Short Communication

Polymorphisms in *PTGS1 (=COX-1)* and Risk of Colorectal Polyps

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Abstract

Two isoforms of prostaglandin H synthase (PTGS = COX) are key enzymes in prostaglandin synthesis and primary targets for aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs). Use of aspirin or other NSAIDs is associated with a lower risk and reduced recurrence of colorectal adenomas, established precursors of adenocarcinoma. This study investigated risk of colorectal adenomatous and hyperplastic polyps associated with several polymorphisms in the coding region of *PTGS1*. Within the Minnesota polyp case-control study, patients with colorectal adenomatous (n = 521) or hyperplastic (n = 194) polyps and n = 621 polyp-free controls were genotyped for four *PTGS1* polymorphisms (R8W, L15-L16del, P17L, L237M); these had been predicted to affect protein function based on sequence-homology software. Age- and sex-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were computed. Whereas there was no appreciable difference in adenoma or hyperplastic polyp risk associated with R8W, P17L, and L237M, an increased risk was observed for individuals heterozygous for the L15-L16del polymorphism (OR = 3.6, 95% CI 1.2–11.2). The variant L15-L16del allele appeared to be associated with a stronger increase in adenoma risk among nonusers of aspirin/other NSAIDs. The reduced risk observed with aspirin/other NSAID use was limited to those wild type for P17L [PP users: OR = 0.6 (0.5–0.8) *versus* PP nonusers: 1.0 (referent) (P interaction = 0.03)]. To our knowledge, this study represents the first investigation of polymorphisms in *PTGS1* and risk of colorectal polyps. The L15-L16del variant allele may result in an increased risk of colorectal adenomas, whereas P17L may be relevant to the pharmacogenetics of aspirin. These preliminary findings require confirmation in larger studies of colorectal neoplasia. (Cancer Epidemiol Biomarkers Prev 2004;13(5):889–93)

Introduction

Prostaglandin H synthase (PTGS) catalyzes the formation of prostaglandin G_2, and, through its peroxidase activity, prostaglandin H_2 (1). The latter serves as the precursor for a number of important prostanoids, including prostaglandin E_2. Two PTGS isoforms, PTGS1 and PTGS2, have been well characterized and share approximately 60% homology. PTGS1 is constitutively expressed and appears to be important in maintaining prostanoid levels for ''housekeeping'' functions; PTGS2 is inducible and plays a critical role in many inflammatory responses (2). There is strong evidence that use of PTGS inhibitors, such as aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs), is associated with a reduced risk of colorectal polyps and cancer (3, 4), and decreases the recurrence of colorectal adenomas (5, 6). There is also some evidence connecting regular NSAID use to lower risks of cancers of the digestive tract, breast, and lung cancer (3, 4, 7–12). Evidence from mouse experiments implicates not only PTGS2, but also PTGS1 in colorectal carcinogenesis (13, 14), potentially through effects on prostaglandin E_2 levels (15, 16). We recently identified several polymorphisms in the coding region of *PTGS1* (17), predicted that several of these may affect protein function based on sequence homology software applications (R8W, P17L, L237M), and show here that some of these variants affect risk of colorectal adenomas, possibly in interaction with aspirin or other NSAID use.

Methods

Study Subjects. Participant recruitment for this case-control study has been described previously (18). Briefly, cases with colorectal adenomatous and/or hyperplastic polyps and polyp-free control subjects were recruited through a large multiclinic private gastroenterology practice in metropolitan Minneapolis. Patients aged 30–74 years who were scheduled for a

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colonoscopy between April 1991 and April 1994 were recruited before colonoscopy so as to blind patients and recruiters to the final diagnosis. The study was approved by the internal review boards of the University of Minnesota and each endoscopy site. Written informed consent was obtained.

Cases were identified as meeting eligibility criteria (see Ref. 18) and having a first diagnosis of colon or rectal adenomatous (n = 521) or hyperplastic polyp (n = 194) at the time of the colonoscopy. Control subjects were free of polyps during colonoscopy (n = 621). Patients for whom the colonoscopy did not reach the cecum were ineligible; removed polyps were examined histologically using standard diagnostic criteria (19). Information on use of aspirin and NSAIDs, lifestyle factors and diet, anthropometry, demographics, and medical information, including family history of cancer and polyps, were obtained by questionnaire. The participation rate for all colonoscoped patients was 68%.

Genotyping. Genomic DNA was extracted from peripheral white blood cells using the Puregene kit (Gentra Systems, Minneapolis, MN). *PTGS1* genotyping was performed at the Core Laboratory of the Public Health Sciences Division of the Fred Hutchinson Cancer Research Center (J.B.). RFLP was used to genotype the *PTGS1* R8W polymorphism as described (17). Genotyping of the P17L, and L237M polymorphisms was performed by sequencing, which resulted in the discovery of a new polymorphism, L15-L16del (17). For quality control purposes, 94 randomly selected samples were genotyped twice for each polymorphism. There were no discrepancies. The genotype frequencies did not deviate from Hardy-Weinberg equilibrium at the 0.05% significance level.

Statistical Data Analysis. Logistic regression analysis was used to estimate odds ratios (ORs) and corresponding 95% confidence intervals (CIs) comparing cases (with adenomatous or hyperplastic polyps), to polyp-free controls in association with *PTGS1* genotypes, adjusting for age and sex. Multivariate adjustment by previously identified risk factors (body mass index, race, physical activity, dietary intakes of alcohol, fiber, or kilocalories, hormone replacement therapy, or smoking) did not alter the ORs appreciably (data not shown). For evaluating interaction between *PTGS1* polymorphisms and NSAID use, the respective interaction terms were included in the logistic regression models. All statistical tests were two sided and analyses were undertaken with SAS 8.02 (SAS Institute, Cary, NC).

Haplotype frequencies were estimated using the expectation maximization algorithm as implemented in the program EH (20). Permutation testing (1000 replications) was used to evaluate the significance of (*a*) differences between observed and expected haplotype frequencies in controls, and (*b*) differences between observed haplotype frequencies in cases and controls (21).

Results

Characteristics of the study population and risk factors for colorectal polyps have been described previously (18, 22–24). Briefly, adenoma cases were older than individuals with hyperplastic polyps or polyp-free controls and more likely to be male.

We investigated four polymorphisms within the coding region of *PTGS1*, R8W (variant allele frequency among controls = 0.07), L15-L16del (allele frequency = 0.01), P17L (allele frequency = 0.07), and L237M (allele frequency = 0.03). Genotype frequencies among case groups and controls are given in Table 1. The R8W, P17L, and L237M variants were selected because of their likely phenotypic impact based on predictions using sequence homology software (25, 26), and expected allele frequency ($\geq$0.03) in the Minnesota Caucasian population (17). A new polymorphism, L15-L16del (a deletion of two leucines), was discovered as part of genotyping for this study. Whereas R8W, L15-L16del, and P17L are found in the *PTGS1* signal peptide sequence which targets the protein for translocation into the lumen of the endoplasmic reticulum, L237M is located near the PTGS1 dimer interface connecting two identical monomeric subunits (27).

In this population, no haplotypes were estimated to carry two of the variant *PTGS1* alleles. We observed no evidence for linkage disequilibrium between the variant alleles, neither considering haplotypes based on all four polymorphisms (P = 0.10) nor considering two polymorphisms at a time (all six P values > 0.05). However, due to the rarity of the variant alleles, a much larger sample size would be needed to reliably test for linkage disequilibrium.

Table 1. *PTGS1* genotype frequencies among adenoma cases, hyperplastic polyp cases, and controls

PTGS1 polymorphism	Genotype	Adenomas *N* (%)	Hyperplastic polyps *N* (%)	Controls *N* (%)
R8W				
	Wt/wt	445 (85.4)	164 (84.5)	539 (86.8)
	Wt/var	73 (14.0)	30 (15.5)	79 (12.7)
	Var/var	3 (0.6)	0 (0.0)	3 (0.5)
L15-L16del				
	Wt/wt	510 (97.9)	191 (98.5)	616 (99.2)
	Wt/var	11 (2.1)	3 (1.5)	5 (0.8)
P17L				
	Wt/wt	451 (86.6)	173 (89.2)	527 (84.9)
	Wt/var	63 (12.1)	20 (10.3)	90 (14.5)
	Var/var	7 (1.3)	1 (0.5)	4 (0.6)
L237M				
	Wt/wt	493 (94.6)	183 (94.3)	585 (94.2)
	Wt/var	28 (5.4)	11 (5.7)	36 (5.8)

Table 2. Risk of colorectal adenomatous and hyperplastic polyps associated with *PTGS1* polymorphisms (age- and sex-adjusted ORs)

PTGS1 polymorphism	Genotype	Number of cases/controls	OR	95% CI
Adenomas				
R8W	Wt/wt	445/539	1.0	Ref
	Wt/var or var/var	76/82	1.1	(0.8–1.6)
L15-L16del	Wt/wt	510/616	1.0	Ref
	Wt/var	11/5	3.6	(1.2–11.2)
P17L	Wt/wt	451/527	1.0	Ref
	Wt/var or var/var	70/94	0.9	(0.6–1.2)
L237M	Wt/wt	493/585	1.0	Ref
	Wt/var	28/36	0.8	(0.5–1.4)
Hyperplastic polyps				
R8W	Wt/wt	164/539	1.0	Ref
	Wt/var or var/var	30/82	1.2	(0.8–1.9)
L15-L16del	Wt/wt	191/616	1.0	Ref
	Wt/var	3/5	2.1	(0.5–9.1)
P17L	Wt/wt	173/527	1.0	Ref
	Wt/var or var/var	21/94	0.7	(0.4–1.1)
L237M	Wt/wt	183/585	1.0	Ref
	Wt/var	11/36	1.0	(0.5–2.0)

Haplotype frequencies did not differ between either one of the case groups and controls ($P = 0.56$ and $P = 0.63$, respectively). ORs estimating the relative risk associated with each *PTGS1* variant are shown in Table 2. On the basis of the risk patterns observed, individuals with at least one variant allele of R8W and P17L were grouped together. There was no appreciable difference in adenoma or hyperplastic polyp risk associated with *PTGS1* polymorphisms R8W, P17L, and L237M. A statistically significant increase in risk was seen for those heterozygous for the L15-L16del polymorphism (age and sex-adjusted OR = 3.6, 95% CI 1.2–11.2). This risk was perhaps further increased among those with synchronous adenomatous and hyperplastic polyps

Table 3. Risk of colorectal adenomas associated with *PTGS1* polymorphisms stratified by use of aspirin or other NSAIDs (age- and sex-adjusted ORs)

PTGS1 polymorphism	Genotype	Regular, current use of aspirin or other NSAIDs	
		No	Yes
		OR (95% CI) (*N* cases/*n* controls)	OR (95% CI) (*N* cases/*n* controls)
R8W	Wt/wt	1.0 (ref) 278/302	0.7 (0.5–0.9) 167/237
	Wt/var or var/var	1.3 (0.8–2.1) 50/42	0.6 (0.4–1.1) 26/40
		P interaction = 0.31	
L15-L16del	Wt/wt	1.0 (ref) 320/343	0.7 (0.5–0.9) 190/273
	Wt/var	12.8 (1.4–115) 8/1	1.0 (0.2–4.9) 3/4
		P interaction = 0.12	
P17L	Wt/wt	1.0 (ref) 287/288	0.6 (0.5–0.8) 190/273
	Wt/var or var/var	0.6 (0.4–0.97) 41/56	0.8 (0.5–1.4) 29/38
		P interaction = 0.03	
L237M	Wt/wt	1.0 (ref) 314/324	0.6 (0.5–0.9) 179/261
	Wt/var	0.6 (0.3–1.3) 14/20	0.8 (0.4–1.6) 14/16
		P interaction = 0.22	

(OR = 5.8, 95% CI 1.2–28.3) (data not shown). However, due to the small number of individuals carrying the L15-L16del allele, all of these estimates were quite imprecise. For all variants, there were no appreciable differences based on stratification by age group (>50 years *versus* younger), or polyp location.

Regular aspirin and NSAID use (>1/week) have been previously identified in this population as associated with reduced adenoma risk (24) [age and sex-adjusted OR = 0.70 (0.53–0.92) and 0.65 (0.53–0.92), respectively]. We evaluated whether associations with current regular use of aspirin or NSAIDs differed depending on *PTGS1* genotypes (Table 3). There was indication that the L15-L16del polymorphism was associated with a stronger increase in adenoma risk among non-users of aspirin/other NSAIDs. Furthermore, the P17L polymorphism modified risk of adenomas associated with regular current use of aspirin/other NSAIDs: among those with 17PP genotypes (wt/wt) regular use of aspirin/other NSAIDs was associated with a significantly decreased risk [OR = 0.6 (0.5–0.8)]; yet, among individuals carrying at least one variant 17 L allele, no risk reduction was observed [compared to the reference group of wt/wt non-users: wt/var or var/var non-users OR = 0.6 (0.5–1.0) *versus* wt/var or var/var users OR = 0.8 (0.5–1.4) (P interaction = 0.03)]. Similarly, the inverse association observed with aspirin/NSAID use was limited to those wild type for the L237M polymorphism [users: OR = 0.6 (0.5–0.9) *versus* nonusers: 1.0 (ref)], although this interaction was not statistically significant (P = 0.22). However, these preliminary findings may be spurious and clearly require confirmation in a larger population. Risk patterns for hyperplastic polyps were generally similar to those for adenomas (data not shown).

In addition to investigating genotypes at each polymorphic site in direct comparison (*e.g.*, wt/wt *versus* wt/het), we also undertook analyses restricting the reference group to individuals wt/wt for all four polymorphisms (wt/wt haplotypes). ORs were virtually identical, although with wider CIs, attributable to the smaller number of individuals in the reference category.

Discussion

To our knowledge, this study represents the first investigation of polymorphisms in *PTGS1* and risk of colorectal polyps. Although more attention has been given to *PTGS2* as a key player in colorectal carcinogenesis, recent experiments among *Min* mice, an animal model for intestinal polyp formation, implicate *PTGS1* as equally important for intestinal carcinogenesis (14). *PTGS1* knock-out mice showed a similar reduction in polyp burden as *PTGS2* knock-outs compared to the wild-type *PTGS1* (+/+) or *PTGS2* (+/+) *Min*/+ mouse (14). Epidemiological studies of aspirin intake show reduced risks of colorectal adenomas and cancer associated with regular use (3), and effective prevention of adenoma recurrence (6, 28). These reduced risks are seen with low-dose and low-frequency aspirin, most likely insufficient to inhibit COX-2, and suggest that mechanisms other than direct inhibition of COX-2 may be involved. One hypothesis is that COX-1 activity in platelets releases lipid and protein mediators that may induce COX-2 via a paracrine mechanism in the formation of neoplasia (3). Thus, both animal and epidemiological studies support the concept that both PTGS1 and PTGS2 are involved in colorectal carcinogenesis.

We here report that a variant in the signal peptide of PTGS1 (L15-L16del) may result in an elevated risk of colorectal adenomas. Risk associated with this genotype appeared stronger among individuals currently not using other NSAIDs or aspirin on a regular basis. The consistency of the findings for L15-L16del for both hyperplastic and adenomatous polyps provides support for a true association. Yet, given the overall rarity of the variant *PTGS1* alleles, our findings could also be attributable to chance and should be interpreted with caution until confirmed.

Furthermore, the P17L polymorphism appeared to modify the effects of regular aspirin/other NSAID use on colorectal polyp risk. Halushka *et al.* recently presented data indicating that this variant (which exists in complete linkage disequilibrium with the promoter polymorphism A-842G) may slightly increase the inhibitory effect of aspirin on platelet cyclooxygenase activity (29). This in vitro finding is seemingly at odds with the observation in our study population of a benefit of aspirin/other NSAIDs limited to those with the wild-type PP genotype. Yet, the effects of the P17L variant on cyclooxygenase inhibition by aspirin in vitro may not be conclusive, as they were based on changes in the levels of prostaglandin F2a, which is a minor cyclooxygenase metabolite in platelets (30). Clearly, evaluation of the pharmacogenetic relevance of this *PTGS1* polymorphism will require further experimental and pharmacological data.

We targeted our study to *PTGS1* polymorphisms located in regions encoding amino acids that are highly conserved among PTGS1 proteins of different species, and have been predicted to exert phenotypic effects by two sequence-based algorithms (17, 25, 26). There is now some limited experimental evidence suggesting that the P17L variant has functional consequences, whereas R8W was not found to alter the formation of prostaglandin $F_{2\alpha}$ in human platelets *in vitro* (31). Unfortunately, L15-L16del and L237M were not evaluated for functional impact in these experimental studies. The L15-L16del polymorphism results in the deletion of two amino acids in the PTGS1 signal peptide, which targets the protein for translocation into the lumen of the endoplasmic reticulum (32). Evaluation of the PTGS1 signal peptide polymorphisms with the SignalP software program, which predicts the likelihood of an NH_2-terminal segment being recognized as a signal peptide and the site of signal peptidase cleavage (33), suggested that the polymorphisms would have little impact.[2] These findings reduce but do not abolish the likelihood that the L15-L16del polymorphism could cause alterations in protein location as a result of miscompartmentalization. A signal peptide may have multiple functions and consequences of genotypic variation in signal peptides are not completely understood (34).

In conclusion, the L15-L16del polymorphism in the *PTGS1* gene may result in an increased risk of colorectal adenomas, particularly among non-aspirin/other NSAID

[2] R. Kulmacz, personal communication.

users. Our finding of effect modification between the *PTGS1* P17L variant and aspirin/other NSAID use could potentially be of pharmacogenetic relevance. Yet in light of the rarity of the variant alleles of all *PTGS1* polymorphisms, our findings should be considered preliminary. Larger studies of both colorectal adenomas and cancer should be undertaken investigating these *PTGS1* variants and their interaction with NSAID use.

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