Prostacyclin Synthase and *Arachidonate 5-Lipoxygenase* Polymorphisms and Risk of Colorectal Polyps

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Abstract

Prostacyclin synthase (PGIS) and arachidonate 5-lipoxygenase (ALOX5) are enzymes relevant to prostaglandin and leukotriene synthesis, both important pathways for colon cancer risk. We hypothesized that genetic variation altering the function of these enzymes would modify risk of colorectal polyps. In a Minnesota-based case-control study of adenomatous (n = 517) or hyperplastic (n = 192) polyps versus polyp-free controls (n = 618), we investigated the role of promoter repeat polymorphisms in *PGIS* and *ALOX5* as well as *ALOX5* −1700 G>A. Having fewer than six repeats on both *PGIS* alleles (<6R/<6R) was associated with an increased risk of adenomas compared with the 6R/6R (wild-type) genotype (OR, 1.90; 95% CI, 1.09-3.30). Having more repeats (>6R/≥6R) reduced risk (OR, 0.73; 95% CI, 0.40-1.35; P_{trend} = 0.03). In allele-based analyses, fewer repeats were associated with a modestly increased risk of adenomas and perhaps hyperplastic polyps. There were no risk differences for either the *ALOX5* VNTR or −1700 G>A polymorphisms. Associations with regular use of aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs) differed by *PGIS* genotype. Among individuals with at least one wild-type allele, NSAID use was associated with a decreased risk; however, those with fewer *PGIS* repeats (<6R/<6R) did not benefit ($P_{interaction}$ = 0.06). There was also evidence of an interaction between the *COX-2* −765 G>C and *ALOX5* −1700 G>A genotypes ($P_{interaction}$ = 0.07). The *PGIS* promoter polymorphism may affect risk of colorectal polyps and modify the effects of NSAID use on polyp risk. A more comprehensive investigation of genetic variability in prostaglandin synthesis in relation to risk of colorectal neoplasia and NSAID pharmacogenetics is warranted. (Cancer Epidemiol Biomarkers Prev 2006;15(3):502–8)

Introduction

Arachidonic acid is metabolized by the colon through two main pathways, the prostaglandin and leukotriene cascades (see Fig. 1; ref. 1). Prostaglandins are produced by most cells in the body and participate in inflammatory responses and other cellular activities (2). The central enzymes in the prostaglandin pathway are cyclooxygenases 1 and 2 (COX-1 and COX-2), which form prostaglandin H_2, the precursor of all other prostaglandins (2).

Prostaglandins have been found to be up-regulated in colon cancer (3-5) and genetic polymorphisms in both *COX-1* and *COX-2* have previously been associated with colon neoplasia risk by our group and others (6-10). COX-1 and COX-2 are the primary targets of nonsteroidal anti-inflammatory drugs (NSAIDs; ref. 11), which are known to prevent colorectal neoplasia (12-15).

Although COX-1 and COX-2 have been the focus of much research, the effects of genetic variants in other enzymes in the prostaglandin pathway have not been as thoroughly examined. One of these enzymes, prostaglandin I_2 synthase (prostacyclin synthase or PGIS), acts downstream of COX-1 and COX-2 to catalyze the formation of prostaglandin H_2 into prostaglandin I_2 (or prostacyclin; see Fig. 1). Prostacyclin may play a number of roles in preventing cancer, including suppression of inflammation and cell proliferation, promotion of apoptosis, prevention of metastasis, and reduced growth of established metastases (16-19). The cardiovascular toxicity caused by COX-2-specific inhibitors may be in part due to reduced production of prostacyclin, which has antithrombotic activity (20). As the enzyme responsible for the production of prostacyclin, PGIS may be important in cancer prevention. PGIS is expressed in a wide array of tissues, including ovaries, heart, skeletal muscle, lung, and prostate, suggesting that PGIS has a variety of functions (21).

Prostacyclin levels can be reduced in colorectal cancer (4). A study of murine colon adenocarcinomas reported reduced tumor incidence and burden in transgenic mice engineered to overexpress *PGIS* (22). *In vitro* studies of human colon cancer cell lines have shown evidence that increased levels of prostacyclin may prevent metastases (23). In addition, several studies have examined the role of PGIS in lung cancer with comparable results, reporting reduced tumor incidence and burden among mice overexpressing *PGIS* (24, 25) and no expression of both *PGIS* or prostacyclin in human lung tumors (26).

Another major pathway for the metabolism of arachidonic acid is via its conversion by lipoxygenases [5-lipoxygenase (ALOX5), ALOX12, and ALOX15] to other signaling molecules, specifically to leukotrienes via ALOX5, to 12-hydroperoxyeicosatetrenoic (12-HPETE) via ALOX12, and to 15-HPETE via ALOX15. ALOX5 and ALOX12 have been described as procarcinogenic lipoxygenases and are both up-regulated in several cancer types whereas ALOX15 seems to be anticarcinogenic, possibly through its role in the metabolism of linoleic acid or through inhibition of the products of the ALOX5 and ALOX12 cascades (27).

Arachidonate 5-lipoxygenase (ALOX5) is a dual-function protein that catalyzes the oxygenation of arachidonate to 5-HPETE and its subsequent conversion to leukotriene A4 (28). Downstream products of ALOX5 have been shown to enhance cell proliferation and increase cell survival (29). In addition, ALOX5 is up-regulated in colon cancer (27, 30), as well as several other cancer types, including esophageal (31), breast (32), prostate (33), and pancreatic cancers (34). ALOX5

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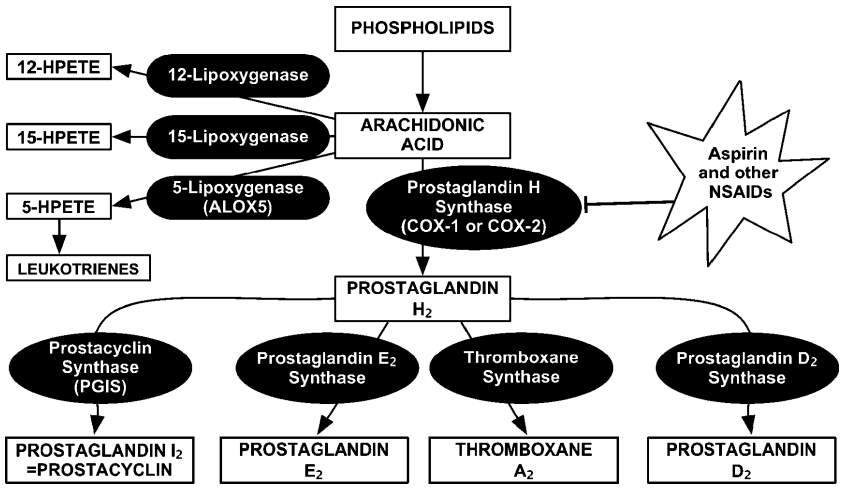

Figure 1. ALOX5 and PGIS in the prostaglandin and eicosanoid synthesis pathway.

has also been shown to play a role in angiogenesis in mouse models of cigarette smoke–induced inflammatory tumorigenesis (35).

Genetic polymorphisms in both *PGIS* and *ALOX5* have been identified. A 9-base variable number of tandem repeat (VNTR) polymorphism [−3(CCAGCCCCG)3-8] has been identified in the promoter region of *PGIS* (originally designated *CYP8A1*; refs. 36, 37). In luciferase assays, alleles with fewer repeats (3R, 4R, or 5R) were associated with reduced promoter activity compared with wild-type (6R)—the 4R allele had 3-fold lower promoter activity and the 5R allele had 2-fold lower activity compared with 6R in three cell lines exposed to interleukin-6 stimulation (36, 37). The *PGIS* VNTR polymorphism has been associated with cerebral infarction and hypertension (36, 38).

In the promoter region of *ALOX5*, a 6-base VNTR polymorphism [−176(GGGCGG)2-8] and a single-nucleotide polymorphism (−1700 G>A) have been identified (39). In promoter assays in two cell lines, the 3R allele of the VNTR polymorphism was associated with a 40% to 78% reduction in activity compared with wild-type (5R). The *ALOX5* 4R allele was associated with a 30% reduction whereas the 6R allele was associated with 20% lower activity in one cell line and a 60% increase of activity in another (39, 40). The effects of the *ALOX5* −1700 G>A polymorphism are unclear. The *ALOX5* VNTR polymorphism has not yet been investigated in relation to colorectal cancer whereas the −1700 G>A polymorphism has previously been associated with a decreased risk of colon cancer (8).

Due to the associations of both PGIS and ALOX5 with various types of cancer, we hypothesized that genetic variation in *PGIS* and *ALOX5* could alter the risk of colorectal neoplasms. We investigated associations between the *PGIS* VNTR polymorphism and the two *ALOX5* polymorphisms and risk of colorectal adenomatous and hyperplastic polyps. We further investigated possible interactions with aspirin or other NSAID use and polymorphisms in *COX-1, COX-2*, or *TGF-β1* (an inducer of COX-2; ref. 41).

Materials and Methods

Study Subjects. Participant recruitment for the Minnesota case-control study has previously been described (42). Briefly, cases with colorectal adenomatous and/or hyperplastic polyps and polyp-free controls were recruited through a large multiclinic private gastroenterology practice in metropolitan Minneapolis. Patients of ages 30 to 70 years, who were scheduled to undergo colonoscopy between April 1991 and April 1994, were enrolled before colonoscopy to blind patients and recruiters to the final diagnosis. The internal review boards of the University of Minnesota and each endoscopy site approved this study. Written informed consent was obtained from all participants.

Eligible participants were Twin Cities metropolitan area residents, English-speaking, had no known genetic syndrome associated with predisposition to colonic neoplasia, and had no individual history of cancer (except nonmelanoma skin cancer) or inflammatory bowel disease. Cases had a first diagnosis of colon or rectal adenomatous (n = 521) or hyperplastic polyp (n = 194) at the time of colonoscopy whereas controls were polyp-free (n = 621). Patients for whom colonoscopy did not reach the cecum were excluded. Removed polyps were examined histologically according to standard diagnostic criteria (43).

Information on use of aspirin and other NSAIDs, lifestyle factors and diet, demographics, anthropometry, and medical information, including family history of cancer and polyps, was obtained by questionnaire. The participation rate for all colonoscoped patients was 68%.

Genotyping. Genomic DNA was extracted from peripheral WBC using the Puregene kit (Gentra Systems, Minneapolis, MN). *PGIS* genotyping was done at the Core Laboratory of the Public Health Sciences Division of the Fred Hutchinson Cancer Research Center (J.B.). The promoter repeat polymorphisms of *ALOX5* and *PGIS* were genotyped using GeneScan (Applied Biosystems, Foster City, CA) assays and the *ALOX5* −1700 G>A polymorphism was genotyped by TaqMan assay (Applied Biosystems). The *ALOX5* VNTR reactions consisted of 40 ng genomic template DNA, 1.5 mmol/L $MgCl_2$, 1× PCR Gold Buffer (Applied Biosystems), 0.5 units of Amplitaq Gold Polymerase (Applied Biosystems), 200 nmol/L each of oligonucleotide primers ALOX5sp1-F 5′-6FAM-AGGAACAGACACCTCGCTGAGGAGAG-3′ and ALOX5sp1-R 5′-GAGCAGCGAGCGCCGGGAGCCTCGGC-3′ (ref. 44; Applied Biosystems), 150 μmol/L each of dATP, dCTP, and dTTP, 75 μmol/L each of dGTP and 7-deaza-2′-dGTP (Roche Diagnostics GmbH, Mannheim, Germany), and 8% (v/v) DMSO (Sigma, St. Louis, MO) in 20 μL. Cycling was at 94°C for 10 minutes, followed by 30 cycles of 94°C for 30 seconds, 62°C for 45 seconds, 72°C for 1 minute, and finally 72°C for 5 minutes. The observed amplicon lengths ranged from 257 bp (2 repeats) to 293 bp (8 repeats). The *PGIS* 9-base promoter repeat reactions consisted of 40 ng genomic template DNA, 1.2 mmol/L $MgCl_2$, 1× PCR Gold Buffer (Applied Biosystems), 0.5 units of Amplitaq Gold Polymerase (Applied Biosystems), 200 nmol/L each of oligonucleotide primers PGIS-F 5′-6FAM-CACATTTTCCATCAGGCCTGAGCTG-3′ and PGIS-R 5′-GGGATACTGGAGCGGGACTCGG-3′ (Applied Biosystems), 150 μmol/L each of dATP, dCTP, and dTTP, 75 μmol/L each of dGTP and 7-deaza-2′-dGTP (Roche), 2% (v/v) formamide (Applied Biosystems), and 6% (v/v) DMSO (Sigma) in 20 μL.

Cycling was at 94°C for 15 minutes, followed by 35 cycles of 94°C for 30 seconds, 64°C for 45 seconds, 72°C for 1 minute, and finally 72°C for 5 minutes. The observed amplicon lengths ranged from 411 bp (3 repeats) to 456 bp (8 repeats). Amplicons from both reactions were combined and run on the ABI 3100 Genetic Analyzer. Data were analyzed with Genotyper software version 3.7 and manually reviewed. The *ALOX5* −1700 G>A polymorphism was genotyped using a TaqMan assay in which 3.75 ng of genomic DNA were amplified with a TaqMan Core Reagent Kit with AmpErase UNG (Applied Biosystems). The 20-μL reactions were 5.0 mmol/L $MgCl_2$, 100 nmol/L each of oligonucleotide primers ALOX5 −1700F 5′-CCCCAAATTGATCTACAGCTTCA-3′ and ALOX5 −1700R 5′-TTGTCCATGCATCATTTGTTGA-3′, and 200 nmol/L each of antisense oligonucleotide probes with nonfluorescent MGB quencher, ALOX5 −1700G 5′VIC-TAG-TCCACTGATCTGTAAT-3′ and ALOX5 −1700A 5′-6FAM-CTAGTCCATTGATCTGTAA-3′, and a 360-bp amplicon was created. Cycling consisted of 2-minute incubation at 50°C, 10-minute denaturation at 95°C, and 45 cycles of 95°C for 15 seconds and 60°C for 3 minutes. All cyclings were done on Perkin-Elmer Gene Amp PCR System 9700. All results were repeated for 94 randomly selected samples for quality control, with no discrepancies. All results were confirmed in a separate previously described population (45).

Statistical Data Analysis. Logistic regression analysis was used to estimate odds ratios (ORs) and corresponding 95% confidence intervals (95% CI); *PGIS* and *ALOX5* status, both specific genotypes and alleles (number of repeats on one allele), among cases with adenomatous or hyperplastic polyps was compared with polyp-free controls. Multivariate adjustment for previously identified risk factors (age, sex, body mass index, dietary intakes of fiber, alcohol, energy, postmenopausal hormone use, and smoking) was used largely because of some confounding in the stratified analyses. Possible effect modification by NSAID use or polymorphisms in *COX-1, COX-2*, or *TGF-β1* was evaluated by including the respective multiplicative interaction term in the logistic regression models. All statistical tests were two sided and analyses were done with SAS 9.1 (SAS Institute, Cary, NC).

Results

Characteristics of the study population have previously been described (42, 46, 47). Briefly, adenoma cases were older than those with hyperplastic polyps and polyp-free controls and were more likely to be male. Regular use of aspirin or other NSAIDs was somewhat more common among controls compared with both case groups. To evaluate risk based on specific genotypes present in the population, the *PGIS* and *ALOX5* repeat polymorphism genotypes were grouped according to putative phenotype (amount of gene expression relative to wild-type). Genotype and allele frequencies among case groups and controls are listed in Table 1; phenotypic groupings are shown in Table 2. All polymorphisms were in Hardy-Weinberg equilibrium in both cases and controls.

ORs for the *PGIS* and *ALOX5* genotypes and alleles are shown in Table 2. For the *PGIS* polymorphism, having fewer than 6 repeats on both alleles (<6R/<6R) was associated with an increased risk of adenomas compared with the 6R/6R (wild-type) genotype (OR, 1.90; 95% CI, 1.09-3.30). Although not statistically significant, this pattern of an increased risk with fewer repeats was also seen for hyperplastic polyps (OR, 1.86; 95% CI, 0.91-3.81). Having more repeats [>6R/≥6R, i.e., at least 6 (= wild-type) repeats on one allele and more than 6 on the other] was associated with a nonsignificantly decreased risk of adenomas (OR, 0.73; 95% CI, 0.40-1.35). There was a statistically significant trend of decreasing adenoma risk with increasing number of *PGIS* repeats (P = 0.03). Associations in the allele-based analysis were more modest, although the trends were similar (P_{trend} = 0.04), such that the *PGIS* allele with 4 repeats was associated with an increased adenoma risk (OR, 1.30; 95% CI, 1.01-1.68). Similar results were observed for hyperplastic polyps.

There were no significant differences in polyp risk for either of the *ALOX5* polymorphisms. For the *ALOX5* VNTR polymorphism, carrying an allele with any number of repeats other than wild-type (5 repeats) was associated with decreased risk of both adenomas and hyperplastic polyps, but these results were not statistically significant and no clear trends were observed (Table 2).

Regular aspirin or other NSAID use (>1/wk for at least 1 year) has previously been associated with a decreased risk of adenomas in this study population (for aspirin, OR, 0.63; 95% CI, 0.44-0.90; for other NSAIDs, OR, 0.50; 95% CI, 0.31-0.82; ref. 47). Because the primary targets of NSAIDs are the COX-1 and COX-2 enzymes in the initial synthesis of prostaglandins from arachidonic acid, we evaluated whether these protective associations were altered by *PGIS* or *ALOX5* genotype.

The associations with regular use of aspirin or other NSAIDs differed by *PGIS* genotype (Fig. 2; Table 3). Among individuals

Table 1. Genotype and allele frequencies among adenoma cases, hyperplastic polyp cases, and controls

	Adenomas *n* (%)	Hyperplastic polyps *n* (%)	Controls *n* (%)
PGIS VNTR polymorphism—genotype*			
3/6 repeats	0 (0)	1 (1)	7 (1)
4/4 repeats	16 (3)	4 (2)	13 (2)
4/5 repeats	20 (4)	8 (4)	12 (2)
4/6 repeats	120 (23)	44 (23)	135 (22)
4/7 repeats	5 (1)	0 (0)	5 (1)
5/5 repeats	4 (1)	2 (1)	4 (1)
5/6 repeats	67 (13)	26 (13)	81 (13)
5/7 repeats	5 (1)	0 (0)	1 (0)
6/6 repeats (wild-type)	255 (49)	94 (48)	332 (53)
6/7 repeats	21 (4)	13 (7)	33 (5)
6/8 repeats	2 (1)	0 (0)	0 (0)
7/7 repeats	1 (0)	0 (0)	2 (0)
PGIS VNTR polymorphism—allele*			
3 repeats	0 (0)	1 (0)	0 (0)
4 repeats	177 (17)	60 (16)	178 (14)
5 repeats	100 (10)	38 (10)	102 (8)
6 repeats	720 (70)	274 (71)	913 (74)
7 repeats	33 (3)	13 (3)	43 (3)
8 repeats	2 (0)	0 (0)	0 (0)
ALOX5 VNTR polymorphism—genotype*			
2/5 repeats	1 (0)	1 (1)	0 (0)
3/3 repeats	1 (0)	0 (0)	4 (1)
3/4 repeats	3 (1)	0 (0)	1 (0)
3/5 repeats	5 (1)	0 (0)	8 (1)
4/4 repeats	13 (2)	6 (3)	15 (2)
4/5 repeats	143 (27)	53 (27)	184 (29)
4/6 repeats	2 (0)	0 (0)	6 (1)
5/5 repeats (wild-type)	336 (64)	126 (65)	382 (61)
5/6 repeats	15 (3)	6 (3)	16 (3)
5/7 repeats	3 (1)	1 (1)	4 (1)
5/8 repeats	0 (0)	0 (0)	1 (0)
ALOX5 VNTR polymorphism—allele*			
2 repeats	1 (0)	1 (0)	0 (0)
3 repeats	10 (1)	0 (0)	17 (1)
4 repeats	174 (17)	67 (17)	221 (18)
5 repeats	839 (80)	313 (81)	977 (79)
6 repeats	17 (2)	6 (2)	22 (2)
7 repeats	3 (0)	1 (0)	4 (0)
8 repeats	0 (0)	0 (0)	1 (0)
ALOX5 −1700 G>A			
GG	357 (69)	135 (70)	416 (67)
GA	150 (29)	53 (27)	192 (31)
AA	14 (3)	6 (3)	16 (3)

*N for genotype-based analysis is the number of subjects, whereas for allele-based analysis N is the number of alleles (i.e., twice the number of subjects).

Table 2. Association between *PGIS* and *ALOX5* polymorphisms and risk of adenomatous and hyperplastic polyps

	Controls (*N*)	Adenomas		Hyperplastic polyps	
		Cases (*N*)	OR (95% CI)	Cases (*N*)	OR (95% CI)
PGIS VNTR polymorphism—genotype*					
<6/<6 repeats†	29	40	1.90 (1.09-3.31)	14	1.86 (0.91-3.81)
<6/6 repeats‡	216	187	1.07 (0.80-1.42)	70	1.11 (0.75-1.63)
6/6 repeats (reference)	332	255	1.00 (reference)	95	1.00 (reference)
>6/≥6 repeats§	35	24	0.74 (0.40-1.35)	13	1.25 (0.59-2.65)
			P_{trend} = 0.03		P_{trend} = 0.3
<6/>6 repeats‖ (mixed)	6	10	2.47 (0.79-7.71)	0	— (—)
PGIS VNTR polymorphism—allele*					
4 repeats	178	177	1.30 (1.01-1.68)	60	— (—)
5 repeats	102	100	1.19 (0.86-1.64)	38	1.32 (0.86-2.03)
6 repeats (reference)	913	720	1.00 (reference)	273	1.00 (reference)
7 repeats	43	33	0.89 (0.51-1.53)	13	0.98 (0.48-1.99)
8 repeats	0	2	— (—)	0	— (—)
			P_{trend} = 0.04		P_{trend} = 0.36
ALOX5 VNTR polymorphism—genotype*					
<5/≤5 repeats¶	213	167	0.85 (0.64-1.12)	61	0.75 (0.51-1.10)
5/5 repeats (reference)	382	337	1.00 (reference)	126	1.00 (reference)
5/>5 repeats**	21	18	0.70 (0.33-1.47)	7	0.85 (0.32-2.26)
			P_{trend} = 0.5		P_{trend} = 0.2
ALOX5 VNTR polymorphism—allele*					
2 repeats	0	1	— (—)	1	— (—)
3 repeats	17	10	0.60 (0.20-1.79)	0	— (—)
4 repeats	221	174	0.90 (0.70-1.14)	67	0.85 (0.60-1.21)
5 repeats (reference)	977	839	1.00 (reference)	313	1.00 (reference)
6 repeats	22	17	0.65 (0.33-1.26)	6	0.74 (0.26-2.07)
7 repeats	4	3	0.90 (0.18-4.49)	1	0.73 (0.09-5.90)
8 repeats	1	0	— (—)	0	— (—)
			P_{trend} = 0.58		P_{trend} = 0.31
ALOX5 −1700 G>A*					
GG (reference)	416	357	1.00 (reference)	135	1.00 (reference)
GA	192	150	0.87 (0.66-1.16)	53	0.74 (0.50-1.09)
AA	16	14	1.05 (0.46-2.37)	6	1.05 (0.36-3.10)
			P_{trend} = 0.5		P_{trend} = 0.3

NOTE: Multivariate adjustment for age, sex, smoking (pack-years), alcohol, hormone use, caloric intake, body mass index, and fiber intake.
*N for genotype-based analysis is the number of subjects, whereas for allele-based analysis N is the number of alleles (i.e., twice the number of subjects).
†Includes 4/4, 4/5, and 5/5 genotypes.
‡Includes 4/6 and 5/6 genotypes.
§Includes 6/7, 6/8, and 7/7 genotypes.
‖Includes 4/7 and 5/7 genotypes.
¶Includes 2/2, 3/3, 3/4, and 4/5 genotypes.
**Includes 5/6, 5/7, and 5/8 genotypes.

with at least one wild-type allele, NSAID use was associated with a decreased risk; in contrast, among those with fewer repeats (<6R/<6R), there was no reduction in risk ($P_{interaction}$ = 0.06; Table 3). In a direct comparison (i.e., excluding all other genotype groups) of individuals with fewer repeats on both alleles (<6R/<6R; lower *PGIS* expression) compared with those who were wild-type (6R/6R), there was a statistically significant interaction (P = 0.03), suggesting that individuals with reduced *PGIS* expression may not benefit from NSAID use. There was no statistically significant interaction between either of the *ALOX5* polymorphisms and regular NSAID use.

To elucidate the combined effects of genetic variability in the prostaglandin synthesis pathway (see Fig. 1), we investigated gene-gene interactions between *PGIS* and *ALOX5* and polymorphisms in *COX-1* (R8W, P17L, L15-L16del, and L237M), *COX-2* (−765 G>C), and *TGF-β1* (L10P). There is evidence for the functional relevance of these polymorphisms (48-51) and we have previously shown that they can alter risk of polyps or modify NSAID associations (6, 7, 52). No statistically significant interactions between *PGIS* or *ALOX5* and *TGF-β1* or *COX-1* were observed, nor was there evidence for an interaction between *PGIS* and *COX-2*. Due to the low allele frequencies, in particular for *COX-1* polymorphisms, our statistical power was limited. However, there was a suggestion of an interaction between the *COX-2* promoter polymorphism (−765 G>C) and *ALOX5* −1700 G>A (Table 4). Among those who had the wild-type *ALOX5* genotype, variant *COX-2* alleles were associated with a decrease in adenoma risk; among those with *ALOX5* GA or AA genotype, adenoma risk increased with variant *COX-2* alleles ($P_{interaction}$ = 0.07).

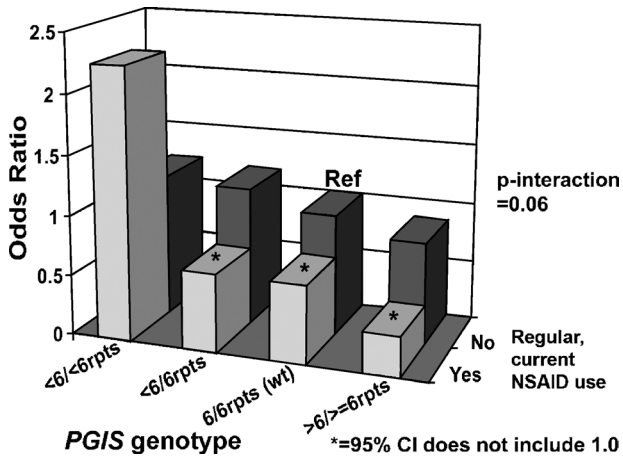

Figure 2. Risk of colorectal adenomas by *PGIS* VNTR genotype and NSAID use.

Table 3. Association between *PGIS* genotype and risk of adenomatous polyps, stratified by NSAID use

	Aspirin or other NSAID use*					
	No			Yes		
	Controls (*N*)	Cases (*N*)	OR (95% CI)	Controls (*N*)	Cases (*N*)	OR (95% CI)
PGIS VNTR polymorphism						
<6/<6 repeats†	19	20	1.19 (0.57-2.49)	10	20	2.26 (0.97-5.26)
<6/6 repeats‡	115	118	1.15 (0.79-1.67)	101	69	0.65 (0.43-0.98)
6/6 repeats (reference)	188	164	1.0 ref.	144	92	0.65 (0.44-0.95)
>6/≥6 repeats§	19	8	0.84 (0.39-1.79) P_{trend} = 0.3	16	6	0.33 (0.11-0.99) P_{trend} = 0.03
Mixed‖	3	0	— (—)	3	3	1.42 (0.22-9.15) $P_{interaction}$ = 0.06

NOTE: Multivariate adjustment for age, sex, smoking (pack-years), alcohol, hormone use, caloric intake, body mass index, and fiber intake.
*Regular aspirin and/or other NSAID use is defined as >1/wk for at least 1 year.
†Includes 4/4, 4/5, and 5/5 genotypes.
‡Includes 4/6 and 5/6 genotypes.
§Includes 6/7, 6/8, and 7/7 genotypes.
‖Includes 4/7 and 5/7 genotypes.

Discussion

This study illustrates that genetic variability in enzymes relevant to the formation of prostaglandins and other eicosanoids can affect the risk of colorectal neoplasia and be potentially relevant for the pharmacogenetics of NSAIDs. We hypothesized that individuals with fewer *PGIS* repeats would be at increased risk of colorectal polyps given that increasing numbers of repeats have been associated with increasing *PGIS* promoter activity. As discussed previously, mouse genetic and human association studies suggest a cancer-preventive role of prostacyclin (36-38). Our findings, showing an association between fewer *PGIS* repeats and polyp risk, support a possible role of prostacyclin in colon carcinogenesis and are consistent with the findings of previous studies and proposed biological mechanisms (24). In addition, a recent study showed that tissue samples from colon cancer and adenoma cases had hypermethylated *PGIS* promoter regions compared with normal colon tissue, further supporting the hypothesis that *PGIS* may play a role in cancer prevention (53).

Our results suggest that the risk reduction afforded by NSAID use varies by *PGIS* genotype. Specifically, we observed that only individuals with at least one wild-type allele showed a reduced risk with regular NSAID use. This may be an important observation pharmacogenetically because it is an indication that not all individuals benefit from regular NSAID use. Our preliminary finding of an increased risk of adenoma and hyperplastic polyps among those with the <6/<6 genotype who used aspirin or other NSAIDs warrants further investigation.

NSAID pharmacogenetics is complex and seems to involve central enzymes in prostaglandin synthesis (6, 7) as well as the metabolism of NSAIDs (47). In light of recent concerns of toxicity associated with COX-2-specific inhibitors, genetic studies may be important to predict who is more likely to benefit from COX-2-specific inhibitor use and who is more prone to cardiovascular side effects.

To our knowledge, this is the first study of the *ALOX5* VNTR polymorphism in relation to risk of colorectal neoplasia. We hypothesized that fewer repeats would be associated with decreased risk of colorectal polyps because fewer repeats have been associated with lower gene expression and *ALOX5* has procarcinogenic properties (27, 39, 40). Although not statistically significant, our findings are consistent with this hypothesis.

One other group has evaluated the *ALOX5* −1700 G>A polymorphism in relation to colon cancer risk, reporting that, among Caucasians, both the GA and AA genotypes were associated with a decreased risk of colon cancer, although this was not statistically significant among those with the AA genotype (8). We did not observe these patterns. Our study provided some evidence that the effects of regular NSAID use may differ by *ALOX5* genotype; however, several of the categories were quite small and we may not have had adequate statistical power to detect a true association.

We found evidence that the association with the *ALOX5* −1700 G>A polymorphism may differ by *COX-2* −765 G>C genotype. Both ALOX5 and COX-2 use arachidonic acid and an interaction due to ''substrate shunting'' is possible. The *COX-2* −765 G>C polymorphism is associated with decreased gene expression (48, 54). Our findings suggest that a comprehensive analysis of genetic variability in this pathway in relation to colorectal neoplasia is warranted.

For comparison, we performed both genotype-based and allele-based analyses for the *PGIS* and *ALOX5* repeat polymorphisms. Whereas a genotype corresponds to the biological entity of the cell, containing two chromosomes, allele-based

Table 4. *ALOX5* genotype, *COX-2* −765 G>C genotype, and risk of adenomatous polyps

	COX-2 (−765 G>C)								
	GG			GC			CC		
	Controls (*N*)	Cases (*N*)	OR (95% CI)	Controls (*N*)	Cases (*N*)	OR (95% CI)	Controls (*N*)	Cases (N)	OR (95% CI)
ALOX5 −1700 G>A									
GG (reference)	260	239	1.00 ref.	111	94	0.88 (0.61-1.28)	14	6	0.36 (0.13-1.02)
GA or AA	145	103	0.73 (0.52-1.02)	47	44	0.93 (0.57-1.53)	5	4	1.37 (0.25-7.39) $P_{interaction}$ = 0.07

NOTE: Multivariate adjustment for age, sex, smoking (pack-years), alcohol, hormone use, caloric intake, body mass index, and fiber intake.

analyses are increasingly used in haplotype analyses (e.g., the effects of one haplotype are explored without taking the coexisting second haplotype into account). Our results for the *PGIS* promoter polymorphism illustrate that risk patterns are similar regardless of which approach is used. However, the genotype-based analysis reveals much stronger increases in risk for individuals who carry fewer than 6 repeats on both alleles (<6R/<6R, OR, 1.90; 95% CI, 1.09-3.30) than does the allele-based analysis (OR, 1.30 for 4 repeats; OR, 1.19 for 5 repeats). This suggests that an allele-based approach may fail to fully capture the biological significance of two risk alleles within one cell and that associations may be diluted due to a mixing of effects. The effect of one allele is likely to depend on the characteristics of the second allele of the cell and only a genotype-based approach can capture this information.

One limitation of this study is that the relatively small number of cases with hyperplastic polyps reduced the statistical power to detect associations, especially in stratified analyses. In general, the patterns observed in the hyperplastic polyps agree with the patterns observed among the adenomas. Larger sample sizes would detect potential risk differences in this group with more precision.

Further, the promoter polymorphism we have investigated in this study is not the only identified genetic variant in *PGIS*. One common synonymous single-nucleotide polymorphism (R373R) and six rare coding mutations have been reported (37, 55). However, these rare mutations had minor allele frequencies of <1%. We chose to use the promoter polymorphism because it has functional relevance, has been associated with other diseases, and is the best characterized of the known *PGIS* mutations.

Similarly, there are several known polymorphisms in *ALOX5*, including three synonymous coding single-nucleotide polymorphisms (T7T, T90T, and P576P) and one nonsynonymous coding single-nucleotide polymorphism (E254K; refs. 8, 39). We focused our analyses on *ALOX5* polymorphisms likely to have functional effect. Another promoter single-nucleotide polymorphism, −1753 G>A, is known to be in tight linkage disequilibrium with −1700 G>A and has also been associated with a decreased risk of colon cancer (8). Due to the linkage disequilibrium between these two single-nucleotide polymorphisms, the evaluation of either one should be sufficient.

In summary, results from the present study suggest that genetic variability in *PGIS* alters risk of colorectal polyps; the increased risks observed with fewer repeats are consistent with a cancer-preventive role of prostacyclin. In addition, risks associated with NSAID use differed by *PGIS* genotype. This result could have pharmacologic implications, indicating that there are genetically defined subgroups that are more likely to benefit from NSAID chemoprevention, a finding that is consistent with our past reports (6, 7, 47). Both *PGIS* and *ALOX5* may interact with *COX-2*, indicating that the aggregation of genetic variation in eicosanoid synthesis may be relevant for colorectal neoplasia. Finally, our results emphasize the necessity to go beyond an allele-based approach and consider genotypes (e.g., the combination of two alleles) to better define risk patterns in studies of molecular epidemiology.

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