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Genetic Variation in Inflammatory Pathways is Related to Colorectal Cancer Survival

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Abstract

Purpose—Colorectal cancer (CRC) patient prognosis is associated with systemic inflammation, and anti-inflammatory drugs can reduce both CRC incidence and mortality. Genetic variation in pro-inflammatory pathways can affect an individual's CRC risk. However, few studies have investigated the prognostic importance of this genetic variation in CRC patients.

Experimental Design—We investigated the association between CRC survival and genetic variation in pro-inflammatory pathways among patients from the Puget Sound SEER registry. Single nucleotide polymorphisms were genotyped in five genes (*PTGS-1*, *PTGS-2*, *MRP4*, *NFκB*, *IκBKβ*). Vital status was ascertained through linkage to the National Death Index. Cox proportional hazards regression was used to calculate hazard ratios and 95% confidence intervals. The false discovery rate method of Benjamini and Hochberg was applied to address multiple testing.

Results—Four *PTGS-1* variants were associated with CRC survival. One, G>A intron 9 (rs1213266), was associated with ~50% lower CRC mortality (HR_{AA/AG} vs. GG:0.48; 95% CI 0.25-0.93). Three variants, including L237M, resulted in significantly elevated CRC mortality risk, with hazard ratios ranging from approximately 1.5-2.0. Two variants in *IκBKβ*, including R526Q, were significantly associated with CRC survival. Correction for multiple testing indicated that variants in both *PTGS-1* and *IκBKβ* are reproducibly associated with CRC survival.

Conclusion—Our findings suggest that genetic variation in pro-inflammatory pathways may be important for CRC prognosis. This investigation represents one of the first descriptions of the relationship between inherited polymorphisms and mortality in CRC patients and provides a starting point for further research.

Keywords

prostaglandins; *IκBKβ*; COX-1; colorectal cancer prognosis

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Introduction

Inflammation has consistently been associated with colorectal cancer development and prognosis in clinic and population studies.^{1, 2} The role of inflammation in prognosis may be mediated through influences on processes crucial for tumor progression, including metastasis and invasion.³⁻⁵ Medications that inhibit inflammation such as non-steroidal anti-inflammatory drugs (NSAIDs), which interact with the prostaglandin synthesis pathway, decrease the risk of colorectal neoplasia.⁶⁻⁸ Consistent with inflammation's role not only in cancer development but also cancer progression, NSAIDs have also been associated in large, population-based studies with improved survival of colorectal cancer patients.⁹⁻¹¹

The prostaglandin synthesis pathway is critical for regulation of inflammatory processes and plays a well-defined role in colorectal carcinogenesis.^{12, 13} Prostaglandin H synthases (COX-1 and COX-2) are pivotal enzymes in this pathway;^{14, 15} upregulation of prostaglandins results in cellular proliferation, angiogenesis, and increased cellular motility.¹⁶⁻¹⁸ The nuclear factor kappa B (NFκB) pathway represents another important pro-inflammatory pathway associated with colorectal cancer; NFκB is a transcription factor with multiple targets involved in inflammatory signaling and carcinogenesis, including prostaglandin synthases.¹⁹⁻²² The NFκB transcription factor plays a role not only in regulating cellular growth signals but also in regulating apoptosis and the survival of cancer cell populations.^{23, 24}

A study conducted in a Spanish population²⁵ investigated variation in two *PTGS-2* (COX-2) single nucleotide polymorphisms (SNPs) (−765 G>C and 3618 A>G) among 284 colorectal cancer patients in relation to tumor characteristics and disease prognosis. 3618 A>G was found to be a prognostic indicator for colorectal cancer patients, with carriers of the variant allele experiencing approximately 60% improved survival compared to wild type patients. To our knowledge, this has been the only population study to date examining polymorphisms in genes involved in the prostaglandin synthesis pathway in relation to colorectal cancer survival. However, multiple studies have noted that variation in genes encoding both prostaglandin synthases and the NFκB transcription factor is associated with colorectal cancer risk.²⁶⁻²⁹ We therefore hypothesized that variation in these important inflammation-associated genes would affect the survival of colorectal cancer patients.

We examined SNPs in genes involved in both the prostaglandin synthesis pathway (*PTGS1*=*COX-1*, *PTGS2*=*COX-2*, *MRP4*) and the NFκB pathway (*NFκB*, *IκBKβ*) in relation to colorectal cancer survival among patients identified from the population-based Seattle Colon Cancer Family Registry. This investigation represents one of the first descriptions of the relationship between inherited genetic polymorphisms and survival after a diagnosis of colorectal cancer.

Materials and Methods

Study Population

The Colon Cancer Family Registry (Colon CFR) is a six-site international collaboration established to investigate the genetic epidemiology of colorectal cancer. This report describes the Seattle Colon CFR, where patients with incident, invasive colorectal cancer occurring from 1997-2002 from 3 counties in Western Washington State were ascertained from the population-based Puget Sound Surveillance Epidemiology and End Results registry.³⁰

Colorectal cancer patients from the Seattle Colon CFR who were genotyped as part of a Colon CFR-wide study of candidate SNPs³¹ were included in our survival analysis. No patients reported multiple primary tumors. This study utilized a case/unaffected sibling control design, selecting colorectal cancer cases from each Colon CFR study site who had unaffected siblings also enrolled in the Colon CFR.

SNP Selection and Genotyping

Selection of tagSNPs and SNP genotyping methods for the parent study have been published previously.³¹ Briefly, tagSNPs for *PTGS-1*, *PTGS-2*, *MRP4*, *NFκB* and *IkBKβ* were selected using Haploview Tagger and the following criteria: minor allele frequency (MAF) >5%, pairwise r^2 of >0.95, and distance from closest SNP of >60 base pairs (bp). The 5' and 3' untranslated regions (UTR) for each gene were extended to include the most up- or downstream SNP within the linkage disequilibrium (LD) block (~10 kb upstream and 5kb downstream). In regions of no or low LD, SNPs with an MAF >5% at a density of ~ 1 per kb were selected from either HapMap or dbSNP.

We investigated the following tagSNPs: 17 in *PTGS-1*, 8 in *PTGS-2*, 41 in *NFκB*, 9 in *IkBKβ*, and 62 in *MRP4*. SNPs were genotyped on the Illumina platform in the laboratory of Dr. Duggan at Translational Genomics Research Institute (Phoenix, AZ). SNPs were excluded based on the following criteria: GenTrain score of <0.4, 10% GC score of <0.25, ABT Dev of >0.1239, call rate of <0.95, or more than two P-P-C errors. Interplate and intraplate replicates were included, and SNPs were excluded from the analysis if there were more than two errors on the replicate genotypes. In addition, genotype data from 30 CEPH trios (Coriell Cell Repository, Camden, NJ) were used to confirm reliability and reproducibility of the genotyping. SNPs were excluded from the analysis if more than three genotypes were discovered to be discordant in comparison with the genotype from the International HapMap Project.

Outcome Assessment

Vital status and date and cause of death were ascertained for all cases through linkage to the National Death Index (NDI) records; causes of death were classified using ICD10 codes. The NDI identifies known deaths throughout the United States with a high degree of sensitivity, validity, and completeness.^{32, 33} The primary outcome of interest was mortality from colorectal cancer, assessed from underlying cause of death obtained from the NDI. Time to colorectal cancer mortality was evaluated from SEER-reported date of colorectal cancer diagnosis and NDI-recorded date of death. Patients alive at the time of their last known vital assessment were censored at that date, with the most recent vital status linkage occurring December 31, 2009. Patients dying of causes other than colorectal cancer were censored at their recorded date of death.

Tumor characteristics at the time of diagnosis, including stage and sub-site, were obtained from SEER reports. Advanced disease was defined as colorectal cancer with distant metastasis (n=61); non-advanced disease included localized and regional stage disease (n=379). Sub-site of disease was categorized using ICD10 codes: proximal (C18.0-C18.5); distal (C18.6-18.7); and rectal (C19.9, C20.9, and C21.8). Based upon established guidelines and ten available MSI markers,³⁴ cases were classified as MSI-stable if 0% of loci were unstable, MSI-low if < 30% of loci were unstable, and MSI-high if ≥ 30% of loci were unstable, with unequivocal results for at least four markers required to characterize MSI status.

Statistical Analyses

Cox proportional hazards regression models were used to calculate hazard ratios (HR) and 95% confidence intervals (95% CI) for the association between each SNP and colorectal cancer survival. Regression models assumed a dominant mode of inheritance; the number of events was not sufficient to evaluate unrestricted or log-additive models. Cox models included adjustment for sex, age at diagnosis and self-reported white/non-white race; models additionally including the stage of disease at diagnosis were also run. In exploratory analyses, models were restricted to Caucasians (n=381). Regression models were also run with all-cause mortality as an outcome. Results were considered statistically significant if the two-sided *P*-value <0.05.

To address the issue of conducting multiple tests within each gene, we applied the false discovery rate (FDR) control method of Benjamini and Hochberg.^{35, 36} The control of the FDR using the Benjamini and Hochberg (B & H) method takes a decidedly different approach from the more conservative family-wise error rate (FWER) methods, including the Bonferroni correction, balancing protection against false inference with the ability to detect true associations. The B & H method is a step-up method that requires listing the *P*-values calculated from regression analyses in descending order from highest to lowest (i.e. values closer to 0 listed first). Once a FDR level has been pre-determined, the B & H method takes into account both the total of number of tests done (i.e. number of SNPs tested) and the *P*-values calculated for each test from regression models in order to calculate an adjusted *P*-value for each test (i.e. each SNP). These adjusted *P*-values are compared to the *P*-values calculated directly from regression models, and a list of noteworthy SNPs at the FDR level chosen is identified. Instead of protecting against any type 1 error (i.e. one or more false positives), the B & H method allows for false positive results in the process of discovering true positives and guarantees that under repeated use, the long-run average of false positives will fall at or below some pre-specified FDR level.

For example, if a FDR level (e.g. 25%) is chosen, the following equation is applied to each of the sequentially listed *p*-values in a given gene, beginning from the least to most extreme (i.e. values closest to 0 listed first): $FDR * (P\text{-value order} / \text{total } P\text{-values})$. At a FDR of 25%, the Benjamini-adjusted *P*-value for the 6th ordered SNP out of a list of 20 SNP *P*-values in a given gene would be: $0.25 * (6/20) = 0.08$. If the *P*-value for the *n*th ordered value falls below the Benjamini-adjusted *P*-value, all SNPs with *P*-values equal to or less than that ordered value are considered noteworthy. Returning to the previous example, if the *P*-value (calculated from Cox regression) for the 6th ordered SNP were less than 0.08, the first 6 *P*-values (i.e. first 6 SNPs) would all be considered noteworthy at the FDR 25% level. Of these, we would expect that one quarter (less than 2) would be false positives but that the remaining three quarters (at least 4) are true positives.

This false discovery rate method controls the number of false positives so that we have confidence that a certain percentage of the positive results reported are in fact true positives, recognizing at the same time that a certain percentage are false positives. We generated a list of noteworthy SNPs at both the FDR 50% and FDR 25% levels for the five genes investigated.

Results

After an average of 6.5 years (SD=3.1 years) of follow-up after colorectal cancer diagnosis, 153 deaths from any cause were observed. Three quarters of the deaths among patients were due to colorectal cancer (n=115). Patients' ages at diagnosis ranged from 23 to 74, with ~10% of patients under the age of 40. A slightly larger proportion of deceased patients had microsatellite stable tumors and tumors located in the distal colon. As expected, patients

diagnosed with localized tumors had much better overall survival compared to patients with advanced disease, with over 30% of deceased patients being diagnosed with advanced disease, as compared to only 5% of patient who remained alive at the end of study follow up (Table 1).

Genetic variation in both *PTGS-1* (COX-1) and *IKBKβ* was associated with colorectal cancer patient prognosis. These two genes had more SNPs with *P* -values <0.05 than expected by chance (more than 1 SNP for every 20 tested detected as statistically significant). Additionally, of the five genes tested, only *PTGS-1* and *IKBKβ* had SNPs that were noteworthy. All SNPs noteworthy at the FDR 50% level are reported in **Tables 2 and 3**; SNPs noteworthy at the FDR 25% level are denoted in *italics*. The other three genes investigated did not include noteworthy SNPs; for illustrative purposes, *P* -values calculated from Cox regression models and B & H -adjusted *P* -values (at the FDR 50% level) for these three genes are presented in Supplementary Tables.

Four of the seventeen SNPs in *PTGS-1* were statistically significantly associated with colorectal cancer-specific mortality. The presence of the minor allele conferred an approximately 1.5-2 times greater risk of colorectal cancer mortality compared to the wild type for three of the SNPs (rs10306155: G>A intron 2, rs4836885: A>G intron 8, L237M: C>A exon 7). In contrast, patients with the minor allele for rs1213266 (G>A intron 9) had approximately 50% lower mortality compared to wild type patients (HR: 0.48; 95% CI 0.25-0.93) (Table 2). Correction for multiple testing confirmed that genetic variation in *PTGS-1* was associated with colorectal cancer survival, with six SNPs, including all SNPs noted above with *P* -values < 0.05, were noteworthy at the FDR 25% level; the expectation is that one quarter of these SNPs (less than 2) are false positives but that the remaining three quarters (at least 4) may in fact be associated with colorectal cancer patient survival.

Two of the nine SNPs in *IKBKβ* were statistically significantly associated with colorectal cancer mortality. Patients with the minor allele for rs11986055 (A>C intron 19) experienced less than half the mortality due to colorectal cancer compared to wild type patients (HR: 0.39; 95% CI 0.14-1.00) (Table 3). Estimates for R524Q were imprecise since only one patient was observed to carry the minor allele. Correction for multiple testing also indicated that genetic variation in *IKBKβ* was associated with colorectal cancer survival, with two SNPs in *IKBKβ* noteworthy at the FDR 25% level.

When we restricted analyses to Caucasians only (n=381), we obtained similar results to those reported here. For both *PTGS-1* and *IKBKβ*, all SNPs noteworthy when investigating colorectal cancer survival, except one, were also noteworthy when considering the outcome of death from any cause among patients. Effect estimates for the association between these SNPs and all-cause mortality were similar to those specific to colorectal cancer. For example, of the four SNPs in *PTGS-1* that were statistically significantly associated with colorectal cancer-specific mortality, three were also statistically significantly associated with all-cause mortality (rs10306155: G>A intron 2, rs4836885: A>G intron 8, L237M: C>A exon 7) (Table 2).

Adjustment for stage of disease at diagnosis attenuated the statistical significance of the associations for three of the SNPs in *PTGS-1* (rs10306155: G>A intron 2, rs4836885: A>G intron 8, L237M: C>A exon 7), although two of these SNPs, rs10306155 (G>A intron 2) and L237M (C>A exon 7), remained marginally associated with colorectal cancer-specific mortality (HR: 1.48; 95% CI 0.96-2.28; HR: 1.77; 95% CI 0.91-3.45, respectively). For these SNPs, patients with the wild type genotype were statistically significantly less likely than patients with the minor allele to present with advanced stage of disease at diagnosis. For both rs10306155 (G>A intron 2) and rs4836885 (A>G intron 8), ~13% of patients with

the wild type genotype presented with advanced disease, compared to ~19% of patients with the minor allele. For L237M, 14% of patients with the LL genotype presented with advanced tumors compared to 29% of patients with either the LM or MM genotype.

Adjustment for other tumor characteristics, including MSI status and tumor sub-site, did not alter reported effect estimates, and the distribution of these tumor characteristics was similar between cases with and without the variant allele for all except L237M. While approximately 36% and 30% of patients with the LL genotype presented with rectal and distal tumors, respectively, only ~13% of patients with either the LM or MM genotype presented with distal tumors, and 50% presented with rectal tumors.

Discussion

This investigation is one of the first to explore the relationship between inherited genetic polymorphisms and colorectal cancer survival. Genetic variation in both *PTGS-1* (COX-1) and *IKBKβ* was associated with an altered risk of mortality from colorectal cancer. Our confidence in these results is strengthened by the fact that specific polymorphisms in *PTGS-1* and *IKBKβ* showed consistent statistical evidence of an association with colorectal cancer survival. Both genes had more SNPs with statistically significant associations than would be expected by chance; each gene had multiple SNPs that were noteworthy using the B&H FDR control method; and all of the statistically significant SNPs in both *PTGS-1* and *IKBKβ* were also noteworthy SNPs at the FDR 25% level.

The majority of the SNPs identified have not been thoroughly characterized with respect to function, but at least three of the polymorphisms identified result in non-synonymous coding amino acid changes. The presence of the minor allele (A allele) in L237M (rs5789) in *PTGS-1* results in a leucine to methionine change at amino acid position 237; the presence of the minor allele (A allele) in P17L (rs3842787) in *PTGS-1* results in a proline to leucine change at amino acid position 17; and the minor allele (A allele) in R524Q (rs2272736) of *IKBKβ* results in an arginine to glutamine change. The L237M polymorphism has been characterized previously as significantly altering protein expression levels of COX-1.^{37, 38} Future studies are warranted to determine if the other non-synonymous coding change polymorphisms may result in functional changes in protein expression levels.

Multiple SNPs in *PTGS-1* were observed to be in high LD ($r^2 > 0.80$): rs10306155 & rs4836885 ($r^2 = 0.93$); rs10306155 & rs9299280 ($r^2 = 0.87$); rs4836885 & rs9299280 ($r^2 = 0.95$); rs6478565 & 4273915 ($r^2 = 0.91$); rs10306163 & rs3842798 ($r^2 = 0.95$). These SNPs can be grouped into three LD blocks in *PTGS-1*: bin 1 (rs10306155: G>A intron 2, rs4836885: A>G intron 8, & rs9299280: G>A intron 8), bin 2 (rs10306163: A>G intron 8, rs3842798: A>G exon 7), and bin 4 (rs6478565: A>G intron 8, rs4273915: G>C intron 7). Although this may represent some redundancy in the information for any of these given SNPs, at least three distinct SNPs with *P*-values <0.05 and four SNPs noteworthy at the FDR 25% level would remain if only one SNP from each of these bins were included in our analyses; the inference that genetic variation in *PTGS-1* (COX-1) is associated with colorectal cancer mortality would be unchanged. In addition, the observation of multiple noteworthy SNPs within one LD block provides stronger evidence that these particular regions of the prostaglandin synthase 1 gene may be associated with colorectal cancer prognosis.

It is biologically plausible that these genes, which influence inflammation, are involved in colorectal cancer survival. Prostaglandin synthase 2 (COX-2) expression has been linked to colorectal cancer recurrence and to specific processes such as angiogenesis that are crucial for tumor progression.^{39, 40} Prostaglandin synthase 1 (COX-1), which is constitutively

expressed in the colon, has not been as thoroughly investigated, despite synthesizing the same downstream prostanoids and having a demonstrated role in tumorigenesis.^{41, 42} COX-1 is involved in maintaining the colonic mucosa and vasculature;^{43, 44} alterations in the cumulative level of prostaglandins resulting from genetic variation in *PTGS-1* could interrupt these functions and contribute to cancer progression by altering a tumor's ability to promote angiogenesis and cellular extravasation and invasion.

I κ BK β has previously been identified as a crucial link between inflammatory processes and carcinogenesis in laboratory studies.^{45, 46} This role in carcinogenesis is likely due to the inhibition of NF κ B transcriptional activity by I κ BK β and the resulting resolution of NF κ B-mediated inflammation in cells.^{47, 48} Additionally, crucial downstream targets of the NF κ B transcription factor include the prostaglandin synthases;^{19, 22} disruptions in the regulation of NF κ B through variation in *I κ BK β* could lead to altered *PTGS-1* and *PTGS-2* expression, resulting in variation in prostanoid production that could contribute to cancer progression.

Prior genome-wide scans investigating colorectal cancer incidence have not identified these genes as loci related to colorectal cancer initiation. However, investigation of the association between the top variants identified in scans of colorectal cancer risk with respect to the outcome of colorectal cancer survival has yielded null results.⁴⁹ Colorectal cancer incidence and colorectal cancer progression and prognosis, although related, are independent outcomes, and we expect that the opposite may also be true—variants identified as important for disease progression may not be equally important for disease initiation. Inflammation is known to be important for initiation, but an important role also exists for inflammation in the regulation of cellular adhesion, disintegration of the extracellular matrix, and angiogenesis, which all affect tumor invasion and metastatic potential. Our results are novel, and further studies, particularly genome-wide scans, investigating the role of genetic variation in colorectal cancer prognosis may in fact identify new loci that were not identified in scans related to disease incidence.

The associations observed here may be due in part to an association between variation in the investigated genes and the stage at which colorectal cancer is diagnosed in patients. Inherited genetic variation is a lifelong exposure, such that polymorphisms in a given individual may alter the rate at which disease develops and progresses, resulting in colorectal cancer diagnosis at a different stage of disease. If genetic variation alters survival after a diagnosis of colorectal cancer because it alters the stage at which the tumor is diagnosed, then stage may be considered part of the “causal pathway” between genetic variation and colorectal cancer survival. This is consistent with our observations, in that adjustment for stage attenuated the magnitude of observed associations for certain SNPs in *PTGS-1*, and patients with minor alleles for these SNPs were more likely to present with advanced stage of disease at diagnosis.

The B & H method, rather than asking whether any individual test result is a false positive, is designed instead to answer the question of whether any of the positive test results generated may in fact warrant further investigation. Use of this method allowed us to take a gene-by-gene analysis approach, answering the question of whether any variation in each of the selected pro-inflammatory pathway genes, not just in particular SNPs, was associated with mortality after a diagnosis of colorectal cancer. The control of the FDR often has increased statistical power to detect true positives and may arguably be a more suitable method than the more conservative Bonferroni test for studies seeking to generate potential hypotheses for replication in future studies.³⁶ Utilizing a standard FDR level has been suggested to be a potentially more useful method for allowing a uniform comparison of genetic epidemiology studies.⁵⁰

Additional study strengths include accurate exposure measurement and complete and standardized outcome follow-up for all study participants. The potential for population stratification was examined by restriction of analyses to Caucasian patients only, with no differences in associations observed. The average time between diagnosis and study enrollment for cases in the Seattle Colon CFR was 8 months (95% CI: 3-13), such that our study did not suffer from long lag times between diagnosis and enrollment that can result in patient loss, particularly loss of patients with more advanced stages of disease, and limit generalizability of results.

This is one of the first investigations of inherited genetic variation and colorectal cancer survival; additional studies with larger sample sizes and more ethnically diverse study populations are required to confirm our findings and to further characterize the specific nature of the associations between the identified genes and patient survival. Future studies should also include more detailed treatment information. We were only able to consider first-line treatment data in our analysis; although this data did not alter observed associations, the examination of more detailed treatment information could shed light on potential interactions between inherited genetic variation and treatment responses. Finally, patients originated from a population-based cancer registry, but the design of the parent genetic association studies required that each colorectal cancer patient had to have a sibling that was not affected by colorectal cancer to participate. The minor allele frequencies observed in this study population were higher than would be expected in a population that was not enriched with a first-degree family history of colorectal cancer. Although the direction of the potential bias introduced by this selection is difficult to predict, future studies should be conducted in true population-based samples to maximize generalizability.

Very little is known about the role of genetic variation in altering patient survival after a diagnosis of colorectal cancer. Our findings suggest that variation in genes involved in crucial inflammatory pathways may be important for disease prognosis. This study begins to shed light on specific pro-inflammatory genes that should be investigated further; both *PTGS-1* (COX-1) and *IkBK β* should be top priority genes for inclusion in future studies of colorectal cancer outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Translational Relevance

The presence of common genetic variation can refine prediction of patient outcome for colorectal cancer and help guide the management and risk assessment for individual patients. This study suggests that inflammatory pathway-associated genetic variation may possibly be useful for improving outcome prediction for colorectal cancer patients. These results improve our understanding of colorectal cancer progression, confirming that key cellular pathways involved in colorectal cancer incidence also play a role in disease progression. Identified genes provide a good starting point for further research and potential targets for colorectal cancer therapy, including pharmacogenetic research on COX-inhibitors.

Table 1

Characteristics of colorectal cancer cases, stratified on vital status ^a

	Total Colorectal Cancer Cases (n=426)		Deceased (n=151)		Alive (n=275)	
	N	%	N	%	N	%
Age						
<50 years	203	47.7	57	37.8	146	53.1
50-59.9 years	86	20.2	29	19.2	57	20.8
60-69.9 years	94	22.1	39	25.8	55	20.0
≥ 70 years	43	10.1	26	17.2	17	6.2
Sex						
Female	198	46.5	68	45.0	130	47.3
Male	228	53.5	83	55.0	145	52.7
Race						
Caucasian	381	89.4	136	90.1	245	89.1
Non-Caucasian	45	10.6	15	9.9	30	10.9
Screening Endoscopy ^b						
No	395	92.7	140	92.7	255	92.7
Yes	31	7.3	11	7.3	20	7.3
History of Polyps						
No	231	54.2	82	54.3	149	54.2
Yes	192	45.1	67	44.4	125	45.5
MSI Status ^c						
Stable	196	72.9	78	75.7	118	71.1
Low	36	13.4	15	14.6	21	12.7
High	37	13.8	10	9.7	27	16.3
Tumor Stage						
Localized	146	34.5	30	20.1	116	42.3
Regional	216	51.1	72	48.3	144	52.6
Distant	61	14.4	47	31.5	14	5.1

	Total Colorectal Cancer Cases (n=426)		Deceased (n=151)		Alive (n=275)	
	N	%	N	%	N	%
Site of Tumor						
Proximal	123	28.9	40	26.5	97	35.3
Distal	137	32.2	50	33.1	73	26.6
Rectal	158	37.1	59	39.1	99	36.0

^a Vital Status is defined as the vital status for each colorectal cancer patient at their last date of follow up, with 34% of colorectal cancer patients deceased during the course of follow-up and 66% alive at last date of contact.

^b Endoscopy: Sigmoidoscopy or colonoscopy received at least two years prior to colorectal cancer diagnosis.

^c MSI status of tumors was available for ~63% of patients. Percentages reported are out of the total cases with MSI status available.

Table 2

Associations between COX-1 polymorphisms^a, colorectal cancer mortality, and all-cause mortality

Common Name	dbSNP ID	MAF%	Colorectal Cancer Mortality				All-Cause Mortality			
			Alive/Censored	Colorectal Cancer Deaths	HR and 95% CI for CRC Mortality	p-value	Alive	All Deaths	HR and 95% CI for All-Cause Mortality	p-value
G>A Intron 9	rs1213266									
	GG	15.2	254	103	1.00	--	226	131	1.00	--
	AG/AA		58	11	0.48 (0.25-0.93)	0.02	49	20	0.71 (0.43-1.15)	0.16
G>A Intron 2	rs10306155 <i>a,b</i>									
	GG	26.4	242	80	1.00	--	215	107	1.00	--
	AG/AA		70	34	1.62 (1.06-2.49)	0.03	60	44	1.63 (1.13-2.36)	<0.01
A>G Intron 8	rs4836885 <i>a,c</i>									
	AA		231	77	1.00	--	205	103	1.00	--
	AG/GG	26.4	76	37	1.55 (1.02-2.34)	0.04	67	46	1.47 (1.02-2.12)	0.04
C>A Exon 7	L237M									
	CC		298	104	1.00	--	263	139	1.00	--
	CA/AA	5.0	14	10	2.09 (1.07-4.06)	0.05	12	12	2.08 (1.14-3.82)	0.02
G>A Intron 8	rs9299280 <i>b,c</i>									
	GG		232	77	1.00	--	205	104	1.00	--
	AG/AA	26.6	80	37	1.48 (0.98-2.25)	0.07	70	47	1.44 (1.00-2.07)	0.05
A>G Intron 8	rs6478565 <i>d</i>									
	AA		207	68	1.00	--	183	92	1.00	--
	AG/GG	33.0	105	46	1.44 (0.97-2.13)	0.08	92	59	1.37 (0.97-1.94)	0.07
A>G Intron 8	rs10306163 <i>e</i>									
	AA		192	64	1.00	--	170	86	1.00	--
	AG/GG	38.1	118	50	1.33 (0.89-1.97)	0.16	103	65	1.29 (0.92-1.82)	0.14

Common Name	dbSNP ID	MAF%	Colorectal Cancer Mortality				All-Cause Mortality			
			Alive/Censored	Colorectal Cancer Deaths	HR and 95% CI for CRC Mortality	p-value	Alive	All Deaths	HR and 95% CI for All-Cause Mortality	p-value
G>C Intron 7	rs4273915 ^d									
	GG		210	73	1.00	--	186	97	1.00	--
	GC/CC	30.9	102	41	1.30 (0.87-1.95)	0.20	89	54	1.29 (0.91-1.83)	0.15
A>G Intron 9	rs12551233									
	AA		257	98	1.00	--	225	130	1.00	--
	AG/GG	15.9	55	16	0.72 (0.41-1.27)	0.24	50	21	0.68 (0.41-1.12)	0.13
A>G Intron 7	rs3842798 ^e									
	AA		196	68	1.00	--	174	90	1.00	--
	AG/GG	35.6	116	46	1.23 (0.83-1.84)	0.30	101	61	1.24 (0.88-1.75)	0.23
G>A Intron 2	rs12353214									
	GG		250	86	1.00	--	223	113	1.00	--
	AG/AA	23.9	62	28	1.26 (0.80-1.98)	0.34	52	38	1.36 (0.92-2.00)	0.12
A>G Exon 2	P17L									
	GG		275	97	1.00	--	243	129	1.00	--
	AG/AA	12.3	55	16	1.33 (0.76-2.34)	0.34	30	20	1.22 (0.73-2.03)	0.45 [†]

Results in italics were noteworthy at the FDR 25% level (6 SNPs for colorectal cancer-specific and 5 SNPs for all-cause mortality).

Results in table above are adjusted for age, sex, and reported race at enrollment.

Minor allele frequencies (MAF) were calculated among unaffected siblings of cases.

[†] The SNPs were 'noteworthy' at an FDR 50% level when investigating both colorectal cancer-specific and all-cause mortality unless otherwise noted.

[‡] SNP was not 'noteworthy' at the FRD 50% level for all-cause mortality

^a SNPs are in high LD ($r^2=0.93$)

^b SNPs are in high LD ($r^2=0.87$)

^c SNPs are in high LD ($r^2=0.95$)

SNPs are in high LD ($r^2=0.91$)
 p
SNPs are in high LD ($r^2=0.95$)

Table 3
Associations between IκBκ polymorphisms[†], colorectal cancer mortality, and all-cause mortality

Common Name	dbSNP ID	MAF%	Colorectal Cancer Mortality				All-Cause Mortality			
			Alive/Censored	Colorectal Cancer Deaths	HR and 95% CI for CRC Mortality	p-value	Alive	Death from Any Cause	HR and 95% CI for All-Cause Mortality	p-value
A>C Intron 19	rs11986055									
	AA		281	109	1.00	--	247	142	1.00	--
	AC/CC	9.5	31	5	0.39 (0.14-1.00)	0.04	28	8	0.44 (0.19-1.02)	0.06
G>A Exon 15	R526Q									
	GG		312	113	1.00	--	275	150	1.00	--
	AG/AA	0.2	0	1	23.3 (2.62-207.7)	0.04	0	1	50.03 (5.7-437.2)	<0.01
C>T	rs6474387									
	CC		263	101	1.00	--	230	134	1.00	--
	CT/TT	15.0	48	12	0.57 (0.28-1.13)	0.08	44	16	0.57 (0.32-1.04)	0.07
T>A Intron 1	rs3747811									
	TT		84	39	1.00	--	74	49	1.00	--
	AT/AA	69.8	228	75	0.69 (0.46-1.04)	0.09	201	102	0.79 (0.55-1.14)	0.21
G>A Intron 19	rs10958713									
	GG		138	54	1.00	--	121	71	1.00	--
	AG/AA	55.9	173	60	0.80 (0.54-1.18)	0.26	153	80	0.86 (0.62-1.21)	0.40 [‡]
A>G Intron 5	rs9694958									
	AA		264	101	1.00	--	233	132	1.00	--
	AG/GG	14.6	48	13	0.72 (0.39-1.33)	0.27	42	19	0.76 (0.45-1.27)	0.29
G>A Intron 5	rs2272733									
	GG		247	94	1.00	--	217	124	1.00	--
	AG/AA	19.6	65	20	0.77 (0.45-1.29)	0.30	58	27	0.76 (0.48-1.19)	0.23

Results in italics were also noteworthy at the FDR 25% level (2 SNPs for colorectal cancer-specific and 3 SNPs for all-cause mortality).

Results in table above are adjusted for age, sex, and reported race at enrollment.

Minor allele frequencies (MAF) were calculated among unaffected siblings of cases.

[‡]The SNPs were 'noteworthy' at an FDR 50% level when investigating both colorectal cancer-specific and all-cause mortality unless otherwise noted.

[‡]SNP was not 'noteworthy' at the FRD 50% level for all-cause mortality