Increased Cyclooxygenase-2 Expression in Duodenal Compared with Colonic Tissues in Familial Adenomatous Polyposis and Relationship to the −765G ⟶ *C COX-2* Polymorphism

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Abstract

Background: Colorectal cancers arising in patients with familial adenomatous polyposis (FAP) can be largely prevented by polyp surveillance and prophylactic colectomy. As a result, duodenal adenocarcinoma has become a leading cause of death in patients with FAP. Cyclooxygenase 2 (COX-2) inhibition is effective against colorectal polyposis in FAP, but is less effective in treating duodenal polyps. We compared the expression of COX-2 in duodenal and colorectal adenomas from patients with FAP and from patients with sporadic neoplasms and correlated expression to a *COX-2* promoter polymorphism (−765G→C) that is reported to influence COX-2 expression.

Methods: The study population included 36 FAP patients with colonic adenomas, 22 FAP patients with duodenal adenomas, 22 patients with sporadic duodenal adenomas, and 17 patients with sporadic duodenal adenocarcinoma. Neoplastic and corresponding normal tissue COX-2 expressions were determined using immunohistochemistry on tissue microarrays. The prevalence and ethnic distribution of a polymorphism in the *COX-2* promoter that influences COX-2 expression (−765G ⟶ C) were determined in DNA from 274 individuals by real-time quantitative PCR.

Results: Among patients with FAP, histologically normal duodenal mucosa showed higher COX-2 expression than normal colonic mucosa ($P < 0.02$), and duodenal adenomas had higher COX-2 expression than colonic adenomas ($P \leq 0.01$). In addition, the normal duodenum of patients with FAP showed higher COX-2 expression than the normal duodenal mucosa of patients with sporadic adenomas ($P < 0.05$). COX-2 expression was significantly higher in the normal-appearing ($P < 0.01$) mucosa of patients with FAP carrying the −765GG genotype compared with those carrying the −765GC or −765CC genotypes. The −765C genotype was more common in African Americans than in Caucasians (52% versus 33%, $P < 0.01$).

Conclusions: High COX-2 expression in the normal and adenomatous duodenal mucosa of patients with FAP may explain the poorer response of these neoplasms to chemoprevention with COX-2 inhibitors.

Familial adenomatous polyposis (FAP) is caused by germ-line mutations in the adenomatous polyposis coli (*APC*) gene, which leads to the development of innumerable adenomatous polyps throughout the colorectum. Without colectomy, colorectal carcinoma is almost inevitable usually by the fifth decade of life. In recent decades, colorectal cancer screening and prophylactic surgery have significantly improved the survival of patients with FAP. However, the life expectancy of patients with FAP is still below that of the general population, largely due to the risk of developing upper gastrointestinal tract malignancy.

The duodenum is the second commonest site of adenoma development in patients with FAP, and ~5% of patients with FAP will develop duodenal cancer during their lifetime (1–3). Currently, the main management options for patients with duodenal adenomatosis are endoscopic surveillance and selective surgical resection. Duodenal surgery, either a pancreas-preserving duodenectomy or pancreaticoduodenectomy, is indicated for patients with either severe duodenal polyposis or duodenal carcinoma. However, these therapeutic options do not adequately manage the duodenal neoplasia that arises in the setting of FAP. Colorectal adenomas occurring in patients with FAP have been shown to regress with sulindac, a non-steroidal anti-inflammatory drug, and with cyclooxygenase 2 (COX-2) inhibitors (4, 5). These drugs have been targeted towards treating duodenal adenomas but results have been

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modest and conflicting. Some investigators have shown that sulindac and COX-2 inhibitors can reduce small duodenal adenomas (6, 7), but not large adenomas (8), whereas others have not (9, 10).

The rationale behind using nonsteroidal anti-inflammatory drugs as chemopreventive agents is their inhibitory effect on the COX enzymes. Two isoforms of COX exist, COX-1 and COX-2, which are involved in the conversion of arachidonic acid into prostaglandins. Prostaglandins, in turn, regulate cellular functions such as angiogenesis and cell proliferation and have been associated with progression of tumor development in a size-dependent manner (11). In the colorectum, COX-2 is increasingly up-regulated with progression from adenoma to carcinoma (12–16). The importance of COX-2 expression in the pathogenesis of colorectal neoplasia is dramatically illustrated by the marked reduction of polyps seen in APC^{MIN} mice crossed with *COX-2* knockout mice (17). The mechanisms by which COX-2 overexpression may contribute to tumorigenesis have been extensively studied and include antiapoptotic, proangiogenic, and proinvasive effects (18). Other gastrointestinal tract neoplasms are associated with increases in COX-2 expression including esophageal and gastric neoplasia (19, 20). However, duodenal neoplasms in the setting of FAP have not, to our knowledge, been investigated for COX-2 expression.

Therefore, to provide information concerning the potential to prevent the progression of duodenal neoplasms in patients with FAP using COX-2 inhibitors, we evaluated the expression of COX-2 in duodenal adenomas of patients with FAP compared with that in duodenal tissues from patients with sporadic duodenal adenomas and duodenal carcinomas as well as with that in colonic mucosa from patients with FAP. In addition, we examined the association between intestinal COX-2 expression and the presence of a common *COX-2* promoter polymorphism (−765C→G; refs. 21, 22).

Materials and Methods

Patients. Patients were selected by searching the Johns Hopkins Surgical Pathology archives for patients with FAP and colonic and duodenal polyps and for patients with sporadic duodenal adenoma and/or duodenal carcinoma. Thirty-six patients with FAP had colon polyps (19 male, mean age: 34.5 ± 18.1 (SD) years; 17 female, mean age 33.8 ± 13.9 years) and 22 patients with FAP had duodenal polyps (7 male, mean age 48.4 ± 11.5 years; 15 female, mean age 44.8 ± 11.7 years). Two patients with FAP had colorectal adenocarcinoma and two had duodenal adenocarcinoma in association with colonic and duodenal polyposis, respectively. In addition, 22 patients with sporadic duodenal adenomas (11 male, mean age: 66.1 ± 12.5 years; 11 female, mean age: 61.2 ± 17.1 years) and 17 patients with sporadic duodenal carcinomas (11 male, mean age: 66.2 ± 9.7 years; 7 female, mean age: 54.1 ± 14.9 years) were included in the analysis (Table 1). To investigate the correlation between the −765G/C *COX-2* promoter genotype and COX-2 expression, normal duodenal mucosa was obtained from 93 patients who underwent pancreaticoduodenectomy for pancreatic adenocarcinoma. To determine the prevalence of the *COX-2* promoter polymorphism by ethnicity, 219 unselected Caucasian patients and 50 African American patients were genotyped (206 with pancreatic cancer, 46 with familial adenomatous polyposis, 17 with benign gallbladder disease, and 5 with chronic pancreatitis). The study was approved by the Institutional Review Board of the Johns Hopkins University.

Cyclooxygenase-2 measurement

Tissue microarrays. Formalin-fixed paraffin embedded tissues were collected to generate 13 tissue microarrays (Table 1). For tissue microarray construction, representative areas containing morphologically defined normal mucosa, adenoma, or carcinoma were identified on an H&E-stained reference slide by an experienced pathologist (C.A.I.D.) and encircled on the paraffin block. Tissue microarrays were constructed using a manual Tissue Puncher/Arrayer (Beecher Instruments, Silver Spring, MD). The diameter of the punched core was 1.4 mm. Serial sections were cut from these tissue microarrays, one of which was stained with H&E as a reference.

Table 1. Tissue microarrays and patient characteristics

Tissue microarray	Number	Adenoma	Normal	Carcinoma	Mean age ± SD	
					Male	Female
FAP colon*	5	133 different polyps from 36 different patients	Matched normal mucosa from 30 patients	Matched carcinoma from 2 patients	34.5 ± 18.1 (n = 19)	33.8 ± 13.9 (n = 17)
FAP duodenum*	1	49 different polyps from 22 FAP patients	Matched normal mucosa from 13 patients	Matched carcinoma from 2 patients	48.4 ± 11.5 (n = 7)	44.8 ± 11.7 (n = 15)
Sporadic duodenum adenoma	1	36 different sporadic polyps from 22 patients	Matched normal mucosa from 15 patients		66.1 ±12.5 (n = 11)	61.2 ± 17.1 (n = 11)
Sporadic duodenum carcinoma	2	Matched adenoma from 7 patients	Matched normal mucosa from 15 patients	17 sporadic duodenal carcinomas from 17 patients	66.2 ± 9.7 (n = 11)	54.1 ±14.9 (n = 7)
Normal duodenum from patients with pancreatic adenocarcinoma	3		Normal duodenum mucosa from 93 patients		67.1 ±10.8 (n = 51)	65.8 ± 11.2 (n = 42)

*COX-2 expression was also determined in colonic mucosa of 9 and duodenal mucosa of 2 FAP patients using individual paraffin sections of their tissues.

Table 2. Immunohistochemical scoring of COX-2 expression in familial adenomas of the duodenum and colon and sporadic duodenal adenomas and carcinomas

		COX-2 expression*					
		Absent (%)	Weak (%)	Moderate (%)	Strong (%)	Very strong (%)	*P*
FAP colon	Normal (*n* = 30)	0	3 (10)	22 (73)	3 (10)	2 (7)	0.0019†
	Adenoma (*n* = 36)‡	0	4 (11)	22 (61)	8 (22)	2 (6)	0.00045§
	Carcinoma (*n* = 2)	0	0	0	0	2 (100)	
FAP duodenum	Normal (*n* = 13)	1 (8)	1 (8)	3 (23)	2 (15)	6 (46)	‖
	Adenoma (*n* = 22)¶	1 (5)	0	11 (50)	2 (9)	8 (36)	**
	Carcinoma (*n* = 2)	0	0	0	1 (50)	1 (50)	
Sporadic duodenal adenoma	Normal (*n* = 15)	1 (7)	0	12 (80)	1 (7)	1 (7)	0.0224††
	Adenoma (*n* = 22)‡‡	0	1 (4)	7 (32)	7 (32)	7 (32)	
Sporadic duodenal carcinoma	Normal (*n* = 17)	0	0	12 (71)	4 (23)	1 (6)	0.024†
	Adenoma (*n* = 9)§§	0	0	6 (67)	2 (22)	1 (11)	
	Carcinoma (*n* = 17)	0	0	5 (29)	5 (30)	7 (41)	
All sporadic duodenal adenomas‖‖	Normal (*n* = 32)	1 (3)	0	24 (75)	5 (16)	2 (6)	
	Adenoma (*n* = 31)	0	1 (3)	13 (42)	9 (29)	8 (26)	

*The highest score was used in statistical analysis if multiple cores from multiple polyps, normal mucosa or carcinoma from the same patient were present.
† Carcinoma versus normal.
‡ One-hundred thirty-three different polyps from 36 different FAP patients.
§Carcinoma versus adenoma.
‖Normal FAP duodenal mucosa shows higher levels of COX-2 than normal-appearing FAP colonic mucosa ($P = 0.014$) and normal duodenal mucosa from patients with sporadic duodenal adenomas ($P = 0.037$).
¶Forty-nine different polyps from 22 different FAP patients.
**Duodenal adenomas from patients with FAP show higher levels of COX-2 than colonic adenomas from patients with FAP ($P = 0.01$).
†† Normal versus adenoma.
‡‡ Thirty-six different polyps from 22 different patients.
§§Thirteen different polyps from nine different patients.
‖‖Pooled adenomas from patients with sporadic duodenal adenomas and adenomas that were found in patients with sporadic duodenal carcinoma.

Immunohistochemistry. Immunohistochemistry for COX-2 was done on unstained 4 μm sections as previously described (23). An anti–COX-2 monoclonal antibody (Cayman Chemical, Ann Arbor, MI) was used at a dilution of 1:100. Two independent observers (C.A.I.D. and L.A.A.B.) scored the intensity of epithelial COX-2 staining in a semiquantitative manner on a five-grade scale: absent, weak, moderate, strong, or very strong COX-2 labeling (24, 25). Stromal staining was scored separately. In assigning scores, the observers assessed all of the tissue cores on the tissue microarrays. In most cases, multiple cores from multiple polyps and multiple cores from normal mucosa or carcinoma were present. The highest score was used in the statistical analysis.

−765G/C genotyping

DNA isolation. Genomic DNA was obtained from deparaffinized formalin-fixed paraffin-embedded tissue of 46 patients with FAP using TK buffer [200 μg/mL of proteinase K and 0.5% Tween 20, 50 mmol/L Tris (pH 9), 1 mmol/L NaCl, 2 mmol/L EDTA]. After overnight incubation in 50 μL TK buffer at 56°C, tubes were incubated at 95°C for 10 minutes to inactivate the proteinase K (26). In addition, DNA was isolated using Qiagen Tissue Kits (Qiagen, Valencia, CA) from fresh-frozen normal duodenum or normal pancreas tissue of 206 patients with pancreatic adenocarcinoma who had undergone Whipple resection, as well as formalin-fixed paraffin-embedded gall bladder tissue from 17 patients with benign gall bladder disease who had undergone cholecystectomy and 5 patients with chronic pancreatitis who had undergone Whipple resection.

Real-time PCR. The −765G → C promoter polymorphism was detected in the SmartCycler1 (Cepheid, Sunnyvale, CA) using the following primers: *COX2RealTimeFor*: 5′-cattaactatttacagggtaactgcttagg-3′ and *COX2RealTimeRev*: 5′-ccccctccttgtttcttgga-3′. Fluorescent MGB probes (Applied Biosystems), which were used to detect the *G* allele (probe 1, 765G: 6-FAM-5′-ctttcccgcctctct-3′) and the *C* allele (probe 2, 765C: TET-5′-ctttccccctctct-3′; ref. 27). Samples were assayed in a 26 μL reaction mixture containing 12.5 μL Quantitect Buffer (Qiagen), 1.25 μL of each primer (final concentration 0.5 μmol/L), 0.25 μL of each probe (final concentration 0.1 μmol/L), 9.5 μL diethyl pyrocarbonate–treated H_2O, and 50 ng of sample genomic DNA. PCR reactions were done starting with 94°C for 15 minutes to activate HotStarTaq DNA polymerase, followed by 45 cycles of 94°C for 15 seconds and 60°C for 30 seconds. Two samples with known genotype and a water control were simultaneously assayed in each run.

Sequencing. Fourteen samples were sequenced to validate the single nucleotide polymorphism real-time PCR assay. After initial amplification of the promoter region containing the single nucleotide polymorphism of interest (primers, *COX2For*: 5′-gcatacgttttggacatttag; *COX2Rev*: 5′-ctaccttcagtgtacatagc), the PCR product was purified using the QIAquick PCR Purification Kit (Qiagen). Subsequently, samples were sequenced using an internal forward primer (*COX2IntFor*: 5′-gttttggacatttagcgtcc) and the Applied Biosystems 3730 DNA Analyzer.

Statistics. Nonparametric χ^2 tests were used to assess differences between groups and to assess the correlation between COX-2 expression and *COX-2* genotype. In addition, χ^2 tests were used to compare the observed genotype prevalence with the expected prevalence of each genotype for a population in Hardy-Weinberg equilibrium.

Results

Cyclooxygenase-2 expression in the colon of patients with familial adenomatous polyposis. COX-2 was expressed in the normal-appearing and adenomatous colonic epithelium of all

patients. The level of COX-2 expression in normal colonic mucosa was similar to levels found in colorectal adenomas from patients with FAP. The colon carcinomas from patients with FAP had significantly higher COX-2 expression than normal-appearing (χ^2 = 14.9, P = 0.0019) and adenomatous mucosa (χ^2 = 17.9, P = 0.00045; Table 2; Fig. 1A, C, and E).

Cyclooxygenase-2 expression in the duodenum of patients with familial adenomatous polyposis. COX-2 was similarly expressed in the normal duodenal mucosa, duodenal adenomas, and duodenal carcinomas (Table 2; Fig. 1B, D, and F). Normal-appearing duodenal mucosa from FAP patients showed significantly higher levels of COX-2 than normal-appearing FAP colonic mucosa (Fig. 1B versus A; χ^2 = 12.5, P = 0.014). Similarly, duodenal adenomas from patients with FAP showed higher levels of COX-2 than colonic adenomas from patients with FAP (χ^2 = 13.3, P = 0.01; Fig. 1C and D).

Cyclooxygenase-2 expression in sporadic and familial adenomatous polyposis duodenal adenomas. Normal duodenal mucosa was available from 15 of 22 patients that had sporadic duodenal adenomas. The majority (80%) showed moderate COX-2 expression in normal duodenal mucosa whereas COX-2 was expressed in all duodenal adenomas. Furthermore, sporadic duodenal adenomas had significantly higher COX-2 expression than normal duodenal mucosa (χ^2 = 11.4, P = 0.0224) with most adenomas exhibiting strong COX-2 expression (Table 2). Moreover, normal duodenal mucosa from FAP patients showed statistically significantly higher levels of COX-2 than normal duodenal mucosa from patients with sporadic duodenal adenomas (χ^2 = 10.2, P = 0.037). This observation is further supported by the finding that 69.2% of matched normal and adenomatous duodenal mucosa of FAP patients showed the same COX-2 intensity, whereas only 33.3% of sporadic duodenal adenomas showed the same level of COX-2 expression in their normal duodenal mucosa (χ^2 = 8.4, P = 0.015; Table 3). These results could not be explained by differences in the grade of adenoma or how the adenoma was obtained (by resection, polypectomy, or biopsy) between patients with FAP and those with sporadic duodenal adenomas.

Cyclooxygenase-2 expression in sporadic duodenal carcinomas. All 17 patients with sporadic duodenal carcinoma expressed COX-2 in normal duodenal mucosa, mostly with moderate labeling intensity. Most of the 17 sporadic duodenal carcinomas exhibited strong or very strong COX-2 immunoreactivity. In addition, all nine adenomas associated with duodenal carcinoma displayed COX-2 immunostaining (Table 2). Duodenal carcinomas had statistically significantly higher levels of COX-2 expression than normal duodenal mucosa (χ^2 = 7.5, P = 0.024).

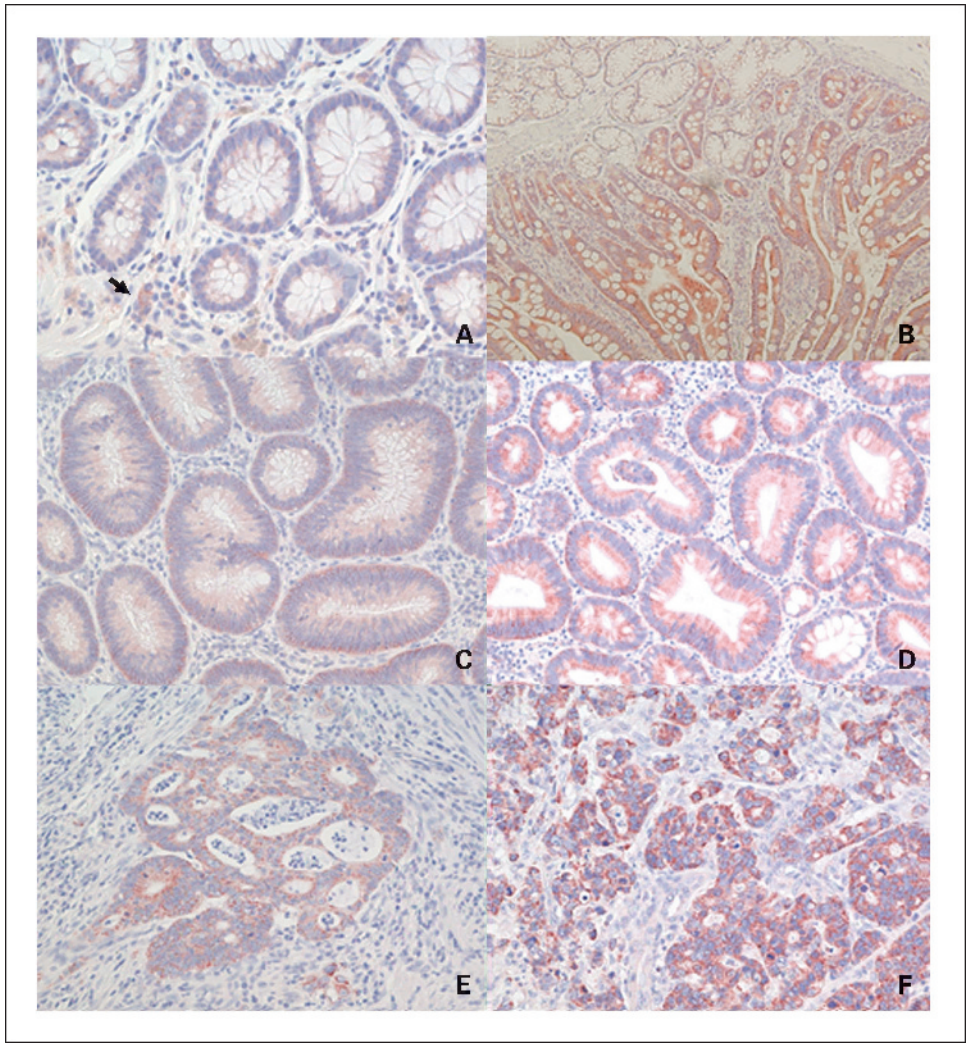

Fig. 1. COX-2 immunoreactivity in duodenum and colon. *A*, normal colon mucosa in a patient with FAP exhibiting weak COX-2 immunoreactivity (×100). Arrow, macrophage labeling. *B*, normal duodenal mucosa from a patient with FAP demonstrating strong COX-2 immunoreactivity. *C*, colon adenoma in FAP showing moderate COX-2 staining (×64). *D*, duodenum adenoma in FAP showing strong COX-2 staining (×64). Colon carcinoma (*E*; ×64) and duodenum carcinoma (*F*; ×64) showing very strong COX-2 immunoreactivity.

Table 3. COX-2 expression in matched normal and adenomatous duodenal mucosa of patients with FAP and with sporadic duodenal adenomas

Patient group	N = P	P › N	N › P
FAP (n = 13)	9 of 13 (69.2%)	2 of 13 (15.4%)	2 of 13 (15.4%)
Patients with sporadic duodenal adenomas (n = 15)	5 of 15 (33.3%)	10 of 15 (66.7%)	0
		$\chi^2 = 8.4$	
		$P = 0.01$	

NOTE: N = P, normal mucosa has the same COX-2 intensity as adenomatous mucosa; P › N, adenoma has higher COX-2 intensity than normal mucosa; N › P, normal mucosa has higher COX-2 intensity than adenoma.

Stromal cyclooxygenase-2 immunoreactivity. Stromal COX-2 labeling was focal. It was observed mainly in macrophages underlying the epithelium (Fig. 1A, *arrow*). COX-2 labeling was strong in eroded areas and seen in macrophages underlying the erosion.

Distribution of the −765G → C polymorphism. In patients with FAP, the GG, GC, and CC genotype frequencies were 65.2%, 28.3%, and 6.5%, respectively (Table 4). The GG, GC, and CC genotypes occurred at 63.2%, 32.4%, and 4.4% in the disease control group, respectively. African Americans were more likely to carry a −765C polymorphism than Caucasians (χ^2 = 10.01, P = 0.0067; Table 4). All genotypic distributions are in Hardy-Weinberg equilibrium ($P \geq 0.05$).

Correlation between −765 genotype and cyclooxygenase-2 expression. Carriers of the −765GG genotype with FAP had higher COX-2 expression in their normal (χ^2 = 9.4, P = 0.009) colonic and duodenal mucosa than the −765GC/CC carriers (Table 5). In contrast, there was no significant difference in COX-2 expression in the adenomatous mucosa of GG carriers (χ^2 = 1.4. P = 0.495). There was also no correlation between the level of COX-2 expression in the normal duodenal mucosa of 93 patients with pancreatic cancer and their −765G/C *COX-2* genotype (data not shown).

Recent studies indicate that COX-2 activity may be influenced in certain tissues by estrogen (28). However, we found no evidence of any difference in COX-2 expression in normal-appearing mucosa or adenomas by gender.

Discussion

In this study, we find that COX-2 levels increase with increasing stage of duodenal neoplasia among patients with sporadic disease. This pattern of COX-2 expression has been described for colorectal and other neoplasms and is consistent with a similar adenoma-carcinoma progression sequence for duodenal neoplasia as has been observed for colorectal and other neoplasms (29).

Second, we also find greater COX-2 expression in the normal duodenal mucosa and duodenal adenomas of patients with FAP than in patients with sporadic duodenal neoplasias. Other investigators have noted that normal duodenal mucosa from patients with FAP exhibits increased cell proliferation and ultrastructural changes in cell adhesion function compared with non-FAP controls (30, 31). In addition, several genes, including *COX-2*, are up-regulated in the normal-appearing colon mucosa of APC^{MIN} mice and in patients with colorectal cancer (32). Increased COX-2 expression in histologically normal mucosa from patients with FAP may result from impaired Wnt signaling through a possible transcription factor 4 binding element in the *COX-2* promoter (33). In addition, gastrin expression is increased by *APC* inactivation, and in certain tissues gastrin expression can increase COX-2 expression (34, 35).

In addition, we also find that among patients with FAP, COX-2 expression is higher in normal duodenal mucosa than

Table 4. Prevalence of −765G/*C COX-2* genotype in patients with FAP and disease controls and ethnic distribution of the −765 *COX-2* polymorphism

	Prevalence				
	FAP [n (%)]	Disease controls* [n (%)]	Caucasian [n (%)]	African American [n (%)]	Other/unknown (n)
−765GG	30 (65.2)	144 (63.2)	146 (66.7)	24 (48)	4
−765GC	13 (28.3)	74 (32.4)	66 (30.1)	20 (40)	1
−765CC	3 (6.5)	10 (4.4)	7 (3.2)	6 (12)	0
	46 (100)	228 (100)	219 (100)	50 (100)	5
	FAP vs disease controls:		Caucasian vs African American:		
	$\chi^2 = 0.606$		$\chi^2 = 10.011$		
	$P = 0.74$		$P = 0.0067$		

*Two-hundred six with pancreatic cancer, 17 with benign gallbladder disease, and 5 with chronic pancreatitis.

Table 5. Correlation between intestinal immunohistochemical COX-2 staining and −765 *COX-2* genotype

	Normal				Adenoma			
	Immunohistochemical score				Immunohistochemical score			
	Absent/weak	Moderate	(Very) Strong		Absent/weak	Moderate	(Very) Strong	
GG	1	15	10	26	1	17	13	31
GC/CC	5	8	1	14	1	11	4	16
				40				47
		$\chi^2 = 9.4$	$P = 0.009$			$\chi^2 = 1.4$	$P = 0.495$	

in normal colonic mucosa. Whereas previous studies have compared COX-2 expression in small intestinal cancers and colorectal cancers, where expression patterns were found to be similar (36), COX-2 expression has not been studied in the duodenal tissues of patients with FAP. The higher expression of COX-2 in duodenal mucosa than in colonic mucosa could explain the lower response of duodenal adenomas compared with colonic adenomas to chemoprevention with nonsteroidal anti-inflammatory drugs and COX-2 inhibitors. Other factors that could contribute to differences between colonic and duodenal polyp responses to COX-2 inhibitors, such as greater resistance of duodenal adenomas to apoptosis or differences in the bioavailability of COX-2 inhibitor drugs in the colon versus the duodenum, require investigation. Interestingly, although it is plausible that treatment responses to standard doses of COX-2 inhibitors would be influenced by the amount of COX-2 protein in target tissues, such a relationship has not been clearly shown. Nonsteroidal anti-inflammatory drugs have been shown to reduce COX-2 expression *in vitro* (37) and in $Apc^{MIN/+}$ mouse (38). Indeed, in a previous study examining molecular correlation of adenoma responses and resistance to sulindac, COX-2 expression was lower (although still present) in sulindac-resistant colonic adenomas than in pretreatment adenomas that subsequently regressed with sulindac (23). Our results raise the possibility that higher dosages of COX-2 inhibitors could be more effective against duodenal adenomas as has been suggested for sulindac-resistant adenomas. Future studies should consider measuring duodenal COX-2 expression in patients with FAP undergoing treatment to determine if expression levels predict response to COX-2 inhibitors.

The mechanism(s) responsible for higher COX-2 expression in duodenal mucosa is not known. Several studies have suggested a role for bile acids, such as the unconjugated bile acid chenodeoxycholate, in the development of duodenal neoplasia in $Apc^{MIN/+}$ mice (39). Also, a correlation has been observed between the site of a patients' duodenal adenoma development and the site of exposure of their mucosa to bile (40). *Ex vivo* experiments have shown that COX-2 expression increases in response to exposure to pulses of bile acids and stomach acid (19, 41). Other studies have indicated a chemopreventive effect for ursodeoxycholic acid (42) and combined sulindac and ursodeoxycholic acid in mouse and rat intestine (43).

Finally, we investigated whether a recently reported single nucleotide polymorphism in the *COX-2* promoter affected the level of COX-2 expression in intestinal mucosa. Papafili et al. (22) showed that the −765C allele had lower *COX-2* promoter activity than the −765G allele. In addition, the −765GC and −765CC genotypes correlate with decreased risk of myocardial infarct and stroke and decreased COX-2 expression in atherosclerotic plaques compared with −765GG (21). We found higher COX-2 expression in the normal, but not in the adenomatous, mucosa of patients with FAP who carried −765GG alleles than in those with the −765GC or −765CC genotype. Because the −765 *COX-2* polymorphism influences COX-2 expression in the normal-appearing gastrointestinal mucosa of patients with FAP, it is possible that this polymorphism could influence the number of adenomas that develop in these patients, similar to the effect observed when COX-2 is knocked out in animal models of FAP (17). However, our results indicate that once polyps have formed, *COX-2* genotype does not influence COX-2 expression, suggesting that *COX-2* genotype may not influence the progression of these neoplasias. Interestingly, we also found that African Americans are significantly more likely to be carriers of −765C alleles, raising the possibility that African Americans could be more prone to the beneficial and adverse effects (such as toxicity from nonsteroidal anti-inflammatory drugs) of having lower COX-2 expression. Further study is needed to assess whether this polymorphism acts as a modifier of FAP phenotype or affects COX-2 expression elsewhere in the gastrointestinal tract, and whether genetic differences in the level of COX-2 expression influence patients' response to COX-2 inhibitors.

There is a need to improve the chemopreventive strategies for duodenal neoplasia occurring in the setting of FAP. In addition to COX-2, several other molecular targets merit consideration in chemoprevention studies including peroxisome proliferator-activated receptors δ and γ (44), epidermal growth factor receptor (45), ornithine decarboxylase (46), and nuclear factor κB pathway (47).

In conclusion, we have found that COX-2 is more highly expressed in the duodenal adenomas and normal duodenal mucosa of patients with FAP and this increased COX-2 expression may contribute to the poorer response of these neoplasms to chemoprevention with COX-2 inhibitors. Further investigation is needed to determine the role of the −765G/C COX-2 promoter polymorphism on *COX-2* gene expression in the gastrointestinal tract and its effect on the response to COX-2 inhibitors.

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References

1. Offerhaus GJ, Giardiello FM, Krush AJ, et al. The risk of upper gastrointestinal cancer in familial adenomatous polyposis. Gastroenterology 1992;102:1980–2.
2. Bjork J, Akerbrant H, Iselius L, et al. Periampullary adenomas and adenocarcinomas in familial adenomatous polyposis: cumulative risks and APC gene mutations. Gastroenterology 2001;121:1127–35.
3. Bulow S, Bjork J, Christensen IJ, et al. Duodenal adenomatosis in familial adenomatous polyposis. Gut 2004;53:381–6.
4. Giardiello FM, Hamilton SR, Krush AJ, et al. Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. N Engl J Med 1993; 328:1313–6.
5. Steinbach G, Lynch PM, Phillips RK, et al. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med 2000;342: 1946–52.
6. Debinski HS, Trojan J, Nugent KP, Spigelman AD, Phillips RK. Effect of sulindac on small polyps in familial adenomatous polyposis. Lancet 1995;345:855–6.
7. Phillips RK, Wallace MH, Lynch PM, et al. A randomised, double blind, placebo controlled study of celecoxib, a selective cyclooxygenase 2 inhibitor, on duodenal polyposis in familial adenomatous polyposis. Gut 2002;50:857–60.
8. Nugent KP, Farmer KC, Spigelman AD, Williams CB, Phillips RK. Randomized controlled trial of the effect of sulindac on duodenal and rectal polyposis and cell proliferation in patients with familial adenomatous polyposis. Br J Surg 1993;80:1618–9.
9. Seow-Choen F, Vijayan V, Keng V. Prospective randomized study of sulindac versus calcium and calciferol for upper gastrointestinal polyps in familial adenomatous polyposis. Br J Surg 1996;83:1763–6.
10. Winde G, Schmid KW, Brandt B, Muller O, Osswald H. Clinical and genomic influence of sulindac on rectal mucosa in familial adenomatous polyposis. Dis Colon Rectum 1997;40:1156–68; discussion 1168–9.
11. Yang VW, Shields JM, Hamilton SR, et al. Size-dependent increase in prostanoid levels in adenomas of patients with familial adenomatous polyposis. Cancer Res 1998;58:1750–3.
12. Khan KN, Masferrer JL, Woerner BM, Soslow R, Koki AT. Enhanced cyclooxygenase-2 expression in sporadic and familial adenomatous polyposis of the human colon. Scand J Gastroenterol 2001;36:865–9.
13. Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology 1994; 107:1183–8.
14. Hao X, Bishop AE, Wallace M, et al. Early expression of cyclo-oxygenase-2 during sporadic colorectal carcinogenesis. J Pathol 1999;187:295–301.
15. Kargman SL, O'Neill GP, Vickers PJ, Evans JF, Mancini JA, Jothy S. Expression of prostaglandin G/H synthase-1 and -2 protein in human colon cancer. Cancer Res 1995;55:2556–9.
16. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. Cell 1996;87:159–70.
17. Oshima M, Dinchuk JE, Kargman SL, et al. Suppression of intestinal polyposis in Apc δ716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 1996; 87:803–9.
18. Gupta RA, Dubois RN. Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2. Nat Rev Cancer 2001;1:11–21.
19. Shirvani VN, Ouatu-Lascar R, Kaur BS, Omary MB, Triadafilopoulos G. Cyclooxygenase 2 expression in Barrett's esophagus and adenocarcinoma: *ex vivo* induction by bile salts and acid exposure. Gastroenterology 2000;118:487–96.
20. Saukkonen K, Rintahaka J, Sivula A, et al. Cyclooxygenase-2 and gastric carcinogenesis. APMIS 2003; 111:915–25.
21. Cipollone F, Toniato E, Martinotti S, et al. A polymorphism in the cyclooxygenase 2 gene as an inherited protective factor against myocardial infarction and stroke. JAMA 2004;291:2221–8.
22. Papafili A, Hill MR, Brull DJ, et al. Common promoter variant in cyclooxygenase-2 represses gene expression: evidence of role in acute-phase inflammatory response. Arterioscler Thromb Vasc Biol 2002; 22:1631–6.
23. Keller JJ, Offerhaus GJ, Drillenburg P, et al. Molecular analysis of sulindac-resistant adenomas in familial adenomatous polyposis. Clin Cancer Res 2001;7:4000–7.
24. van Rees BP, Saukkonen K, Ristimaki A, et al. Cyclooxygenase-2 expression during carcinogenesis in the human stomach. J Pathol 2002;196:171–9.
25. Sano H, Kawahito Y, Wilder RL, et al. Expression of cyclooxygenase-1 and -2 in human colorectal cancer. Cancer Res 1995;55:3785–9.
26. Fukushima NSN, Ueki T, Rosty C, Walter KMYC, Hruban RH, Goggins M. Preproenkephalin and p16 gene CpG island hypermethylation in pancreatic intraepithelial neoplasia (PanIN) and pancreatic ductal adenocarcinoma. Am J Pathol 2002;160: 1573–81.
27. Koh WP, Yuan JM, van den Berg D, Lee HP, Yu MC. Interaction between cyclooxygenase-2 gene polymorphism and dietary n-6 polyunsaturated fatty acids on colon cancer risk: the Singapore Chinese Health Study. Br J Cancer 2004;90:1760–4.
28. Egan KM, Lawson JA, Fries S, et al. COX-2 derived prostacyclin confers atheroprotection on female mice. Science. In press 2004.
29. Spigelman AD, Talbot IC, Penna C, et al. Evidence for adenoma-carcinoma sequence in the duodenum of patients with familial adenomatous polyposis. The Leeds Castle Polyposis Group (Upper Gastrointestinal Committee). J Clin Pathol 1994;47:709–10.
30. Santucci R, Volpe L, Zannoni U, et al. Cell proliferation of the duodenal mucosa in patients affected by familial adenomatous polyposis. Gastroenterology 1997;113:1159–62.
31. Biasco G, Cenacchi G, Nobili E, et al. Cell proliferation and ultrastructural changes of the duodenal mucosa of patients affected by familial adenomatous polyposis. Hum Pathol 2004;35:622–6.
32. Chen LC, Hao CY, Chiu YS, et al. Alteration of gene expression in normal-appearing colon mucosa of APC(min) mice and human cancer patients. Cancer Res 2004;64:3694–700.
33. Araki Y, Okamura S, Hussain SP, et al. Regulation of cyclooxygenase-2 expression by the Wnt and ras pathways. Cancer Res 2003;63:728–34.
34. Abdalla SI, Lao-Sirieix P, Novelli MR, Lovat LB, Sanderson IR, Fitzgerald RC. Gastrin-induced cyclooxygenase-2 expression in Barrett's carcinogenesis. Clin Cancer Res 2004;10:4784–92.
35. Koh TJ, Bulitta CJ, Fleming JV, Dockray GJ, Varro A, Wang TC. Gastrin is a target of the β-catenin/TCF-4 growth-signaling pathway in a model of intestinal polyposis. J Clin Invest 2000;106:533–9.
36. Wendum D, Svrcek M, Rigau V, et al. COX-2, inflammatory secreted PLA2, and cytoplasmic PLA2 protein expression in small bowel adenocarcinomas compared with colorectal adenocarcinomas. Mod Pathol 2003;16:130–6.
37. Xu XM, Sansores-Garcia L, Chen XM, Matijevic-Aleksic N, Du M, Wu KK. Suppression of inducible cyclooxygenase 2 gene transcription by aspirin and sodium salicylate. Proc Natl Acad Sci U S A 1999; 96:5292–7.
38. Boolbol SK, Dannenberg AJ, Chadburn A, et al. Cyclooxygenase-2 overexpression and tumor formation are blocked by sulindac in a murine model of familial adenomatous polyposis. Cancer Res 1996;56: 2556–60.
39. Mahmoud NN, Dannenberg AJ, Bilinski RT, et al. Administration of an unconjugated bile acid increases duodenal tumors in a murine model of familial adenomatous polyposis. Carcinogenesis 1999;20: 299–303.
40. Spigelman AD, Williams CB, Talbot IC, Domizio P, Phillips RK. Upper gastrointestinal cancer in patients with familial adenomatous polyposis. Lancet 1989; 2:783–5.
41. Zhang F, Subbaramaiah K, Altorki N, Dannenberg AJ. Dihydroxy bile acids activate the transcription of cyclooxygenase-2. J Biol Chem 1998;273:2424–8.
42. Earnest DL, Holubec H, Wali RK, et al. Chemoprevention of azoxymethane-induced colonic carcinogenesis by supplemental dietary ursodeoxycholic acid. Cancer Res 1994;54:5071–4.
43. Jacoby RF, Cole CE, Hawk ET, Lubet RA. Ursodeoxycholate/Sulindac combination treatment effectively prevents intestinal adenomas in a mouse model of polyposis. Gastroenterology 2004;127:838–44.
44. Girnun GD, Spiegelman BM. PPARγ ligands: taking Ppart in chemoprevention. Gastroenterology 2003; 124:564–7.
45. Torrance CJ, Jackson PE, Montgomery E, et al. Combinatorial chemoprevention of intestinal neoplasia [In Process Citation]. Nat Med 2000;6: 1024–8.
46. Jacoby RF, Cole CE, Tutsch K, et al. Chemopreventive efficacy of combined piroxicam and difluoromethylornithine treatment of Apc mutant Min mouse adenomas, and selective toxicity against Apc mutant embryos. Cancer Res 2000;60:1864–70.
47. Clevers H. At the crossroads of inflammation and cancer. Cell 2004;118:671–4.