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Common Colorectal Cancer Risk Variants in *SMAD7* are Associated with Survival Among Pre-Diagnostic Non-Steroidal Anti-Inflammatory Drug Users: a Population-Based Study of Post-Menopausal Women

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Abstract

Common single nucleotide polymorphisms (SNPs) in *SMAD7* (18q21) have been linked to colorectal cancer (CRC) risk in genome-wide association studies, but little is known about their effects on survival. *SMAD7* regulates gastrointestinal inflammation by inhibiting transforming growth factor- β (TGFB), which can act as both a tumor suppressor and a promoter of metastasis. Regular use of cyclooxygenase-2 (COX2) inhibitors, such as non-steroidal anti-inflammatory drugs (NSAIDs), reduces the risk of developing CRC. Because COX2 overexpression reduces the growth suppressing effects of TGFB, we hypothesized that survival may depend on both *SMAD7* genotype and pre-diagnostic NSAID use. Post-menopausal women, ages 50–74, diagnosed with incident invasive CRC from 1997–2002 were identified using the Seattle-Puget Sound Surveillance, Epidemiology, and End Results cancer registry. Information on pre-diagnostic NSAID use and other risk factors was ascertained by interview, and women were followed-up for survival through December 31, 2009. 727 cases were genotyped for two GWAS hits in *SMAD7* with minor allele frequency > 30%, one with minor allele associated with decreased risk (rs4939827) and one with minor allele associated with increased risk (rs4464148). 242 deaths occurred, 160 attributable to CRC. Among those without distant disease at diagnosis, CRC-specific survival differed by genotype only for NSAID users: each minor allele of rs4939827 was associated with worse survival (hazard ratio (HR)=2.67, 95% confidence interval (CI): 1.33–5.37) and each minor allele of rs4464148 was associated with better survival (HR=0.41, CI:0.18–0.94). *SMAD7* variants known to be important for CRC risk were associated with disease-specific survival among pre-diagnostic NSAID users.

INTRODUCTION

Mothers against decapentaplegic homolog 7 (*SMAD7*) is a key inhibitor of transforming growth factor- β (TGFB), an inflammatory cytokine that regulates cellular proliferation, motility, and apoptosis (Nakao et al., 1997). Regulation of TGFB by *SMAD7* is crucial to maintaining gastrointestinal homeostasis (Hong, 2010). *SMAD7* overexpression is commonly found in patients with chronic inflammatory conditions of the colon (Monteleone et al., 2004), and may be associated with prognosis in patients with colorectal cancer (CRC)

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(Fukushima et al., 2003; Xie et al., 2003; Xu and Pasche, 2007; Bellam and Pasche, 2010). Loss of SMAD/TGFB signaling fidelity interrupts the principal role of TGFB as a growth inhibitor, allowing unchecked cellular proliferation (Akhurst and Derynck, 2001; Roberts and Wakefield, 2003; Halder et al., 2005; Levy and Hill, 2006). After tumor formation, compensatory TGFB overexpression supports the epithelial to mesenchymal transition (EMT) (ten Dijke and Hill, 2004; Elliott and Blobel, 2005), contributing to the disintegration of the extracellular matrix, reducing cellular adhesion, stimulating angiogenesis through interactions with vascular endothelial growth factor (VEGF) (Nowak et al., 2008), and modulating tumor necrosis factor (TNF)-mediated apoptosis (Hong et al., 2007).

Recent genome-wide association studies (GWASs) have consistently identified genetic variants in *SMAD7* as being modestly associated with the risk of developing CRC (Broderick et al., 2007; Tenesa et al., 2008; Tomlinson et al., 2008; Curtin et al., 2009). In a pooled analysis across four populations, Broderick et al. (2007) estimated a 15% (95% confidence interval (CI): 11-19%) decrease in CRC risk per minor allele of rs4939827 and a 15% (CI: 9-21%) increase in risk per minor allele of rs4464148. These two intronic SNPs, 5,569 base pairs apart, are not in high linkage disequilibrium (LD) and are common in the population. Recently, variants in *SMAD7* have been investigated with respect to survival after diagnosis with inconsistent results (Tenesa et al., 2010; Slattery et al., 2011b).

Cyclooxygenase-2 (COX2) is overexpressed in colorectal adenoma and adenocarcinoma (Gupta and Dubois, 2001), and regular use of COX inhibitors, such as non-steroidal anti-inflammatory drugs (NSAIDs), reduces the risk of developing CRC (Giovannucci et al., 1994; Sandler et al., 2003; Chan et al., 2005; Flossmann and Rothwell, 2007; Cole et al., 2009). Use of NSAIDs may also improve survival following diagnosis, possibly by ameliorating the long-term effects of inflammation on tumor progression, leading to less malignant behavior (Thun et al., 1991; Bardia et al., 2007; Zell et al., 2009; Coghill et al., 2011a, b). NSAIDs may up-regulate the expression of members of the TGFB family that have been linked to antiproliferative activity in colorectal cell cultures (Baek et al., 2001). This is consistent with evidence that suggests that the tumor suppressing effects of TGFB may be attenuated by COX2 overexpression (Enders, 2007).

It is unclear if genetic variation in *SMAD7*, in particular, interacts with COX2, but a large case-control study undertaken to extend the GWAS findings noted a statistically significant interaction between *SMAD7* variants and use of NSAIDs two years prior to diagnosis (rs4939827 was evaluated, but not rs4464148) (Slattery et al., 2010). If NSAID users have enhanced TGFB-mediated tumor suppression, they may also have enhanced TGFB-mediated metastatic promotion, and we were able to examine this relation using *SMAD7* genotype information collected as part of a study of CRC in post-menopausal women from Western Washington State.

MATERIALS AND METHODS

Study Population

The study cohort included incident cases of invasive colorectal adenocarcinoma occurring between 1997 and 2002 from 13 counties in Washington State that report to the Seattle-Puget Sound Cancer Surveillance System, a population-based cancer registry that is part of the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program. Eligible women, ages 50-74 years, also participated in the Seattle Colon Cancer Family Registry (Seattle CCFR) and were originally included as cases in a case-control study of CRC incidence (Newcomb et al., 2007a, b). All cases were prospectively followed up for survival. Vital status, including date and cause of death, was ascertained from the National Death Index. Additional details of the study design are reported elsewhere

(Newcomb et al., 2007b). Informed consent was obtained from all participants, and the study was approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center (FHCRC).

Study Interview

A structured telephone interview that ascertained information on known and suspected CRC risk factors was conducted as soon as possible after a woman's cancer diagnosis was reported to the SEER program. Questions were limited to exposures that occurred prior to two years before diagnosis. Specifically, the interview covered: use of common medications, including NSAIDs and post-menopausal hormone therapy; behaviors such as cigarette smoking; screening endoscopy (colonoscopy or sigmoidoscopy) at least two years prior to diagnosis; history of CRC in first-degree relatives; medical history including height, weight, and inflammatory bowel disease (Crohn's disease or ulcerative colitis); as well as demographics such as age at diagnosis and race. In total, 73% of eligible women with CRC completed the interview (Coghill et al., 2011b). During the interview all women were invited to provide DNA from either blood or a buccal sample (62% agreed). The mean time between the date of diagnosis and the date of interview to determine exposure history was 8.0 months (SD = 3.1).

Regular pre-diagnostic NSAID use was defined as use of any aspirin- or ibuprofen-containing medications on at least two occasions per week for at least four continuous weeks anytime earlier than two years before the date of CRC diagnosis. This definition was chosen to be consistent with previous studies of NSAID use and CRC incidence (Giovannucci et al., 1994). Use of prescription NSAIDs such as sulindac, rofecoxib, and celecoxib, or common non-NSAID pain medications, such as acetaminophen, was not considered.

DNA Samples and Genotyping Procedures

Genomic DNA was extracted using Qiagen's QIAmp DNA extraction kit (Qiagen, Valencia, CA) and quantified by Picogreen (Invitrogen, Carlsbad, CA). Two *SMAD7* SNPs (rs4939827 and rs4464148) were genotyped using the Illumina GoldenGate bead-based genotyping technology at the FHCRC Genomics Shared Resource facility, and the resulting genotype data met the following QC criteria: GenTrain Score >0.4; 10% GC Score > 0.25; call frequency > 0.85; replicate errors < 2; duplicate concordance > 85%; and Hardy-Weinberg *P*-value > 0.0001. Intraplate and interplate replicates at a proportion of 5% were included on all plates, with 100% concordance. External control samples from the HapMap project were included on each plate (NA17116, NA07034; Coriell Cell Repository, Camden, NJ) to confirm reliability and reproducibility of the genotyping.

Ascertainment of Tumor Characteristics

Incident tumors were classified by anatomic subsite according to the International Classification of Diseases for Oncology (ICD-O) 3rd edition (Fritz, 2000). We defined proximal tumors as those located proximal to (and including) the splenic flexure (C18.0, C18.2-C18.5). Distal tumors were located in the descending or sigmoid colon (C18.6, C18.7). Rectal tumors were located in the rectosigmoid junction or rectum and included any overlapping lesion of the rectum, anus, and anal canal (C19.9, 20.9, 21.8). Clinical stage at diagnosis was classified into three categories according to the SEER Summary Stage Manual 2000 (localized, regional, distant) (Young et al., 2001). First-course of treatment (none, chemotherapy only, radiation only, or both chemotherapy and radiation) was obtained from SEER records. In total, 665 (91%) of the genotyped women provided tumor biospecimens, which were classified by degree of microsatellite instability status based on a panel of 10 markers in the mismatch repair pathway (stable if 0% unstable markers, low if < 30% unstable markers, high if ≥ 30% unstable markers) (Lindor et al., 2002).

Statistical Analysis

We plotted Kaplan-Meier survival estimates and evaluated differences in survival distributions with a stratified log-rank test. Proportional hazards regression was used to estimate hazard ratios (HRs) and 95% CIs per copy of the minor allele, assuming a log-additive model for both CRC-specific and overall survival. *P*-values for linear trend were calculated using an ordinal *SMAD7*-polymorphism variable. Each SNP was considered individually in two different models: Model 1 adjusted for age at diagnosis (50-59, 60-69, 70-74 years) and race (Caucasian, non-Caucasian); Model 2 additionally adjusted for: stage at diagnosis (localized, regional, distant); body mass index (BMI; < 25, 25-29, ≥ 30 kg/m²); screening endoscopy (never, ever); smoking status (never, former, recent); post-menopausal hormone therapy use of any type (never, ever); inflammatory bowel disease (no, yes); tumor subsite (proximal, distal, rectal); microsatellite instability status (stable, low, high, unknown); and first-course of treatment (none, chemotherapy only, radiation only, both chemotherapy and radiation, unknown). Because both SNPs have been found to be associated with CRC incidence, and any association of *SMAD7* with survival could be a consequence of stage, we also considered separate models that stratified associations by stage at diagnosis.

Women were classified as recent users of NSAIDs if they reported regular use as of two years prior to diagnosis. Women who had stopped using prior to two years before diagnosis and never users were classified as non-recent users. To determine if the genotype effect differed according to NSAID use, we evaluated the statistical significance of an interaction between an ordinal *SMAD7*-polymorphism variable and binary NSAID-use variable (recent use or no recent use). The proportional hazards assumption was assessed by considering interactions with log-transformed survival time. Survival time was calculated from the date of diagnosis as reported in the SEER record until death or the end of available follow-up (December 31, 2009). In the CRC-specific survival analysis, survival time was censored at the date of death from causes other than CRC.

To assess the potential for bias related to interview timing, we conducted two separate sensitivity analyses: 1) in proportional hazard regression models that calculated survival time starting from the date of diagnosis, we adjusted for the lag-time between diagnosis and interview date; and 2) we considered proportional hazard regression models that calculated survival time starting from the date of interview rather than the date of diagnosis. We also assessed whether findings were robust to categorization of regular pre-diagnostic NSAID use by duration of past use (never, ≤ 2 years of use, > 2 years of use), instead of by recency. All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC). All statistical tests were two-sided, with *P* < 0.05 considered statistically significant.

RESULTS

Characteristics of the Study Population

A total of 727 women with CRC had complete information on rs4939827 and rs4464148 genotypes, including 349 with tumors in the proximal colon and 378 with tumors in the distal colon or rectum. 649 women had localized or regional disease at diagnosis. Through December 31, 2009, a total of 160 deaths attributable to CRC occurred (76 proximal and 84 distal/rectal), as well as 82 deaths from other causes. The median duration of follow-up from the date of CRC diagnosis was 6.4 years.

The women were predominantly Caucasian (93%); most had no family history of CRC in first-degree relatives (83%), and most had not undergone a screening endoscopy two years prior to diagnosis (89%). In total, 355 women (49%) reported regular pre-diagnostic NSAID use, 186 of whom were still using regularly two years prior to diagnosis. Compared with

those who were not recent NSAID users, the women who used recently were older at diagnosis and more likely to be Caucasian, to be obese, use post-menopausal hormone therapy, and to have undergone a screening endoscopy. Recent users of NSAIDs were less likely to still be smoking cigarettes two years before diagnosis (Table 1).

Overall, the median duration of regular NSAID use was 3 years. Recent users were more likely to have used for a longer duration (median duration of 5 years for recent users and 1 year for those women who ceased use before at least 2 years prior to diagnosis). Consistent with the current recommendations for cardiovascular health, the majority of women took aspirin at a relatively low daily frequency. Overall, 210 (59%) women reported regularly using aspirin exclusively, 80 (22%) reported regularly using ibuprofen exclusively, and 68 (19%) regularly used both. Among those who used aspirin exclusively, the median frequency of use was once per day with a median duration of 3 years. In contrast, among those who used ibuprofen exclusively, the median frequency of use was twice per day with a median duration of 10 months.

The observed minor allele frequencies (MAFs) for the two *SMAD7* polymorphisms were 47% for rs4939827 (C allele) and 31% for rs4464148 (C allele), both of which are consistent with the HapMap CEU population. For both SNPs, genotype was unrelated to recent pre-diagnostic NSAID use (chi-square $P = 0.64$ and 0.24 for rs4939827 and rs4464148, respectively) and cancer stage at diagnosis (chi-square $P = 0.89$ and 0.70 for rs4939827 and rs4464148, respectively).

Associations with Survival

Figures 1 and 2 display the Kaplan-Meier survival estimates for each genotype stratified by recent regular use of NSAIDs among women without metastatic disease at diagnosis. In proportional hazard regression models for both SNPs, results for the minimally-adjusted and fully-adjusted models did not substantially differ. Results for both models are displayed in Table 2 for disease-specific survival and Table 3 for overall survival. There was no overall association with risk of death from CRC or any cause for either *SMAD7* polymorphism with and without stage adjustment. As a main effect, there was no statistically significant association between recent use of NSAIDs and survival, although the direction of the estimates suggests a reduced risk of death from CRC for recent users compared to those who were not recent users, consistent with previous reports.

The minor allele of rs4939827 was associated with an increased risk of death from CRC only among recent NSAID users. In particular, this association was most pronounced for CRC-specific survival among those with regional disease. In contrast, each minor allele of rs4464148 was associated with a decreased risk of death from CRC among recent NSAID users. Again, this association was most apparent for CRC-specific survival among those with regional disease at diagnosis. Overall survival did not differ by NSAID use for either *SMAD7* polymorphism. Additionally, the direction of these associations did not differ when stratifying by tumor subsite (proximal vs distal/rectal), but the smaller number of events in each subgroup reduced precision of the estimates (data not shown).

Models that adjusted for the lag-time between diagnosis and interview date, or calculated survival time from the date of interview rather than the date of diagnosis resulted in similar hazard ratios (data not shown). For both polymorphisms, former NSAID users had similar associations with survival per minor allele as never users (data not shown). Also, when categorizing regular pre-diagnostic NSAID use by duration (never, ≤ 2 years of use, > 2 years of use), rather than by recency, we observed associations that were similar in direction to those reported in Tables 2 and 3, but of diminished magnitude and not statistically significant (data not shown).

DISCUSSION

Our findings suggest that disease-specific survival varies according to *SMAD7* genotype, but only in patients who were regularly using NSAIDs before diagnosis. Among women who were using NSAIDs regularly prior to diagnosis, each minor allele of rs4939827 was associated with reduced survival, and each minor allele of rs4464148 was associated with improved survival. No association with survival for either SNP was observed among women who had never used NSAIDs or had discontinued use earlier than two years prior to diagnosis. We observed poorer survival for the minor variant previously shown to be associated with a reduced risk of developing CRC, and improved survival for the minor variant previously shown to be associated with an increased risk of developing CRC.

The contrast between the genetic effects on incidence and those on survival may be a consequence of the two faces of TGFB, which shifts from acting as a tumor suppressor to become an accelerator of metastatic activity after tumor formation (Pardali and Moustakas, 2007). *SMAD7* variants that more completely inhibit TGFB may decrease its tumor suppressing activity (resulting in increased risk), but may also decrease its ability to promote metastatic activity (resulting in slower tumor progression and improved survival). Likewise, *SMAD7* variants that result in up-regulation of TGFB may increase its tumor suppressing activity (resulting in decreased risk), but may also increase its ability to promote metastatic activity (resulting in accelerated tumor progression and reduced survival). Consistent with the role of SMAD/TGFB signaling in facilitating the EMT, this association was specific to women diagnosed with tumors that exhibited some direct tumor extension or lymph node involvement and not specifically local disease, although it should be noted that survival estimates for localized stage are based on only a small number of deaths.

Slattery et al. (2010) noted a more prominent reduction in the risk of developing CRC for those who regularly used NSAIDs two years before diagnosis for rs4939827 (odds ratio comparing CC to TT was 0.60; CI: 0.43-0.85 for recent users and 0.86; CI: 0.68-1.09 for those who were not recent users), and have additionally reported significant risk modifications with pre-diagnostic NSAID use for other SNPs in the SMAD/TGFB pathway (Slattery et al., 2011a). We observed that the association of these *SMAD7* polymorphisms with survival was limited to women who were still using NSAIDs up to two years prior to diagnosis, and was not observed among women who had discontinued use earlier.

SMAD7 plays an essential role in maintaining the inflammatory gastrointestinal state observed in patients with inflammatory bowel disease (IBD) (Monteleone et al., 2004). In animal models for IBD, direct inhibition of *SMAD7* via application of antisense oligonucleotides in vivo resulted in lower production of inflammatory cytokines and less injury to intestinal tissues (Monteleone et al., 2008). Gene expression studies have observed *SMAD7* underexpression in colonic mucosa following treatment with COX2 inhibitors (Glebov et al., 2006), and expression of members of the TGFB superfamily, including NSAID-activated gene 1/growth differentiation factor 15 (NAG1/GDF15), is known to be influenced by COX2 (Iguchi et al., 2009). The molecular mechanisms by which anti-inflammatory medications would interact directly with *SMAD7*, specifically, are not yet apparent, and the ability of COX2 overexpression to alter TGFB functionality may very well take place through SMAD-independent pathways (Enders, 2007). While not yet fully understood, our findings underscore the fact that inflammatory processes and related genetic variability may have important consequences for both disease incidence and progression. For instance, it is possible that use of NSAIDs shortly before clinical presentation could produce tumors with less aggressive characteristics (Masferrer et al., 2000), which are more likely to be impacted by genetic alterations in growth factor signaling.

Our findings may reflect differences in patterns of NSAID use. Recent users were more likely to have used regularly for a longer duration and may be more likely to continue use after diagnosis. It is possible that the distinction we observed between recent and non-recent users actually reflects the impact of *post*-diagnostic NSAID use. Information on post-diagnostic NSAID use was not available for this study, and so it is unclear whether the potential interaction that we observed reflects an association with pre- or post-diagnostic behavior or both. Further research is needed to consider whether *SMAD7* genotype can inform a more specific recommendation of post-diagnosis NSAID use for improved CRC survival. Clinically, it may be advantageous to target anti-inflammatory medications only to those who will benefit, and an opposition between the effects on incidence and the effects on survival suggests that the use of NSAIDs may require careful orchestration before and after a diagnosis of CRC in a manner that may depend, in part, on *SMAD7* genotype.

Our population-based cohort of post-menopausal women diagnosed with incident CRC was well-characterized. The SEER records provided standardized ascertainment of several clinically-relevant covariates that could account for differential prognosis including stage, subsite, and treatment. Complete, standardized, and up-to-date vital-status information was obtained from the National Death Index, which has been shown to be valid for research purposes (Calle and Terrell, 1993). Our analyses adjusted for important risk factors for the disease and those associated with survival after diagnosis.

This study has several limitations. Our study included only post-menopausal women. Case-control studies undertaken to extend the findings from GWASs have sometimes been inconsistent by sex. Slattery and colleagues found that the association between rs4939827 and incidence of colon cancer did not differ between men and women (P for interaction = 0.97) (Slattery et al., 2010). However, Thompson et al. (2009) noted a more marked decrease in risk for rs4939827 (P for interaction = 0.02) and an increase in risk for rs4464148 (P for interaction = 0.08) among women compared to men. Accordingly, it is not clear if the associations we observed are sex-specific or if they can be generalized to men.

Because regular NSAID use is generally recommended for those with cardiovascular risk factors, we were not able to exclude the possibility that an excess of deaths from cardiovascular causes accounts for the lack of CRC deaths for certain *SMAD7* genotypes, although among the 186 women using NSAIDs two years prior to CRC diagnosis, only 7 of the 24 deaths attributed to causes other than CRC were from cardiovascular-related conditions. A limited number of deaths, in general, prevented us from specifically examining whether the association between these *SMAD7* polymorphisms and pre-diagnostic NSAID use was related to usage characteristics such as frequency, timing, duration of use, type of medication, as well as whether the association differed by anatomic tumor subsite.

The susceptibility loci in *SMAD7* suggested from GWASs are intronic, and may only be associated with disease risk by being in LD with yet to be discovered functional variants of 18q21. Pittman et al. (2009) identified a polymorphism of 18q21 that may alter *SMAD7* expression by modifying transcription-factor binding. Other such SNPs may also be discovered in subsequent investigations. Additional variants of 18q21 were not available from the parent study, nor were other SNPs in the SMAD/TGFB pathway. Polymorphisms in *TGFB1*, *TGFBRI*, and *SMAD3*, for example, have recently been linked to CRC survival (Försti et al., 2010; Slattery et al., 2011b), and a more comprehensive analysis encompassing additional genes in this pathway may lead to further insights about the roles of inflammation, growth factor signaling, and survival outcomes.

In our study, we focused specifically on loci in *SMAD7* which, unlike variants in *TGFB1*, *TGFB1*, and *SMAD3*, have been modestly, yet consistently, linked to CRC risk in previous GWASs. Additionally, *SMAD7*'s involvement in colorectal carcinogenesis is thought to be independent of its association with other genes in the SMAD/TGFB pathway, based on observations that adenocarcinoma cases have significantly lower *SMAD7* expression than cases with adenomatous polyps, regardless of 18q21 copy number (Pittman et al., 2009). Lastly, we did not have information on any expression status in tumor samples. Future investigations should evaluate whether differential survival for NSAID users according to *SMAD7* genotype can be explained protein expression or mRNA levels of *SMAD7*, *TGFB*, or *COX2* in tumor samples. If replicated, our findings should motivate further research on the functional role of these genetic variants of *SMAD7*, and, more broadly, on the mechanism by which anti-inflammatory medications could influence disease prognosis.

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Figure 1. Kaplan-Meier estimates for CRC-specific survival by genotype of rs4939827 according to pre-diagnostic NSAID use among women without metastatic disease.

Figure 2.
Kaplan-Meier estimates for CRC-specific survival by genotype of rs4464148 according to pre-diagnostic NSAID use among women without metastatic disease.

TABLE 1

Baseline Characteristics of Women Diagnosed with Colorectal Cancer

Characteristic	n	(%)	Recent NSAID Use ^d		
			No (n = 528)	Yes (n = 186)	n (%)
CRC Risk Factors					
Age at diagnosis (years)					
50 - 54	107	(14.7)	88 (16.7)	16 (8.6)	
55 - 59	112	(15.4)	82 (15.5)	29 (15.6)	
60 - 64	129	(17.7)	98 (18.6)	30 (16.1)	
65 - 69	174	(23.9)	118 (22.4)	52 (28.0)	
70 - 74	205	(28.2)	142 (26.9)	59 (31.7)	
Race					
Caucasian	674	(92.7)	484 (91.7)	178 (95.7)	
African American	14	(1.9)	11 (2.1)	3 (1.6)	
Asian/Pacific Islander	22	(3.0)	20 (3.8)	2 (1.1)	
Other	17	(2.3)	13 (2.5)	3 (1.6)	
Pre-diagnostic BMI (kg/m ²)					
< 25	275	(37.9)	216 (41.0)	54 (29.2)	
25 - 29	236	(32.6)	170 (32.3)	62 (33.5)	
≥ 30	214	(29.5)	141 (26.8)	69 (37.3)	
1 st degree family history of CRC					
No	600	(82.5)	443 (83.9)	146 (78.5)	
Yes	127	(17.5)	85 (16.1)	40 (21.5)	
History of screening endoscopy					
Never ^b	649	(89.3)	478 (90.5)	159 (85.5)	
At least 2 years before diagnosis	78	(10.7)	50 (9.5)	27 (14.5)	
Cigarette smoking status					
Never	338	(46.5)	241 (45.6)	86 (46.2)	
Former	280	(38.5)	199 (37.7)	80 (43.0)	
Recent	109	(15.0)	88 (16.7)	20 (10.8)	

Characteristic	n	Total (n = 727)	Recent NSAID Use ^d		n	Yes (n = 186)
			No (n = 528)	Yes (n = 186)		
			(%)	(%)		(%)
Hormone therapy use						
Never	312	(43.4)	243	(46.5)	64	(34.8)
Only E	226	(31.4)	153	(29.3)	71	(38.6)
Only E + P	37	(5.2)	32	(6.1)	5	(2.7)
Both E and E + P	144	(20.0)	95	(18.2)	44	(23.9)
Inflammatory bowel disease						
No	700	(96.6)	508	(96.4)	179	(96.8)
Yes	25	(3.4)	19	(3.6)	6	(3.2)
Clinical Characteristics						
Tumor subsite						
Proximal colon	349	(48.0)	246	(46.6)	95	(51.1)
Distal colon	250	(34.4)	179	(33.9)	69	(37.1)
Rectum	128	(17.6)	103	(19.5)	22	(11.8)
Stage at diagnosis						
Localized	308	(42.5)	225	(42.8)	80	(43.0)
Regional	341	(47.0)	251	(47.7)	82	(44.1)
Distant	76	(10.5)	50	(9.5)	24	(12.9)
First-course treatment^c						
None	288	(41.1)	216	(42.5)	69	(37.9)
Chemotherapy only	289	(41.2)	203	(40.0)	80	(44.0)
Radiation only	23	(3.3)	13	(2.6)	10	(5.5)
Chemotherapy + Radiation	101	(14.4)	76	(15.0)	23	(12.6)
Unknown	26	–	20	–	4	–
Microsatellite instability status^c						
MSS	362	(67.7)	263	(67.6)	94	(68.1)
MSI-L	49	(9.2)	40	(10.3)	8	(5.8)
MSI-H	124	(23.2)	86	(22.1)	36	(26.1)
Unknown	192	–	139	–	48	–

		Recent NSAID Use ^d					
		Total (n = 727)		No (n = 528)		Yes (n = 186)	
Characteristic	n	(%)	n	(%)	n	(%)	
<u>SMAD7 Genotype</u>							
rs4939827							
TT	206	(28.3)	144	(27.3)	57	(30.7)	
TC	364	(50.1)	265	(50.2)	91	(48.9)	
CC	157	(21.6)	119	(22.5)	38	(20.4)	
rs4464148							
TT	351	(48.3)	265	(50.2)	81	(43.6)	
TC	300	(41.3)	213	(40.3)	82	(44.1)	
CC	76	(10.5)	50	(9.5)	23	(12.4)	

Some counts may not sum to total because of missing data. Unknown is included as a category if >2% of total is missing.

^aRegular use as of two years prior to diagnosis

^bNever includes any endoscopy within two years before diagnosis in order to distinguish screening endoscopy from diagnostic endoscopy

^cPercentages exclude unknown

Abbreviations: BMI, body mass index; CRC, colorectal cancer; E, estrogen (unopposed); E+P, estrogen with progesterone MSI-H, microsatellite instability – high; MSI-L, microsatellite instability – low; MSS, microsatellite stable; NSAID, non-steroidal anti-inflammatory drugs

TABLE 2

Adjusted Hazard Ratios (HRs) for CRC-Specific Survival

Model	HR (95% CI) for Recent NSAID Use ^{a,b}	HR (95% CI) for rs4939827 ^c	HR (95% CI) for rs4939827 ^c Recent NSAID Use ^d		P-int ^d	HR (95% CI) for rs4464148 ^c		HR (95% CI) for rs4464148 ^c Recent NSAID Use ^d		P-int ^d
			No	Yes		No	Yes			
All Stages										
Model 1	1.05 (0.72, 1.52)	1.05 (0.85, 1.31)	0.88 (0.68, 1.15)	1.86 (1.17, 2.96)	0.006	0.98 (0.77, 1.25)	1.23 (0.93, 1.62)	0.50 (0.29, 0.87)	0.004	
Model 2	1.07 (0.71, 1.59)	1.07 (0.84, 1.36)	0.96 (0.71, 1.30)	1.37 (0.86, 2.17)	0.22	1.03 (0.80, 1.34)	1.28 (0.94, 1.74)	0.56 (0.32, 0.98)	0.01	
Localized/Regional										
Model 1	0.74 (0.44, 1.25)	1.18 (0.89, 1.57)	0.98 (0.71, 1.35)	2.67 (1.33, 5.37)	0.01	1.00 (0.74, 1.36)	1.26 (0.89, 1.78)	0.41 (0.18, 0.94)	0.01	
Model 2	0.70 (0.40, 1.23)	1.15 (0.84, 1.57)	0.98 (0.69, 1.39)	2.27 (1.08, 4.78)	0.05	1.05 (0.75, 1.47)	1.42 (0.92, 2.19)	0.15 (0.04, 0.68)	0.005	
Localized ^e										
Model 1	1.23 (0.32, 4.74)	1.03 (0.46, 2.30)	1.26 (0.50, 3.18)	1.01 (0.13, 7.78)	0.85	0.80 (0.31, 2.02)	0.58 (0.17, 2.05)	0.42 (0.05, 3.88)	0.80	
Regional										
Model 1	0.70 (0.40, 1.24)	1.20 (0.88, 1.64)	0.89 (0.62, 1.28)	2.84 (1.38, 5.85)	0.005	1.00 (0.72, 1.38)	1.33 (0.93, 1.90)	0.39 (0.16, 0.95)	0.01	
Model 2	0.67 (0.35, 1.26)	1.21 (0.86, 1.71)	0.96 (0.64, 1.43)	2.54 (1.14, 5.65)	0.03	1.11 (0.78, 1.59)	1.55 (0.98, 2.46)	0.15 (0.03, 0.64)	0.003	
Distant										
Model 1	1.31 (0.75, 2.31)	0.99 (0.70, 1.40)	1.02 (0.64, 1.61)	0.93 (0.51, 1.68)	0.82	1.05 (0.72, 1.53)	1.23 (0.76, 2.00)	0.80 (0.40, 1.58)	0.33	
Model 2	1.67 (0.89, 3.82)	0.83 (0.52, 1.32)	0.80 (0.44, 1.46)	0.87 (0.42, 1.82)	0.85	0.98 (0.61, 1.59)	1.27 (0.71, 2.27)	0.60 (0.25, 1.42)	0.16	

Total CRC deaths/at risk: 116/528 with no recent use, 38/186 with recent use; Localized CRC deaths/at risk: 8/225 with no recent use, 3/80 with recent use; Regional CRC deaths/at risk: 67/251 with no recent use, 15/82 with recent use; Distant CRC deaths/at risk: 41/50 with no recent use, 20/24 with recent use. Some counts may not sum to total because of missing data.

Model 1 is adjusted for age at diagnosis and race.

Model 2 is adjusted for age at diagnosis, race, stage, body mass index, screening endoscopy, smoking status, hormone therapy use, inflammatory bowel disease, tumor subsite, microsatellite instability status, and first-course of treatment.

^aRegular use as of two years prior to diagnosis of CRC

^bHR comparing recent NSAID use to no recent NSAID use

^cHR per minor allele (C)

^dP-value for interaction from model that includes SNP, NSAIDs, and SNP × NSAIDs interaction

^eModel 2 was not evaluated due to a limited number of deaths among women with localized disease

Abbreviations: CI, confidence interval; CRC, colorectal cancer; HR, hazard ratio; NSAIDs, non-steroidal anti-inflammatory drugs; SNP, single nucleotide polymorphism

TABLE 3

Adjusted Hazard Ratios (HRs) for Overall Survival

Model	HR (95% CI) for Recent NSAID Use ^{a,b}	HR (95% CI) for rs4939827 ^c	HR (95% CI) for rs4939827 ^c Recent NSAID Use ^a			P-int ^d	HR (95% CI) for rs4464148 ^c			P-int ^d
			No	Yes			No	Yes		
All Stages										
Model 1	1.15 (0.86, 1.55)	1.07 (0.89, 1.28)	0.98 (0.79, 1.21)	1.36 (0.95, 1.94)	0.12	0.96 (0.79, 1.17)	1.07 (0.85, 1.35)	0.71 (0.48, 1.06)	0.08	
Model 2	1.19 (0.87, 1.62)	1.12 (0.92, 1.36)	1.11 (0.88, 1.40)	1.12 (0.79, 1.60)	0.95	0.99 (0.80, 1.22)	1.09 (0.84, 1.40)	0.79 (0.53, 1.19)	0.19	
Localized/Regional										
Model 1	0.96 (0.67, 1.37)	1.13 (0.92, 1.40)	1.06 (0.83, 1.35)	1.38 (0.88, 2.14)	0.30	0.99 (0.79, 1.24)	1.07 (0.82, 1.40)	0.81 (0.50, 1.30)	0.32	
Model 2	0.93 (0.64, 1.37)	1.12 (0.90, 1.40)	1.09 (0.84, 1.42)	1.25 (0.81, 1.94)	0.60	0.99 (0.78, 1.27)	1.07 (0.81, 1.42)	0.81 (0.49, 1.35)	0.34	
Localized ^e										
Model 1	1.12 (0.57, 2.18)	1.22 (0.82, 1.83)	1.26 (0.80, 1.97)	1.40 (0.53, 3.71)	0.85	0.86 (0.55, 1.33)	0.65 (0.37, 1.15)	1.09 (0.47, 2.54)	0.33	
Regional										
Model 1	0.93 (0.60, 1.43)	1.06 (0.82, 1.36)	0.90 (0.66, 1.22)	1.38 (0.86, 2.23)	0.13	1.03 (0.79, 1.34)	1.24 (0.91, 1.66)	0.68 (0.39, 1.21)	0.07	
Model 2	0.89 (0.55, 1.43)	1.09 (0.83, 1.44)	1.00 (0.72, 1.39)	1.25 (0.74, 2.11)	0.47	1.10 (0.82, 1.46)	1.33 (0.95, 1.87)	0.73 (0.39, 1.36)	0.10	
Distant										
Model 1	1.49 (0.86, 2.58)	1.06 (0.76, 1.50)	1.10 (0.70, 1.73)	0.89 (0.51, 1.58)	0.58	0.99 (0.68, 1.44)	1.18 (0.73, 1.91)	0.74 (0.38, 1.45)	0.29	
Model 2	2.00 (0.84, 4.49)	0.94 (0.61, 1.44)	0.87 (0.48, 1.58)	0.92 (0.47, 1.83)	0.90	0.86 (0.54, 1.37)	1.22 (0.62, 2.21)	0.49 (0.21, 1.13)	0.09	

Total all-cause deaths/at risk: 172/528 with no recent use, 62/186 with recent use; Localized CRC all-cause deaths/at risk: 34/225 with no recent use, 12/80 with recent use; Regional CRC all-cause deaths/at risk: 94/251 with no recent use, 28/82 with recent use; Distant CRC all-cause deaths/at risk: 44/50 with no recent use, 22/24 with recent use. Some counts may not sum to total because of missing data.

Model 1 is adjusted for age at diagnosis and race.

Model 2 is adjusted for age at diagnosis, race, stage, body mass index, screening endoscopy, smoking status, hormone therapy use, inflammatory bowel disease, tumor subsite, microsatellite instability status, and first-course of treatment.

^aRegular use as of two years prior to diagnosis of CRC

^bHR comparing recent NSAID use to no recent NSAID use

^cHR per minor allele (C)

^dP-value for interaction from model that includes SNP, NSAIDs, and SNP × NSAIDs interaction

^eModel 2 was not evaluated due to a limited number of deaths among women with localized disease

Abbreviations: CI, confidence interval; CRC, colorectal cancer; HR, hazard ratio; NSAIDs, non-steroidal anti-inflammatory drugs; SNP, single nucleotide polymorphism