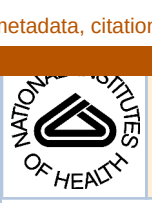

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Cyclooxygenase-2 Polymorphisms, Aspirin Treatment, and Risk of Colorectal Adenoma Recurrence – Data from a Randomized Clinical Trial

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Abstract

Cyclooxygenase-2 (COX-2) catalyzes the rate-limiting step in the production of prostaglandins, potent mediators of inflammation. Chronic inflammation plays an important role in the development and progression of colorectal cancer. Aspirin inhibits COX-2 activity and lowers the risk of colorectal adenomas and cancer. We investigated whether common genetic variation in COX-2 influenced risk of colorectal adenoma recurrence among 979 participants in the Aspirin/Folate Polyp Prevention Study who were randomly assigned to placebo or aspirin and followed for 3 years for the occurrence of new adenomas. Of these participants, 44.2% developed at least one new adenoma during follow-up. Adjusted relative risks (RRs) and 95% confidence intervals (CIs) were calculated to test the association between genetic variation at six COX-2 single nucleotide polymorphisms (SNPs) and adenoma occurrence and interaction with aspirin treatment. Two SNPs were significantly associated with increased adenoma recurrence: for rs5277 homozygous carriers of the minor C allele had a 51% increased risk compared to GG homozygotes (RR=1.51, 95% CI=1.01–2.25), and for rs4648310 heterozygous carriers of the minor G allele had a 37% increased risk compared to AA homozygotes

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(RR=1.37, 95% CI=1.05–1.79). (There were no minor allele homozygotes.) In stratified analyses, there was suggestive evidence that rs4648319 modified the effect of aspirin. These results support the hypothesis that that COX-2 plays a role in the etiology of colon cancer and may be a target for aspirin chemoprevention and warrant further investigation in other colorectal adenoma and cancer populations.

Introduction

Mounting evidence from experimental and observational studies supports the role of inflammation in the development and progression of cancer, including colorectal cancer (1–4). Cyclooxygenase-2 (COX-2), also known as prostaglandin G/H synthase-2 (PTGS-2), is an inducible enzyme which catalyzes the rate-limiting step in the production of prostaglandins and plays a key role in inflammation (5). COX-2 may also have an important role in the etiology of colorectal and other cancers (6,7). It is upregulated in colorectal adenomas and cancer; approximately half of all colorectal adenomas and more than 85% of colorectal cancers have elevated levels of COX-2 (8–11). Increased COX-2 levels have been shown to correlate with later stage, larger tumor size, presence of lymphatic metastases, and risk of recurrence and poorer survival in human colorectal cancers (12–15). Expression and activity of COX-2 is thought to contribute to tumor promotion and carcinogenesis through stimulation of cell proliferation, inhibition of apoptosis, and promotion of angiogenesis and invasiveness (16, 17).

Important additional evidence supporting a role for COX-2 in the inflammation-colon carcinogenesis pathway comes from observational studies and randomized clinical trials demonstrating that nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin (18–21) and selective COX-2 inhibitors (22–25), reduce the risk of colorectal adenoma and cancer. Notably, aspirin use preferentially reduced the risk of colorectal cancers that overexpressed COX-2, suggesting that the anticancer benefit of aspirin is mediated, at least in part, by effects on COX-2 (26).

Twin studies indicate that heritable factors play a relatively large role in the causation of sporadic colorectal cancer, an estimated 35% (27). Although knowledge about the genetic factors that contribute to sporadic cancer is limited, several genome-wide association studies have been published with replicated findings (28) (29). Genetic variation in the COX-2 gene may alter enzyme expression or activity, thereby altering the production of prostaglandins and potentially modulating an individual's inflammatory response and risk of colorectal cancer. In addition, it is possible that polymorphisms in the COX-2 gene could alter an individual's response to NSAIDs, and thus modify the chemopreventive effect of NSAIDs in colorectal neoplasia (30).

Associations of polymorphisms in the COX-2 gene with colorectal adenomas and colorectal cancer have recently been investigated (31–39), including analyses of interactions with aspirin use (33,40–44), and the findings have been mixed. This existing literature is limited by incomplete analysis of genetic variation in the COX-2 gene, small sample sizes and inconsistent and poorly defined measurement of NSAID use. Further investigation into the association between COX-2 genotype and colorectal neoplasia and interaction with NSAID use is needed to clarify the role of genetic variation in COX-2 and may identify individuals who would benefit the most from the use of NSAIDs in the prevention of colon cancer.

The present analysis was undertaken to investigate the impact of COX-2 polymorphisms on risk of colorectal adenoma recurrence and the interaction between COX-2 genotype and aspirin treatment among participants at high risk of adenoma recurrence who were randomly assigned to placebo or aspirin in a large adenoma chemoprevention trial (19). By providing a more

comprehensive analysis of the genetic variation in a relatively large population with a precisely defined and randomized exposure to aspirin treatment, this investigation overcomes many of the limitations of the existing literature.

Materials and Methods

Study Design and Population

We conducted a cohort analysis of the association between COX-2 genotypes and colorectal adenoma recurrence among participants from the Aspirin/Folate Polyp Prevention Study (19, 45). Eligible participants were aged 21–80 with no history of colorectal cancer or any familial colorectal cancer syndrome but with a recent history of a histologically confirmed colorectal adenoma and a complete colonoscopy within three months before enrollment with no remaining colorectal polyps. At enrollment, data was collected on potential risk factors for colorectal cancer, including cigarette smoking, alcohol use and body mass index (BMI). After a run-in period during which compliance with study procedure was assessed, a total of 1121 subjects were randomized to aspirin treatment (placebo, 81 mg or 325 mg daily) and folic acid treatment (placebo or 1 mg daily) in a three-by-two factorial design and followed until a subsequent colonoscopy was performed. The study was completed by 1084 participants (96.7% of 1121 randomized) who had a follow-up colonoscopy at least one year after randomization (mean intervention period ± standard deviation, 32.7 ± 3.8 months). Of these participants, 979 (90.3%) had DNA available for genotyping and are included in the analyses presented here. This work was approved by the institutional review boards at all participating institutions, including the National Cancer Institute, where genotyping was performed, and each of the collaborating clinical centers. All subjects provided written informed consent.

Genotyping

Genomic DNA was isolated from whole blood cells using proteinase K digestion and phenol-chloroform extraction. The COX-2 polymorphisms were genotyped with the 5'nuclease TaqMan allelic discrimination assay using the ABI 7900 Sequence Detection System (Applied Biosystems, Foster City, CA) as previously described (46). Briefly, oligonucleotide probes were labeled with two fluorescent dyes, FAM and VIC, to distinguish between the two alleles of each biallelic SNP. All assays were designed and developed using Assays-by-Design (Applied Biosystems). All primers and probes were synthesized by Applied Biosystems. Each 384-well assay contained internal quality controls of homozygous wild-type, heterozygous, and homozygous variant alleles for the respective polymorphisms along with no template controls. Assays were set up in 384-well plates using 2.5 μl of the 2X TaqMan Universal Master Mix (no AmpErase UNG) including forward/reverse primers and FAM/VIC-labeled probes and 2–5 ng of DNA in a final volume of 5 μl. The thermal cycling conditions for the ABI 7900HT Sequence Detector included an initial setting of 95°C for 5 minutes followed by 40 cycles each of 92°C for 15 seconds and 60° C for 1 minute. Data output was processed and downloaded electronically into the analysis program. The SDS 2.1 analysis software (Applied Biosystems) was used to determine the genotype calls.

Eight haplotype tagging SNPs were chosen for genotyping based upon their minor allele frequencies (MAFs) of 9% and above as detailed at the SeattleSNPs web site[1]: rs689466 (798), rs20417 (1228), rs2745557 (2331), rs5277 (3355), rs20432 (5229), rs5275 (8494), rs2206593 (9123), and rs4648310 (11027). The numbers in parentheses refer to positions in the gene (Genbank entry AY382629). A total of 19 (2%) blinded replicates were included in the set of DNAs genotyped. As the concordance rate for rs689466 was very low (47%), this SNP was dropped from the analysis. For the remaining seven SNPs the concordance rate was 100% and

[1]http://pga.gs.washington.edu/data/ptgs2/

the completion rates ranged from 96 to 99.6 %. Observed and expected genotype counts among controls (participants with no adenoma recurrence) were evaluated for Hardy-Weinberg equilibrium (HWE) by a one degree of freedom χ^2 test. One SNP, rs2206593, deviated substantially from HWE (P<0.0001), with fewer heterozygotes than expected. In addition, the MAF observed (0.34) was much higher than expected (0.03–0.10) in populations of European ancestry (CEU) based on information in the NCBI database (dbSNP), indicating likely genotyping error. Thus, rs2206593 was also dropped from the analysis. The remaining six SNPs, which all exhibited HWE and allele frequencies similar to expected from available databases, were included in the analyses (see Table 1). Gene coverage was determined using the tagger application in the Haploview software program (47) by downloading CEU genotypes from the HAPMAP database and measuring the % of common variants (defined as SNP MAF >0.03) tagged ($r^2 > 0.8$) by genotyping these six SNPs.

Race and Ethnicity

At enrollment, subjects were asked to complete a questionnaire in which they identified their "race or ethnic background" by selecting one of the following: (1) "white, not of Hispanic origin", (2) "black, not of Hispanic origin", (3) "Hispanic", (4) "American Indian or Alaskan Native", (5) "Asian or Pacific Islander", (6) "other", (7) "uncertain". There were no responses of (7) "uncertain". In Table 1, categories 4 and 6 are not shown because to the very small numbers in these two groups. For multivariable analyses, categories 2–6 were combined into one group to create a dichotomous variable.

Statistical Methods

Comparisons of subject characteristics by adenoma recurrence status were performed by Pearson Chi-square tests for categorical variables and two sample t-tests for continuous variables. The principal outcome measured during the trial was the occurrence of one or more adenomas during randomized treatment; however, we also evaluated the occurrence of one or more advanced lesions (defined as tubulovillous or villous adenomas, adenomas ≥1 cm in diameter, adenomas with severe dysplasia, and invasive cancer). Risk ratios were calculated using an overdispersed generalized linear regression model for the Poisson distribution as an approximation to the bionomial family (48). Relative risks (RRs) and 95% confidence intervals (95% CIs) were used to estimate the association between COX-2 genotype and risk of adenoma recurrence. Unadjusted, minimally adjusted (for age, gender, and race) and maximally adjusted (for age, gender, race, BMI, smoking status, alcohol use, clinical center, follow-up time, aspirin treatment and folate treatment) RRs were calculated. Results for crude and fully adjusted models were nearly identical to the minimally adjusted RRs that are reported in the tables. Homozygosity for the most frequent genotype was set as the referent category and RRs were calculated by comparing the heterozygotes to the referent and the minor allele homozygotes to the referent using indicator variables (i.e. an unrestricted genetic inheritance mode). Where appropriate (i.e. rs5277) a recessive inheritance mode was also assessed by comparing minor allele homozygotes to a referent group including major allele homozygotes and heterozygotes.

Phased haplotypes pairs and probabilities were estimated using Powermarker V3.25 (49) using the EM algorithm (50). Generalized linear regression was used to estimate haplotype association with risk of adenoma recurrence taking haplotype uncertainty (probabilities) into account. The haplotype was modeled as a continuous variable where the number of copies of each allele was multiplied by its probability to obtain a continuous variable which was used as the predictor variable. The most common haplotype was used as the reference and omitted from the model. For each haplotype, the model provides an estimate of the risk associated with each additional copy of the specified haplotype. The most frequent haplotypes (with frequencies above 2%) were analyzed individually and the remaining "rare" haplotypes were pooled.

We also evaluated whether aspirin treatment or participant characteristics interacted with COX-2 genotypes to modify associations with adenoma risk using interaction terms in the regression models and Wald tests. The following participant characteristics were considered: gender, age (above versus below median), BMI (< 25, 25 to < 30, ≥30 kg/m^2), current alcohol use (drinker versus non-drinker) and smoking status (current smoker versus non-smoker). To increase power for tests of effect modification, we combined the heterozygotes and the minor allele homozygotes into one category and compared it to the referent group of the major allele homozygotes. Stratified analyses were used to obtain stratum specific estimates of risk and confidence intervals. Effect modification was only assessed for the risk of any adenoma because of the small numbers of advanced lesions.

Analyses of study treatment with aspirin or folate were conducted according to the intention-to-treat principle. Two-sided P values < 0.05 were considered statistically significant. We did not adjust for multiple testing as per convention in epidemiologic studies testing *a priori* defined hypotheses. Stata (version 9) was used for all analyses.

Results

Participant Characteristics and Gene Frequencies

Demographics and other selected characteristics of the subset of participants with DNA available for COX-2 genotyping are presented in Table 2. Among the 979 participants, 433 (44.2 %) had a recurrence of one or more colorectal adenoma during follow-up. Age, gender and race were all associated with risk of adenoma recurrence: individuals with at least one colorectal adenoma recurrence during follow-up were slightly older than those who did not have a recurrence (59.1 and 56.4 years respectively, p<0.001), males were at greater risk compared to females (p=0.004), and Hispanics and Asians appeared to be at reduced risk compared to Caucasians (p=0.063 and 0.052, respectively). In addition, cigarette smoking, body mass index (BMI) and alcohol use were associated with risk of adenoma recurrence: current smokers were at greater risk compared to non-smokers (p = 0.001), individuals with a BMI between 25 and < 30 kg/m^2 or ≥30 kg/m^2 were at greater risk than those with a BMI <25 kg/m^2 (p = 0.018 and 0.005 , respectively), and those who consumed alcoholic drinks were at greater risk than non-drinkers (p = 0.008) As observed in the full analysis of the trial (19,45), individuals who were randomized to 81 mg/day of aspirin treatment were less likely to have a recurrence compared to those randomized to the placebo arm (p=0.017), whereas 325 mg/day aspirin (p=0.86) and folate had no significant effect (p=0.40). Finally, the mean follow-up time did not differ according to adenoma recurrence status (p=0.32).

MAFs and gene locations for the six COX-2 SNPs that were included in this analysis are shown in Table 1. All six SNPs were in HWE among controls (participants with no adenoma recurrence) and genotype frequencies were similar to those expected for a population with predominantly European ancestry.[2] Notably, the gene frequencies for several of the SNPs (rs5277, rs20432, rs5275) varied statistically significantly by self-reported race and ethnicity (Table 1). Due to linkage disequilibrium, genotyping these six SNPs captured approximately 80% of the common genetic variants (SNPS with MAF > 0.03) in the COX-2 gene.

Association of COX-2 Genotypes and Haplotypes with Risk of Colorectal Adenoma Recurrence

We investigated whether any of the six COX-2 SNPs were associated with the recurrence of any adenoma or with advanced lesions (Table 3). Two COX-2 SNPs were associated with a statistically significant increased risk of adenoma recurrence: rs5277 and rs4648310. For

[2]http://www.hapmap.org

rs5277, minor allele homozygotes (CC genotype) had a 49% increased risk of any adenoma compared to the common GG genotype (unadjusted absolute risk 63.6% vs. 46.7%, adjusted RR = 1.49, 95% CI = 1.00–2.23). Similar results were seen when the analysis was restricted to Caucasians only (adjusted RR = 1.61, 95% CI = 1.08–2.39). The pattern seen with rs5277 was indicative of a recessive inheritance mode, since heterozygotes did not have an increased risk, and was statistically significant when analyzed as such (adjusted RR = 1.48, 95% CI = 1.00–2.21, P = 0.05, not shown). For rs4648310 there were no minor allele homozygotes, however, the heterozygotes (AG genotype) had a statistically significant 35% increased risk of any adenoma compared to those with the common AA genotype (unadjusted absolute risk 60.4% vs. 43.3%, adjusted RR = 1.35, 95% CI = 1.03–1.77). There was also a non-significant 40% increase risk of an advanced lesion among these heterozygotes (adjusted RR = 1.40, 95% CI = 0.67–2.91). Similar results were seen when the analysis was restricted to Caucasians only for any adenoma (adjusted RR = 1.34, 95% CI = 1.01–1.76) or advanced lesions (adjusted RR = 1.42, 95% CI = 0.68–2.97). There was no evidence for heterogeneity between the results for advanced vs. non-advanced adenomas for any of the COX-2 genotypes analyzed (data not shown). In addition, in stratified analyses, there was no evidence for interaction between any COX-2 genotype and subject characteristics, including gender, age, BMI, alcohol use or smoking status (data not shown).

The r2 correlation between the two SNPs associated with risk (rs5277 and rs4648310) was 0.16 indicating that they are not in strong linkage disequilibrium. In addition, when both SNPs were included in the same regression model their associations with risk (RRs) remained substantially similar to those seen in the single SNP models (in Table 3) although the associations were no longer statistically significant due to reduced power (data not shown).

Haplotype analysis revealed six common haplotypes with a frequency above 2% (Table 4). The 5 most common haplotypes were not associated with risk of adenoma recurrence. However, the 6^{th} most common haplotype, with a frequency of 2.7%, was associated with a statistically significant 37% increased risk per copy of the haplotype relative to the most common haplotype (adjusted RR = 1.37 , 95% CI = 1.03–1.82). Notably, this haplotype contained variant alleles at rs5277 and rs4648310, both of which were associated with deleterious effects at the genotype level (see Table 3). For advanced adenomas (data not shown), there was an increased risk of similar magnitude that was not statistically significant (adjusted RR = 1.36, 95% CI = 0.62–2.96).

Interaction with Aspirin Treatment

Next, we evaluated whether there was evidence for an interaction between any COX-2 genotype and aspirin treatment on risk of adenoma recurrence (Table 5). There was suggestive evidence for an interaction between rs4648310 genotype and 81 mg of aspirin treatment. As observed in the overall population, 81 mg of aspirin treatment daily was associated with a protective effect among major allele homozygotes (AA genotype) (adjusted RR = 0.76, 95% CI = 0.63–0.91). However, 81 mg of aspirin was not protective among heterozygotes (AG genotype): RR = 1.55 (95% CI = 0.98–2.46) compared to AA homozygotes given placebo (Table 5) and RR = 1.26 (95% CI = 0.61–2.57) compared to AG heterozygotes given placebo (not shown). This interaction did not reach statistical significance (P = 0.11) unless the placebo and 325 mg aspirin groups were combined (P = 0.037, not shown). Similar results were obtained when this analysis was restricted to Caucasians (not shown).

Discussion

In this analysis of a randomized aspirin trial, we investigated the impact of common COX-2 variants on risk of colorectal adenoma recurrence and aspirin chemoprevention. We observed statistically significant increased risks of adenoma recurrence of 49% for the rs5277 CC

genotype and 35% for the rs4648310 AG genotype relative to the homozygous wild type genotypes. Notably, for each copy of a common haplotype containing both variant alleles there was a statistically significant 37% increased risk of adenoma recurrence. In addition, there was suggestive evidence that the protective effect of treatment with 81 mg/day aspirin on risk of colorectal adenoma recurrence was modified by the rs4648310 genotype because the aspirin protective effect was not seen among AG heterozygotes.

A recent review summarized the associations of COX-2 polymorphisms with risk of colorectal adenoma and cancer (51). Although results on 17 COX-2 polymorphisms from 14 studies were reviewed, the ability to reach conclusions was hampered by small sample sizes and other significant limitations in the studies and the analyses (51). Four of the COX-2 polymorphisms that we examined in the current study have been investigated previously for associations with colorectal adenoma, including rs20417, rs20432 and rs5275 (for which we observed no association) and rs5277 (for which we observed an increased risk). For rs20417, pooled analysis showed no association with adenomas, but a trend towards increased risk for cancer that reached statistical significance only among Asians (51). For rs5275, pooled analyses showed a non-significant trend towards a protective effect for adenomas and no association with cancer (51). For rs20432, no associations were found for adenomas or cancer (51). For rs5277, a synonymous SNP in exon 3, we found a statistically significant *increased* adenoma risk, whereas in the pooled analysis (51) there was a non-significant trends towards a *reduced* adenoma risk based on two previous studies (42,44) and towards an increased cancer risk cancer based on two previous studies(52) (34). Thus, our data regarding rs5277 appears to be more consistent with the previous cancer data than the adenoma data. We also examined two COX-2 polymorphisms not previously investigated: rs2745557 and rs4648310. We did not detect an association for rs2745557 with adenoma risk. However, rs4648310, located in the 3' untranslated region, was associated with an increased risk for any adenoma and a non-significant increased risk for advanced adenoma.

Another recent review examined interactions of COX-2 polymorphisms with NSAID use in preventing colorectal neoplasia (53). A total of 17 polymorphisms have been investigated across six studies, but no statistically significant interactions have been reported, possibly due to limited sample sizes (53). A borderline significant interaction was reported for rs20417 (40), but was not confirmed in a different study (42) or in the present analysis. In the current study, we found suggestive evidence for an interaction of aspirin treatment with rs4648310, which also had a main effect on adenoma risk. However, the power to detect interactions was limited in the present analysis and it will be important to follow-up on this finding in other populations because of the potential clinical implication for aspirin chemoprevention. Nonetheless, at the population level, the impact will be limited due to the low frequency of the variant allele. As discussed previously, aspirin is not thought to inhibit Cyclooxygenase-2 at the doses used here; however, it may suppress the expression of COX-2 (19). Regardless, the mechanism is not clear and this finding may be due to chance. Finally, it is worth noting that aspirin's effects are likely to involve multiple pathways in addition to COX-2, including COX-1 and other non-COX mechanisms (54,55).

The functional effects of the two SNPs associated with adenoma risk, rs4648310 and rs5277, are not yet known. In addition, it can not be determined from this investigation whether they represent causative SNPs or whether they are simply markers in linkage disequilibrium with unknown or unmeasured causative SNPs. However, based on their association with increased risk, one possibility is that they may either cause or be associated with an increase in COX-2 activity or expression. Notably, rs4648310 was also recently associated with an increased risk of aggressive prostate cancer, and this effect was modified by dietary omega-3 fatty acids (56).

There are several potential limitations to consider in the current analysis. The generalizability of the results may be limited as the study was conducted on predominantly Caucasian volunteers in a clinical trial with a prior history of adenoma. The endpoint of the study was occurrence of new adenomas rather than colorectal cancer. In addition, we had limited power to investigate risk of advanced adenomas, which were uncommon during follow-up (10% of the population), and interactions with two levels of aspirin treatment. Finally, the associations detected were modest and our findings may be due to chance. Nonetheless, there are several important strengths associated with this study, including the relatively large sample size and the prospective study design, which makes recall or selection bias unlikely. In addition, the outcome (adenoma) was uniformly assessed by a single study pathologist who was blinded to study treatment assignment. Importantly, exposure to aspirin was by randomized treatment with excellent compliance and follow-up. Finally, our analysis encompassed approximately 80% of the common genetic variants in the gene.

In summary, we observed an increased risk of colorectal adenoma recurrence in association with two variants (rs5277 and rs4648310) in the COX-2 gene as well as a possible interaction between aspirin use and one of these variants (rs4648310). Although these results provide support for the hypothesis that COX-2 plays a role in the etiology of colon cancer and may be a target for aspirin chemoprevention, the associations were modest and may be due to chance. Thus, it is important to interpret these results with caution and to replicate them in other colorectal adenoma and cancer populations.

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Table 1

COX-2 polymorphisms: location and minor allele frequency by race and ethnicity

SNP ID	Major/minor allele	Location*	Minor Allele Frequencies					p-value†
			All	Caucasians	African Americans	Hispanics	Asians	
rs20417	G/C	5'-flanking Promoter	0.16	0.16	0.21	0.20	0.19	0.43
rs2745557	C/T	Intron 1	0.18	0.19	0.10	0.14	0.09	0.09
rs5277	G/C	Exon 3 synonymous	0.15	0.16	0.04	0.11	0.03	0.002
rs20432	T/G	Intron 5	0.17	0.15	0.47	0.19	0.22	<0.001
rs5275	T/C	Exon 10 3'-UTR	0.35	0.34	0.61	0.30	0.31	<0.001
rs4648310	A/G	3'-flanking	0.03	0.03	0	0.01	0	0.49

*UTR = untranslated region; flanking = untranscribed region.

†Fisher's exact test for comparison across all groups.

Table 2

Selected characteristics of the study participants with COX-2 genotype data

Characteristic	Total	No Adenoma Recurrence	Adenoma Recurrence	p-value*
No. of subjects	979 (100)	546 (55.8)	433 (44.2)	
Age at enrollment (mean ± SD), years	57.6 ± 9.6	56.4 ± 9.7	59.1 ± 9.3	<0.001
Gender				
Male, No. (%)	630 (64.4)	330 (60.4)	300 (69.3)	
Female, No. (%)	349 (35.6)	216 (39.6)	133 (30.7)	0.004
Race and ethnicity, No. (%)				
Caucasian	864 (88.3)	473 (86.6)	391 (90.3)	
African American	45 (4.6)	22 (4.0)	23 (5.3)	0.44
Hispanic	45 (4.6)	31 (5.7)	14 (3.2)	0.063
Asian	18 (1.8)	14 (2.6)	4 (0.9)	0.052
Other	7 (0.7)	6 (1.1)	1 (0.2)	0.10
Body mass index (kg/m$^{2)}$, No. (%)				
<25	309 (31.6)	193 (35.4)	116 (26.9)	
25–30	455 (46.6)	245 (45.0)	210 (48.6)	0.018
≥30	213 (21.8)	107 (19.6)	106 (24.5)	0.005
Current cigarette smoker, No. (%)				
No	841 (86.2)	487 (89.4)	354 (82.1)	
Yes	135 (13.8)	58 (10.6)	77 (17.9)	0.001
Current alcohol use, No. (%)				
No	293 (31.2)	181 (34.7)	112 (26.7)	
Yes	647 (68.8)	340 (65.3)	307 (73.3)	0.008
Aspirin treatment group, No. (%)				
Placebo	329 (33.6)	174 (31.9)	155 (35.8)	
81 mg aspirin	330 (33.7)	205 (37.6)	125 (28.9)	0.017
325 mg aspirin	320 (32.7)	167 (30.6)	153 (35.3)	0.86
Folate treatment group, No. (%)				
Placebo	449 (45.9)	258 (47.3)	191 (44.1)	
1 mg folate	452 (46.2)	247 (45.2)	205 (47.3)	0.40
Not randomized to folate	78 (8.0)	41 (7.5)	37 (8.6)	0.42
Follow-up time (mean ± SD), months	32.7 ± 3.5	32.6 ± 3.3	32.8 ± 3.8	0.32

* Tests for comparison between group with no adenoma recurrence and group with adenoma recurrence using two sample t-test for continuous variables and Pearson chi-square test for categorical variables.

Table 3

Association of COX-2 genotype with risk of adenoma recurrence

SNP ID*		Any adenoma		Advanced Lesions†	
		Occurrence/Total (%)	RR‡ (95% CI)	Occurrence/Total (%)	RR‡ (95% CI)
rs20417	GG	290/670 (43.3)	1.00 (referent)	69/670 (10.3)	1.00 (referent)
	GC	113/239 (47.3)	1.11 (0.94–1.31)	24/239 (10.0)	0.98 (0.63–1.53)
	CC	13/31 (41.9)	0.97 (0.64–1.46)	3/31 (9.7)	0.92 (0.31–2.77)
rs2745557	CC	292/660 (44.2)	1.00 (referent)	70/660 (10.6)	1.00 (referent)
	CT	128/286 (44.7)	0.99 (0.85–1.16)	25/286 (8.7)	0.82 (0.53–1.26)
	TT	11/26 (42.3)	0.90 (0.58–1.42)	2/26 (7.7)	0.68 (0.18–2.61)
rs5277	GG	303/706 (42.9)	1.00 (referent)	68/706 (9.6)	1.00 (referent)
	GC	112/240 (46.7)	1.05 (0.89–1.24)	27/240 (11.3)	1.15 (0.75–1.77)
	CC	14/22 (63.6)	1.49 (1.00–2.23)	2/22 (9.1)	0.96 (0.25–3.66)
rs20432	TT	288/664 (43.4)	1.00 (referent)	68/664 (10.2)	1.00 (referent)
	TG	114/246 (46.3)	1.09 (0.93–1.29)	22/246 (8.9)	0.88 (0.55–1.39)
	GG	19/38 (50.0)	1.21 (0.85–1.72)	5/38 (13.2)	1.29 (0.54–3.08)
rs5275	TT	190/423 (44.9)	1.0 (referent)	40/423 (9.5)	1.00 (referent)
	TC	176/407 (43.2)	0.96 (0.82–1.12)	38/407 (9.3)	0.97 (0.64–1.48)
	CC	61/133 (45.9)	1.04 (0.84–1.29)	19/133 (14.3)	1.50 (0.89–2.53)
rs4648310§	AA	398/919 (43.3)	1.0 (referent)	89/919 (9.7)	1.00 (referent)
	AG	32/53 (60.4)	1.35 (1.03–1.77)	7/53 (13.2)	1.40 (0.67–2.91)

*No. subjects missing genotype data: 39 for rs20417, 7 for rs2745557, 11 for rs5277, 31 for rs20432, 16 for rs5275 and 7 for rs4648310.

†Advanced lesions include invasive carcinoma or adenomas with at least 25% villous component, high-grade dysplasia, or size ≥ 1 cm.

‡Adjusted for age, gender and race.

§There were no minor allele homozygotes (GG).

Table 4

Association of COX-2 haplotypes with risk of adenoma recurrence

COX-2 Haplotype*	Haplotype Frequency (%)	Occurrence/Total (%)	RR† (95% CI)	p-value‡
GCGTTA	32.11	262/631 (41.9%)	1.00 (referent)	
GCGTCA	18.23	150/355 (42.3%)	0.99 (0.85–1.15)	0.86
GTGTTA	17.28	150/338 (44.4%)	1.01 (0.87–1.18)	0.86
CCGGCA	15.12	136/298 (45.6%)	1.08 (0.93–1.26)	0.33
GCCTTA	11.81	107/229 (46.7%)	1.08 (0.91–1.29)	0.36
GCCTTG	2.73	32/53 (60.4%)	1.37 (1.03–1.82)	0.03
Rare§	2.71	27/48 (56.3%)	1.35 (1.01–1.80)	0.04

*SNP order according to Table 2.

†Adjusted for age, gender and race.

‡Wald test for comparison to most frequent haplotype.

§Combination of haplotypes with a frequency of less than 2%.

Table 5

Association of COX-2 genotype and aspirin treatment group with risk of adenoma recurrence

Polymorphism		Placebo		81 mg aspirin		325 mg aspirin		p-value[†]
		Occurrence/Total (%)	RR[*] (95% CI)	Occurrence/Total (%)	RR[*] (95% CI)	Occurrence/Total (%)	RR[*] (95% CI)	
rs20417	GG	109/226 (48.2%)	1.00 (referent)	84/233 (36.1%)	0.73 (0.59–0.91)	97/211 (46.0%)	0.93 (0.76–1.15)	0.53
	GC + CC	40/87 (46.0%)	0.96 (0.73–1.26)	35/85 (41.2%)	0.85 (0.64–1.13)	51/98 (47.9%)	1.08 (0.84–1.39)	
rs2745557	CC	105/218 (48.2%)	1.00 (referent)	83/227 (36.6%)	0.75 (0.60–0.93)	104/215 (48.4%)	0.99 (0.81–1.21)	0.52
	CT + TT	50/109 (45.9%)	0.93 (0.73–1.20)	42/100 (42.0%)	0.84 (0.64–1.10)	47/103 (45.6%)	0.91 (0.70–1.18)	
rs5277	GG	107/236 (45.3%)	1.00 (referent)	92/240 (38.3%)	0.83 (0.67–1.02)	104/230 (45.2%)	0.97 (0.79–1.19)	0.65
	GC + CC	47/90 (52.2%)	1.09 (0.84–1.41)	32/84 (38.1%)	0.80 (0.59–1.07)	47/88 (53.4%)	1.13 (0.87–1.46)	
rs20432	TT	106/225 (47.1%)	1.00 (referent)	87/227 (38.3%)	0.80 (0.64–0.99)	95/212 (44.8%)	0.92 (0.75–1.14)	0.32
	TG + GG	42/92 (45.7%)	0.99 (0.76–1.29)	36/93 (38.7%)	0.83 (0.62–1.10)	55/99 (55.6%)	1.19 (0.93–1.51)	
rs5275	TT	72/142 (50.7%)	1.00 (referent)	57/145 (39.3%)	0.75 (0.58–0.97)	61/136 (44.9%)	0.88 (0.68–1.14)	0.44
	TC + CC	81/184 (44.0%)	0.87 (0.68–1.10)	67/179 (37.4%)	0.74 (0.57–0.95)	89/177 (50.3%)	0.96 (0.76–1.21)	
rs4648310[‡]	AA	148/315 (47.0%)	1.00 (referent)	113/313 (36.1%)	0.76 (0.63–0.91)	137/291 (47.1%)	0.99 (0.83–1.18)	0.11
	AG	7/12 (58.3%)	1.23 (0.70–2.19)	11/15 (73.3%)	1.55 (0.98–2.46)	14/26 (53.9%)	1.06 (0.70–1.60)	

[*] Adjusted for age, gender and race.

[†] Wald test for interaction between genotype and aspirin treatment.

[‡] There were no minor allele homozygotes (GG).