pocket in murine PTGS2. Three conservative amino acid changes in the mouse enzyme leave the pocket virtually unchanged, compared with sheep PTGS1 (Ser 521 to Thr, Phe 503 to Leu, and Met 525 to Leu). However, the latter two substitutions produce a small hole (18 Å³). D, close-up view of the normal hydrophobic pocket in human PTGS2. At position 525, the human enzyme now contains Val, which still fills the pocket despite a smaller molecular volume. E, close-up view of the proposed variant PTGS2 hydrophobic pocket. Substitution of Ala for Val 525 produces a large cavity (53 Å³) and a smaller adjacent hole (20 Å³).
Thus, there was a suggestion of a protective effect for V511A in all three of the case-control populations.

V511A Effects Apart from NSAID Use. We analyzed V511A separate from NSAID use, because NSAIDs alone may prevent some cases of colorectal neoplasia. Data on NSAID use were available for 180 subjects (75%) in the USC/Kaiser study and 111 subjects (79%) in the UNC study. NSAID users were defined as subjects who took such medication at least once per week for some period of time during the 1-year or the 5-year period before the interview, for the USC/Kaiser or UNC studies, respectively. Excluding NSAID users, the OR for adenomas with V511A was 0.29 (95% CI, 0.08–1.08; Table 4B).

Data on NSAID use were available for 368 subjects (93%) from the Multiethnic Cohort Study. NSAID users were defined as subjects who ever took such medications at least twice per week for at least 2 years. Excluding NSAID users, the OR for cancer among subjects with V511A was 1.19 (95% CI, 0.39–3.61; Table 5B).

NSAID Effects Apart from V511A. We also analyzed NSAIDs separate from V511A, to check effects of these drugs alone. Comparing NSAID users to nonusers in the combined USC/Kaiser and UNC sample, the OR for adenomas when V511A carriers were excluded was 0.56 (95% CI, 0.32–0.96; P = 0.035; Table 4C). The result is compatible with the recognized protective effect of NSAIDs.

Among NSAID users in the Multiethnic Cohort Study, the OR for colorectal cancer was 0.82 when V511A carriers were excluded (95% CI, 0.50–1.33; Table 5C). Again, none of these ORs was statistically significant.

V511A and/or NSAID Use. Finally, we compared subjects who were V511A carriers and/or NSAID users to noncarriers who were nonusers. The analysis may represent low versus high PTGS2 activity. For adenomas, ORs for this comparison were 0.52 in both the USC/Kaiser study (95% CI, 0.28–0.95; P = 0.034) and the combined adenoma study (95% CI, 0.31–0.86; P = 0.011; Table 4D). For colorectal cancer, comparison of V511A carriers and/or NSAID users to noncarriers who were nonusers gave an OR of 0.78 (95% CI, 0.49–1.23; Table 5D).

Discussion

PTGS2 Variants. We found seven variants in PTGS2 coding or flanking intron regions. These DNA variants are among the 46 variants seen previously in or around the PTGS2 gene.\(^4\) Reported variants that change the coding sequence include I1M (Ile1Met), R228H (Arg228His), E488G (Glu488Gly), and V511A. Of these, only V511A was observed in our survey, possibly because heteroduplex analysis may be only 90% sensitive (25).

Our allele frequency (0.045; 95% CI, 0.033–0.060) for V511A in African-Americans was close to a reported frequency of 0.083 (95% CI, 0.023–0.20; Ref. 42). The polymorphism has been reported in Asians at an allele frequency of 0.06 (42). However, we found V511A only in Indians, among the six Asian populations screened.

We looked for association of V511A with variation in PTGS2 gene control regions. No variant was found in a 59-bp PTGS2 promoter region immediately 5' of the mRNA cap site, although a minimal PTGS2 promoter may include 100-bp or more (43). Also, the 116-bp, AU-rich region directly after the stop codon has been shown to inhibit protein translation (38). We did not find any variation in this region in 6 (of 6) subjects with V511A. Thus, there is no sign of association between V511A and variation in a gene control region in our study.

Structure and Function of the V511A Polymorphism. Work on PTGS2 variants focused on V511A, because the polymorphism changes an amino acid that interacts with residues at the active site of the enzyme. Despite the potential for causing significant structural changes in the active site, the polymorphism did not produce detectable differences in enzyme kinetic parameters (V_{max} and K_m) or stability for the utilization of arachidonate, when the enzyme was expressed in COS-1 cells (Table 3). Observed K_m values were comparable with the published K_m for human PTGS2 (5.6 \mu M; Ref. 44). Recently, Fritsche et al. (42) also reported that no functional differences were observed between the V511A variant and wild-type PTGS2. They tested three PTGS2 substrates (arachidonic acid, 2-arachidonyl glycerol, and linoleic acid) and measured effects of four different PTGS2 inhibitors.

Such lack of functional changes may reflect assay conditions in vitro, where arachidonate concentrations were 1–100 \mu M. However, in the cell, PTGS2 is believed to use arachidonate released from the nuclear envelope by cytosolic phospholipase A_2 (45, 46). Physiological arachidonate levels may be fairly low and may be associated with different enzyme properties. For example, Swinney et al. (47) found that PTGS2 binds an inhibitor (SC-58125) 26-fold better at 50 \text{nm} arachidonate than at 20 \mu M arachidonate. An allosteric conformation transition at low arachidonate levels was proposed as an explanation.

Moreover, the in vitro assays monitored PTGS2 activity by following oxygen consumption, a method that does not detect altered products (48). PTGS enzymes oxygenate several different polyunsaturated fatty acids other than arachidonate (49, 50) to form bioactive products. Subtle changes in enzyme structure, because of amino acid substitution, can affect the turnover of alternate substrates, whereas only marginally affecting arachidonate utilization (49, 50). Hence, it may be premature to conclude that there is no functional difference between the V511A variant and wild-type PTGS2, until a more thorough enzymological analysis is done.

Colorectal Neoplasia in Relation to the V511A Polymorphism. Case-control analysis of African-Americans in two studies showed a ~0.5 OR for colorectal adenomas among V511A carriers (Table 4B). Similarly, a third study suggested protection against colorectal cancer (OR ~0.7; Table 5B). The consistency among the three studies tends to support potential validity of a protective effect. Larger sample sizes are needed to confirm our interpretation.

To additionally analyze our small numbers of V511A carriers, we combined V511A carriers with NSAID users and compared them with noncarriers who were nonusers. NSAID users are presumed to have low PTGS2 activity because of inhibition of the enzyme by such drugs. Therefore, the comparison corresponds to low versus high PTGS2 activity, if V511A lowers enzyme activity in vivo. Results were compatible with a protective effect against colorectal adenomas for V511A and/or NSAID use (OR, 0.52; 95% CI, 0.31–0.86; P = 0.011; Table 4D). Additional studies are needed to confirm the result.

Possible effects of V511A, if any, were smaller in the Multiethnic Cohort Study than in the adenoma studies. For example, the OR for colorectal cancer was 1.19 when NSAID...
users were excluded. However, results are not inconsistent, because the CIs were wide and overlapped with those for the adenoma studies. These apparent differences may reflect differences between cancer and adenomas or different definitions of NSAID use. Alternatively, the results may indicate lack of a V511A effect.

Wiesner et al. (51) tested the PTGS2 locus for linkage to colon neoplasia by use of sib pairs. No evidence for linkage was

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<th>USC/Kaiser</th>
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<tr>
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<td>57 (48%)</td>
<td>12 (29%)</td>
<td>54 (45%)</td>
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<td>60.8</td>
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<td>V511A allele frequency (95% CI)</td>
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<td>0.036 (0.0074–0.10)</td>
<td>0.050 (0.026–0.085)</td>
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NSAID usage

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NSAID usage and V511A

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NSAID users versus nonusers

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<td>77</td>
<td>73</td>
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NSAID users and V511A+ and/or NSAID users versus V511A− and nonusers

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<th>USC/Kaiser</th>
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<td>77</td>
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NSAID users and/or V511A− and nonusers

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<td>Nonusers</td>
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<td>44</td>
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<tr>
<td>Both studies</td>
<td>38</td>
<td>74</td>
<td>87</td>
<td>92</td>
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a ORs were adjusted for age and gender.

b NSAID users were defined as subjects who took NSAIDs ≥1 time per week for some time during the 1 year (USC/Kaiser) or 5 years (UNC) before the interview. Data on NSAID use were missing for 29 subjects (21%) from the UNC study and for 60 subjects (25%) from the USC/Kaiser study.
Mechanisms by which PTGS2 and NSAIDs may affect colon cancer are being studied. Prescott and White (52) concluded that PTGS2 overexpression occurs after loss of both APC alleles and after formation of the early polyp in Min mice. They suggested that PTGS2 promotes tumors through prostaglandin receptor signaling. The prostaglandin receptor EP2 appears to be one of the mediators (53). Peroxisome proliferator-activated receptor γ (a nuclear receptor and tumor suppressor) may also be involved (54–58). Lower numbers of intestinal adenomas in Apc Min/H11001, cPLA2/H11002/H11002 mice, which lack the enzyme for the first step in prostanoid synthesis, also support a prostaglandin mechanism for tumorigenesis (59). Angiogenic factors (60) or c-MYB (61) may also play a role.

Each of the three studies used here to analyze the V511A polymorphism have specific strengths. For example, advantages of the sigmoidoscopic case-control study were screening asymptomatic individuals for a first-time diagnosis of adenomas and participation of >80% of cases and control individuals. Awareness by interviewers of case (30%) or control (13%) status was not expected to introduce bias, because interviewers were well trained, and NSAIDs were not a major topic of the interview. A weakness was that adenomas proximal to the left colon cannot be seen with sigmoidoscopy. Roughly 50% of colon cancers occur in the proximal segment (62). Studies show that up to 15–17% of control individuals who have no polyp within view of the sigmoidoscope may have a polyp in the right colon (63). However, our analyses included 140 patients who underwent full colonoscopy, in addition to the 240 subjects screened by sigmoidoscopy. Findings in the colonoscopy group were similar to those in the sigmoidoscopy group (Table 4). Furthermore, misclassification of controls because of incomplete colon screening by sigmoidoscopy would bias the study toward the null. Our results would actually underestimate the true effect of the PTGS2 variant under these circumstances.

Table 5

Subject characteristics (A), ORs\(^a\), and 95% CIs for colon cancer in relation to the V511A PTGS2 polymorphism (B, C, and D) for African-Americans in the Multiethnic Cohort Study

A. Subjects

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<th>Controls</th>
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<tbody>
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<td>Number of subjects</td>
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<td>258</td>
<td></td>
</tr>
<tr>
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<td>67 (48%)</td>
<td>102 (40%)</td>
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<tr>
<td>Male</td>
<td>71</td>
<td>156</td>
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<tr>
<td>Average age, years</td>
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<tr>
<td>V511A allele frequency</td>
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<td>0.041 (0.025–0.062)</td>
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<tr>
<td>Nonusers</td>
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B. V511A+ vs V511A–

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<td>V511A+</td>
<td>V511A–</td>
</tr>
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<tr>
<td>Excluding NSAID users</td>
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<td>73</td>
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C. NSAID users vs NSAID nonusers

<table>
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D. V511A+ and/or NSAID users vs V511A– and nonusers

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<td>V511A+</td>
<td>and/or users</td>
<td>V511A–</td>
<td>and nonusers</td>
</tr>
<tr>
<td>53</td>
<td>73</td>
<td>109</td>
<td>133</td>
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\(^a\) ORs were adjusted for age and gender.

\(^b\) NSAID users were defined as subjects who ever took NSAIDs or NSAID-containing medication ≥2 times per week for 2 years or longer, as indicated on the baseline questionnaire. Data on NSAID use and duration were missing for 28 subjects (7%).
Multiethnic Cohort Study should not be subject to selection biases that may be associated with colonoscopy referrals. In summary, in three large, ethnically diverse studies, we found the suggestion of an inverse association between a variant form of PTGS2 and colorectal neoplasia. Larger sample sizes are needed to assess our initial report of possible protection against colon neoplasia by the V511A polymorphism. The results are consistent with epidemiologic studies and may suggest the importance of PTGS2 inhibitors in reducing the risk of colorectal cancer.
Prostaglandin H Synthase 2 Variant (Val511Ala) in African Americans May Reduce the Risk for Colorectal Neoplasia
