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Variation in the *CYP19A1* gene and risk of colon and rectal cancer

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Abstract

CYP19A1, or aromatase, influences estrogen-metabolizing enzymes and may influence cancer risk. We examine variation in the *CYP19A1* gene and risk of colorectal cancer using data from population-based case-control studies (colon $n = 1,574$ cases, 1,970 controls; rectal $n = 791$ cases, 999 controls). Four SNPs were statistically significantly associated with colon cancer and four were associated with rectal cancer. After adjustment for multiple comparisons, the AA genotype of rs12591359 was associated with an increased risk of colon cancer (OR 1.44 95% CI 1.16–1.80) and the AA genotype of rs2470144 was associated with a reduced risk of rectal cancer (OR 0.65 95% CI 0.50–0.84). Variants of *CYP19A1* were associated with CIMP+ and CIMP+/KRAS2-mutated tumors. CT/TT genotypes of rs1961177 were significantly associated with an increased likelihood of a MSI+ colon tumor (OR 1.77 95% CI 1.26–2.37). We observed statistically significant interactions between genetic variation in *NFKB1* and *CYP19A1* for both colon and rectal cancer. Our data suggest the importance of *CYP19A1* in the development of colon and rectal cancer and that estrogen may influence risk through an inflammation-related mechanism.

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Keywords

Colon cancer; Rectal cancer; *CYP19A1*; *NFκB1*; Aspirin; BMI; CIMP+; *KRAS2*; MSI

Introduction

The association between estrogen and risk of colon and rectal cancer is well documented [1–3]. In both observational studies and clinical trials women taking HRT have been shown to be at reduced risk of colorectal cancer (CRC) [1, 2, 4]. Estrogen status also has been shown to modify associations with BMI in some studies [5], linked to MSI+ tumors [6], and shown to influence survival after diagnosis with colon cancer [7]. The mechanisms by which estrogen influences risk of colon and rectal cancer are thought to go beyond those of estrogen as a sex steroid and include interaction with both insulin- and inflammation-related factors [8–11]. Studies have shown that estrogen status and aspirin use interact to alter colon cancer risk [12]. NFκB and cytokines have been shown to influence estrogen levels [8, 13, 14]. Estrogen working through an inflammation-related mechanism in the etiology of colon and rectal cancer is feasible [15–17].

Few studies have evaluated genetic variation in estrogen-related genes and risk of colon and rectal cancer. We have previously assessed genetic variation in the estrogen receptor alpha and beta (*ESR1* and *ESR2*) and the androgen receptor (*AR*) genes and observed stronger associations for *ESR2* than for *ESR1* and risk of colon cancer [18]. A study by Lin et al. [19] evaluated *ESR1* and *ESR2* and aromatase cytochrome p450 enzyme (*CYP19A1*) which is responsible for the conversion of estrone to estradiol, in 158 incident cases of colon and rectal cancer; they did not have the ability to examine colon and rectal cancer separately. One *CYP19A1* SNP, rs10046, was associated with increased risk of CRC.

Individual variability in estradiol metabolism is influenced by genetic variation in *CYP19A1* [20]. *CYP19A1* influences estrogen-metabolizing enzymes and may importantly influence the effect of estrogen on cancer, including colon and rectal cancer. In this study, we evaluate genetic variation in the *CYP19A1* gene and risk of colon and rectal cancer. We evaluate how variation in *CYP19A1* may interact with variation in other genes involved in regulating estrogen and androgen signaling, *ESR1*, *ESR2*, and *AR*; we have previously reported primary associations with genetic variation in *ESR1*, *ESR2*, and *AR* and colon cancer [18]. We evaluate possible interaction between estrogen status, BMI, recent use of NSAIDs/aspirin, and genetic variation in the *NFκB1* gene to obtain a better understanding of the role of variation in *CYP19A1* in colon and rectal cancer etiology and the possibility that estrogen may be operating through an inflammation-related pathway. We examine specific phenotypes by evaluating associations with specific tumor markers and we determined whether variation in *CYP19A1* influences survival after diagnosis.

Methods

Two study populations are included in these analyses. The first study, a population-based case-control study of colon cancer, included cases ($n = 1,593$) and controls ($n = 1,994$) identified between 1 October 1991 and 30 September 1994 [21] living in the Twin Cities Metropolitan Area, Kaiser Permanente Medical Care Program of Northern California (KPMCP) and a seven-county area of Utah. The second study, with identical data collection methods, included cases with cancer of the rectosigmoid junction or rectum ($n = 790$) and controls ($n = 999$) who were identified between May 1997 and May 2001 in Utah and KPMCP [22]. Eligible cases were between 30 and 79 years of age at time of diagnosis, English speaking, mentally competent to complete the interview, had no previous history of

CRC, and no known (as indicated on the pathology report) familial adenomatous polyposis, ulcerative colitis, or Cohn's disease.

Controls were matched to cases by sex and by 5-year age groups. At KPMCP, controls were randomly selected from membership lists; in Utah, controls 65 years and older were randomly selected from the Health Care Financing Administration lists and controls younger than 65 years were randomly selected from driver's license lists. In Minnesota, controls were selected from driver's license and state-identification lists [23, 24].

Interview data collection

Data were collected by trained and certified interviewers using laptop computers. All interviews were audio-taped as previously described and reviewed for quality control purposes [25]. The referent period for the study was 2 years prior to diagnosis for cases and selection for controls. Detailed information was collected on diet, physical activity, medical history, reproductive history and use of hormone replacement therapy (HRT), regular use of aspirin and non-steroidal anti-inflammatory drugs, and body size.

Tumor registry data

Tumor registry data were obtained to determine disease stage at diagnosis and months of survival after diagnosis. Disease stage was categorized by Surveillance, Epidemiology, and End Results (SEER) staging of local, regional, and distant disease as well as by the American Joint Committee on Cancer (AJCC) staging criteria. Local tumor registries provided information on patient follow-up including vital status, cause of death, and contributing cause of death. Survival-months were calculated based on month and year of diagnosis and month and year of death or date of last contact for those individuals who were still alive.

Tumor marker data

We have previously evaluated tumors for CpG island methylator phenotype (CIMP), microsatellite instability (MSI), *TP53* mutations, and *KRAS2* mutations [26–29] and were therefore able to evaluate genes in relation to tumors with specific characteristics or markers. Details for methods used to evaluate these epigenetic and genetic changes have been described in previous publications [26–29].

TagSNP selection and genotyping

TagSNPs were selected for *CYP19A1* using the following parameters: an $r^2 = 0.8$ defined LD blocks using a Caucasian LD map, minor allele frequency or maf > 0.1, range = -1,500 bps from the initiation codon to +1,500 bps from the termination codon, and 1 SNP/LD bin. All markers were genotyped using a multiplexed bead array assay format based on GoldenGate chemistry (Illumina, San Diego, CA). A genotyping call rate of 99.85% was attained. Blinded internal replicates represented 4.4% of the sample set. The duplicate concordance rate was 100.00%; Supplemental table contains a list of all tagSNPs evaluated for *CYP19A1*. Genotyping methods for *ESR1*, *ESR2*, and *AR* have been previously reported [18]. *NFκB1* was evaluated in the same manner as *CYP19A1*, using the GoldenGate Illumina platform.

Statistical methods

All statistical analyses were performed using SAS® version 9.2 (SAS Institute, Cary, NC) unless otherwise specified. Hardy–Weinberg equilibrium (HWE) testing was performed with the allele procedure using null markers; all SNPs were in HWE. We identified SNPs first by examination of SNPs using an additive model and then applying a stepwise

regression models to identify the individual SNPs and their corresponding inheritance models that contributed uniquely to the overall fit of the model for colon and rectal cancer, for specific tumor markers, and for survival. SNPs identified as important from stepwise were then run with appropriate adjustments to correct for confounding. In addition to minimal adjustments for age, sex, race, and study center, other SNPs returned from stepwise are controlled for within the regression model. Adjustment for multiple comparisons of the 27 *CYP19A1* tagSNPs using the methods of Conneely and Boehnke [30] was performed via R version 2.11.0 (R Foundation for Statistical Computing, Vienna, Austria). Wald *p* values associated with ORs presented in main tables were used for adjustment to determine *p*ACT values that were generated using the Conneely and Boehnke methods.

The haplotype procedure was used to estimate haplotypes by race using the EM algorithm and assuming linkage equilibrium. Haplotype probabilities were assigned to individuals and logistic regression models were weighted accordingly when assessing risk estimates. SAS Genetics routines were also used to test for marker-trait associations within the frameworks of the haplotype procedure. Associations for haplotype were compared to all other haplotypes and are presented for a single copy of the combined alleles. In evaluating haplotypes, we created haplotypes of those tagSNPs that were statistically significantly associated with colon and rectal cancer; since one marker was selected per LD bin, we did not create haplotypes based on LD bins. Minimal adjustments for age, sex, race, and study center are presented; additional adjustments for BMI (kg/m²), use of aspirin or NSAIDs within 2 years of the referent period, and cigarette smoking status (ever or never regularly smoked) did not alter associations.

We evaluated interactions between *CYP19A1* and *ESR1*, *ESR2*, *AR*, and *NFκB1*. Differences in effect by race, sex, age (30–64 or 65–79), recent aspirin or NSAID use, recent estrogen use, BMI (<25, 25–30, >30) were evaluated given the hypothesized mechanisms we proposed for *CYP19A1*. We observed no differences by sex or age. *p* Values for interaction were determined by comparing a full model that included a categorical multiplicative interaction term to a reduced model without an interaction term, using a likelihood ratio test.

Tumors were defined by specific alterations detected; any *TP53* mutation, any *KRAS2* mutation, MSI+, or CIMP+ defined as at least two of five markers methylated. As the proportion of MSI+ tumors in the rectal cases was <3% [31], there was insufficient power to examine these tumor markers with genotype data. Population-based controls were used to assess associations for the population overall when examining multiple outcomes defined by tumor status. Test for heterogeneity were conducted comparing those with a specific tumor phenotype to other cases without that phenotype. This allowed us to determine unique associations with specific tumor molecular phenotype.

Months of survival were determined based on date of diagnosis and date of last contact or death. Associations between SNPs and risk of dying after diagnosis of colorectal cancer were evaluated using stepwise selection and Cox proportional hazards models to estimate multivariate hazard rate ratios (HRRs) and 95% confidence intervals adjusted for age at diagnosis, study center, race, sex, tumor molecular phenotype, and AJCC stage. The false discovery rate for HRRs was controlled for using the methods of Benjamini and Hochberg [32].

Results

The demographics of the study population are shown in Table 1. The majority of participants were male, over 60 at the time of diagnosis, and NHW. Hispanic participants represented the second largest ethnic group.

Four SNPs in the *CYP19A1* gene not in LD were identified as being statistically significantly associated with colon cancer (Table 2); rs11856927 appeared to confound associations with other SNPs although not exhibiting a clear independent association. Evaluation of the haplotypes of remaining four SNPs showed that one copy of 1 common and 1 rarer haplotype were associated with decreased risk of colon cancer. The strongest risk was associated with rs12591359, where an OR of 1.44 (95% CI 1.16–1.80) was observed; the adjusted *p* value for multiple testing for this SNP was 0.02. Adjusted *p* values for other SNPs were 0.10 for rs3751591, 0.12 for rs1961177, and 0.22 for rs17523880. Assessment of specific tumors markers (data not shown in table) showed rs1961177 associated with CIMP+/KRAS, CIMP+, and MSI tumors, with the CT/TT genotypes increasing risk. However, the *p* for heterogeneity was not significant. Additionally, rs12591359 AA and rs17523880 CA/AA genotypes showed a significant 50% increased risk of CIMP+ tumors and rs18523880 CA/AA showed a significant 40% increased risk of MSI tumors; however, the *p* for heterogeneity was not statistically significant for these associations.

For rectal cancer, four SNPs not in LD appeared to be associated independently with disease risk (Table 3). Estimates of risk ranged from 0.66 (95% CI 0.51–0.84) for rs2470144 to 1.32 (95% CI 1.06–1.64) for rs7174997. rs2470144 had a statistically significant *p* value of 0.02 after adjusting for multiple comparisons. The only potentially meaningful difference by race was observed for rs1961177 among non-white participants and rectal cancer, where the OR was 0.39 (95% CI 0.23–0.67), although CI overlap with those presented in Table 2. For rectal cancer three SNPs were uniquely associated with specific tumor markers (data not shown in table): rs12591359 AA genotype was associated with CIMP+/KRAS tumors (OR 3.59 95% CI 1.50–8.61, Wald *p* value 0.004; pACT 0.08, *p* heterogeneity 0.0076); rs10519295 TT/CC genotypes were associated with CIMP+ tumors (OR 0.30 95% CI 0.11–0.84, Wald *p* value 0.023, pACT 0.31, *p* heterogeneity 0.039); and rs1961177 CT/TT genotypes were associated with CIMP+ tumors (OR 0.18 95% CI 0.06–0.51; Wald *p* value 0.001; pACT 0.027; *p* heterogeneity 0.0009). Other SNPs showed roughly a doubling of risk of having a CIMP+/KRAS-mutated tumor (rs700519 CT/TT, rs80251 91 AG/GG, rs17703883 TC/CC, rs1961177 CC, rs2470144 GG/GA, and rs28757184 CT/TT), however, the *p* for heterogeneity was greater than 0.05.

We assessed gene × gene interactions and found no meaningful interactions with *ESR1*, *ESR2*, or *AR*. However, several SNPs in *NFκB1* interacted significantly with *CYP19A1* SNPs in analyses of both colon and rectal cancer (Table 4). For colon cancer rs12591359 and rs3751591 interacted with *NFκB1*. For rectal cancer rs4646 and rs700519 interacted with several *NFκB1* SNPs, of these, the interaction between *CYP19A1* rs700519 and *NFκB1* rs1609798 and rs230510 had pACT values of 0.03.

Assessment of interactions with NSAIDs, estrogen status, and BMI did not yield consistent associations across any *CYP19A1* SNPs for colon cancer. However, for rectal cancer, we saw statistically significant interactions with both NSAID use and BMI. Differences in associations across levels of these variables are shown with haplotypes of SNPs not in LD with each other that interacted significantly with these variables, rs11856927, rs2470144, and rs7174997 (Table 5). The TGG and the GAG haplotypes were significantly associated with both NSAID use and BMI.

After adjusting for AJCC stage at diagnosis and tumor markers, having the TT genotype of rs7174997 increased the likelihood of death after colon cancer diagnosis by 70% (Table 6). For rectal cancer, the A allele for rs12591359 were inversely associated with survival time, as was the GG genotype of rs17601876, rs1902584 AT/TT genotype, rs2470158 TT genotype, and the CA/AA genotype of rs4646.

Discussion

Our data suggest that genetic variation in *CYP19A1* influences risk of developing colon and rectal cancer. The specific molecular pathways of most relevance appear to be those involving CIMP+, KRAS2-mutated, and MSI+ tumors. Given the observed gene–gene and gene–environment interactions, it is reasonable to postulate that the influence of estrogen on colon and rectal cancer risk may involve inflammation-related mechanisms.

We observed several SNPs in the *CYP19A1* gene independently associated with both colon and rectal cancer. Three haplotypes were associated with colon cancer and one haplotype was associated with rectal cancer risk. These haplotypes consisted of SNPs not in LD with each other and added little information beyond that gained by examining-independent SNPs. Of interest is the observation that although *CYP19A1* polymorphisms were associated with colon and rectal cancer, the polymorphisms that were most important differed for colon and rectal cancer. We have frequently observed that associations between colon and rectal cancer differ, suggesting that although the genes are important to both, that the specific mechanism whereby the gene is working may differ. However, we observed greater similarities in the associations when examining molecular tumor phenotypes. For both colon and rectal cancer *CYP19A1* SNPs were associated with CIMP+/KRAS and CIMP+ tumors. Other markers appeared to be important for MSI+ colon tumors, and thus the low frequency of MSI+ rectal tumors could account for the lack of association of these SNPs with rectal cancer overall. Our previous work has suggested that estrogens may be involved in MSI tumors [6]; this work corroborates those earlier findings. MSI+ and CIMP+ tumors often cluster together. Our data suggest that a CIMP+ pathway and CIMP+/KRAS2-mutated tumors may be related to estrogen. Others have suggested that sex is associated with CIMP+ colon tumors [33].

Several variants in *CYP19A1* have been associated with variable circulating estrogen levels [34–37], and haplo-block analysis has shown that this variability can be captured by analyzing tagSNPs [34]. *CYP19A1* haplotypes also have been investigated in association with cancer risk at various sites [38–41]. It should be noted that two of the markers that we demonstrate as being associated with rectal cancers; rs1961177, and with colon cancer; rs7174997, resides within 6 and 9 kb, respectively, of two tightly linked SNPs in the 5' flanking region of *CYP19A1* (rs6493497 and rs7176005) that have previously been shown to be associated with increased circulating estradiol levels [42]. Further investigation will be required to determine if rs7174997 and rs1961177 are directly associated with plasma estradiol levels. However, some associations became evident when interactions with other genes and/or exposures were examined [43, 44].

Data have shown that estrogen can enhance an inflammatory response by activating the NFκB complex pathway [13]. NFκB signaling pathway has been identified as one of the key pathways through which anti-estrogens and aromatase inhibitors exert their effects [45]. We observed statistically significant interactions between genetic variants of *NFκB1* and *CYP19A1* for both colon and rectal cancer. For rectal cancer, we also observed significant interaction between *CYP19A1* variants and regular use of aspirin/NSAIDs and BMI. These observations also suggest that the estrogen associations with colon and rectal cancer may operate via an inflammation-related pathway.

Our data show that variation in the *CYP19A1* gene can influence survival after diagnosis with colon cancer. We have previously reported that HRT use influences colon cancer survival, possible through an MSI-related pathway [6, 7]. Others have shown that estrogen levels in colon tumors are statistically significantly associated with prognostic factors and

may influence the progression of the disease [46]. Our data suggest that genetic factors that may influence estrogen levels also may relate to survival after diagnosis.

The study has many strengths, the data set is rich in exposure data, genetic data, tumor marker data, and survival information. Because of this, we have the ability to thoroughly evaluate *CYP19A1* to determine not only broad independent associations, but also to help identify molecular pathways through which they may operate. In doing this, we made many comparisons. However, several *p* values remained statistically significant even after adjustment for these comparisons. To better understand these findings, functional studies are needed to elucidate the role of *CYP19A1* and colon and rectal cancer.

In summary, our findings support a role of *CYP19A1* and colon and rectal cancer development and survival. Our data further suggest that CIMP+ and MSI+ are important molecular subtypes in this association. Given the significant interactions between variants of *CYP19A1* and *NFκB1*, aspirin, and BMI, we postulate that an estrogen-related inflammation driven pathway is central to carcinogenesis in these CRC subtypes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Description of study population

	Colon				Rectal			
	Male		Female		Male		Female	
	Control n (%)	Case n (%)	Control n (%)	Case n (%)	Control n (%)	Case n (%)	Control n (%)	Case n (%)
Center								
Utah	201 (19.20)	142 (16.32)	177 (19.47)	107 (15.62)	201 (37.15)	156 (34.59)	164 (39.23)	118 (38.94)
KPMCP	439 (41.93)	428 (49.20)	348 (38.28)	316 (46.13)	340 (62.85)	295 (65.41)	254 (60.77)	185 (61.06)
Minnesota	407 (38.87)	300 (34.48)	384 (42.24)	262 (38.25)	NA	NA	NA	NA
Age								
30–39	22 (2.10)	13 (1.49)	18 (1.98)	10 (1.46)	10 (1.85)	12 (2.66)	11 (2.63)	7 (2.31)
40–49	70 (6.69)	56 (6.44)	58 (6.38)	46 (6.72)	55 (10.17)	47 (10.42)	46 (11.00)	49 (16.17)
50–59	169 (16.14)	163 (18.74)	157 (17.27)	127 (18.54)	133 (24.58)	119 (26.39)	110 (26.32)	77 (25.41)
60–69	371 (35.43)	306 (35.17)	302 (33.22)	232 (33.87)	200 (36.97)	161 (35.70)	129 (30.86)	89 (29.37)
70–79	415 (39.64)	332 (38.16)	374 (41.14)	270 (39.42)	143 (26.43)	112 (24.83)	122 (29.19)	81 (26.73)
Race/ethnicity								
NHW	974 (93.03)	799 (91.84)	854 (93.95)	629 (91.82)	458 (84.66)	365 (80.93)	366 (87.56)	260 (85.81)
Hispanics	39 (3.72)	33 (3.79)	36 (3.96)	26 (3.80)	36 (6.65)	37 (8.20)	27 (6.46)	24 (7.92)
Black	34 (3.25)	38 (4.37)	19 (2.09)	30 (4.38)	29 (5.36)	17 (3.77)	14 (3.35)	12 (3.96)
Asian	0	0	0	0	18 (3.33)	32 (7.10)	11 (2.63)	7 (2.31)

Table 2

Associations between *CYP19A1* and risk of colon cancer

	Controls <i>n</i>	Cases <i>n</i>	OR ^a (95% CI)	Wald <i>p</i> value	pACT
<i>CYP19A1</i> (rs12591359)				0.00102	0.02231
GG/GA	1659	1276	1		
AA	297	278	1.44 (1.16–1.80)		
<i>CYP19A1</i> (rs17523880)				0.01525	0.22143
CC	1454	1126	1		
CA/AA	502	430	1.23 (1.04–1.45)		
<i>CYP19A1</i> (rs1961177)				0.00683	0.11669
CC	1547	1161	1		
CT/TT	410	394	1.26 (1.06–1.48)		
<i>CYP19A1</i> (rs3751591)				0.00575	0.10496
TT	1333	1115	1		
TC/CC	624	441	0.81 (0.70–0.94)		
<i>CYP19A1</i> rs12591359(G>A) and rs17523880(C>A) and rs1961177(C>T) and rs3751591(T>C)					

	Frequency	Per copy risk (95% CI)	Global <i>p</i> value
G-C-C-T	0.38	0.89 (0.80–0.99)	0.0059
A-C-C-T	0.25	1.06 (0.94–1.20)	
G-A-C-T	0.09	1.14 (0.96–1.35)	
A-C-C-C	0.09	0.82 (0.69–0.97)	
A-C-T-T	0.05	1.45 (1.17–1.79)	
G-A-C-C	0.04	0.92 (0.71–1.18)	
G-C-T-T	0.04	1.05 (0.84–1.31)	
G-C-C-C	0.02	0.9 (0.65–1.25)	
G-C-T-C	0.01	0.92 (0.59–1.45)	

^a Adjusted for age, center, race, sex, other genotypes, and rs11856927

Table 3Associations between *CYP19A1* and risk of rectal cancer

	Controls <i>n</i>	Cases <i>n</i>	OR ^a (95% CI)	Wald <i>p</i> value	pACT
<i>CYP19A1</i> (rs1961177)				0.0188	0.2713
CC	715	583	1.00		
CT/TT	244	171	0.75 (0.59–0.95)		
<i>CYP19A1</i> (rs2470144)				0.001	0.0214
GG/GA	690	576	1.00		
AA	269	178	0.66 (0.51–0.84)		
<i>CYP19A1</i> (rs7174997)				0.0149	0.233
GG	660	492	1.00		
GT/TT	299	262	1.32 (1.06–1.64)		
<i>CYP19A1</i> rs1961177(C>T) and <i>CYP19A1</i> rs2470144(G>A) and <i>CYP19A1</i> rs7174997(G>T)					
Frequency	Per copy risk (95% CI)	Global <i>p</i> value			
C-G-G	0.37	1.17 (1.02–1.34)	0.4652		
C-A-G	0.33	0.86 (0.74–0.99)			
C-A-T	0.19	1.12 (0.93–1.34)			
T-G-G	0.11	0.83 (0.68–1.01)			

^a Adjusted for age, center, race, sex, and other genotypes

Table 4

Interaction between *CYP19A1* and *NFκB1* and colon and rectal cancer

	Controls	Cases	OR ^a (95% CI)	Controls	Cases	OR (95% CI)	p Interaction	pACT
<i>Colon cancer</i>								
<i>CYP19A1</i> (rs12591359)								
<i>NFκB1</i> (rs3821958)	GG/GA			AA				
AA	572	447	1.00	114	96	1.08 (0.80–1.46)	0.0215	0.2890
AG	787	615	1.00 (0.85–1.18)	142	119	1.07 (0.81–1.41)		
GG	300	213	0.91 (0.73–1.12)	40	63	2.01 (1.32–3.05)		
<i>CYP19A1</i> (rs3751591)								
<i>NFκB1</i> (rs230510)	TT			TC/CC				
AA	454	343	1.00	183	165	1.2 (0.93–1.55)	0.0040	0.0730
AT	615	545	1.19 (0.99–1.42)	310	197	0.84 (0.67–1.06)		
TT	263	226	1.16 (0.92–1.45)	131	79	0.81 (0.59–1.11)		
<i>NFκB1</i> (rs3774964)								
AA	531	450	1.00	261	169	0.76 (0.61–0.96)	0.0190	0.2647
AG	600	516	1.01	287	191	0.78 (0.63–0.98)		
GG	201	148	0.86	76	81	1.26 (0.90–1.77)		
<i>Rectal cancer</i>								
<i>CYP19A1</i> (rs4646)								
<i>NFκB1</i> (rs3774964)	CC			CA/AA				
AA/AG	395	343	1.00	434	279	0.74 (0.60–0.92)	0.0061	0.1908
GG	74	59	0.93 (0.64–1.35)	56	73	1.46 (1.00–2.13)		
<i>CYP19A1</i> (rs700519)								
<i>NFκB1</i> (rs11722146)	CC			CT/TT				
GG	458	299	1.00	27	38	2.02 (1.20–3.41)	0.0026	0.0972
GA	337	294	1.32 (1.06–1.63)	49	34	0.99 (0.62–1.58)		
AA	78	83	1.59 (1.13–2.24)	10	6	0.86 (0.31–2.42)		
<i>NFκB1</i> (rs1609798)								
CC	432	286	1.00	25	37	2.09 (1.22–3.57)	0.0009	0.0398
CT	358	292	1.22 (0.99–1.52)	49	35	1.01 (0.63–1.60)		

	Controls	Cases	OR ^a (95% CI)	Controls	Cases	OR (95% CI)	p Interaction	pACT
TT	81	97	1.77 (1.27–2.47)	12	6	0.72 (0.27–1.96)		
<i>NFKB1</i> (rs1801)								
GG	362	237	1.00	21	34	2.33 (1.31–4.13)	0.0036	0.1235
GC	394	327	1.26 (1.01–1.57)	52	35	0.96 (0.60–1.52)		
CC	117	112	1.44 (1.06–1.95)	13	9	0.98 (0.41–2.35)		
<i>NFKB1</i> (rs230510)								
AA	260	250	1.00	40	26	0.63 (0.37–1.07)	0.0007	0.0328
AT	437	328	0.78 (0.63–0.98)	42	40	0.94 (0.59–1.50)		
TT	176	98	0.58 (0.43–0.79)	4	12	2.98 (0.94–9.38)		
<i>NFKB1</i> (rs4648068)								
AA	417	279	1.00	22	35	2.23 (1.28–3.91)	0.0026	0.0955
AG	364	305	1.23 (0.99–1.53)	53	36	0.94 (0.60–1.48)		
GG	92	92	1.46 (1.05–2.03)	11	7	0.89 (0.34–2.35)		

^a Adjusted for age, center, race, and sex

Table 5

Associations among aspirin, BMI, *CYP19A1*, and rectal cancer risk

<i>CYP19A1</i> rs11856927(T>G) and rs2470144(G>A) and rs7174997(G>T)				
	No recent aspirin use OR (95% CI) ^a	Recent aspirin use OR (95% CI)	<i>p</i> Interaction	
T-G-G	0.81 (0.66–0.98)	1.16 (0.92–1.47)	0.0116	
G-G-G	1.16 (0.94–1.44)	1.27 (0.96–1.67)	0.6567	
G-A-G	1.10 (0.88–1.39)	0.63 (0.47–0.85)	0.0004	
T-A-T	1.09 (0.85–1.40)	1.15 (0.85–1.56)	0.6397	
T-A-G	0.89 (0.69–1.16)	0.81 (0.59–1.13)	0.5768	
G-A-T	1.18 (0.69–2.02)	1.00 (0.49–2.03)	0.7665	
	Normal (<25) OR (95% CI)	Overweight (25–29) OR (95% CI)	Obese (≥30) OR (95% CI)	<i>p</i> Interaction
T-G-G	0.95 (0.73–1.25)	0.81 (0.64–1.01)	1.20 (0.89–1.61)	0.0125
G-G-G	0.86 (0.64–1.15)	1.39 (1.06–1.81)	1.57 (1.12–2.20)	0.0026
G-A-G	1.12 (0.82–1.53)	0.89 (0.67–1.19)	0.68 (0.48–0.95)	0.0082
T-A-T	1.17 (0.83–1.66)	1.25 (0.93–1.70)	0.88 (0.61–1.27)	0.1149
T-A-G	0.96 (0.66–1.40)	0.80 (0.58–1.10)	0.83 (0.57–1.22)	0.1546
G-A-T	1.09 (0.52–2.27)	1.34 (0.69–2.59)	0.75 (0.31–1.84)	0.2901

^a Adjusted for age, center, race, and sex

Associations between *CYP19A1* SNPs and colorectal cancer survival center, race, sex, AJCC stage, tumor markers, and other SNPs

Table 6

	Death/person years	HRR (95% CI)	Wald <i>p</i> value	FDR <i>p</i> value
<i>Colon</i>				
<i>CYP19A1</i> (rs7174997)			0.0403	0.6803
GG/GT	281/6189	1		
TT	17/193	1.7 (1.02–2.82)		
<i>Rectal</i>				
<i>CYP19A1</i> (rs12591359)			0.0005	0.0061
GG	33/1100	1		
GA/AA	117/2143	2.3 (1.45–3.67)		
<i>CYP19A1</i> (rs17601876)			0.0002	0.0059
AA/AG	115/2402	1		
GG	35/851	2.6 (1.57–4.31)		
<i>CYP19A1</i> (rs1902584)			0.01	0.0677
AA	123/2764	1		
AT/TT	27/489	1.78 (1.15–2.77)		
<i>CYP19A1</i> (rs2470158)			0.0084	0.0677
CC/CT	149/3242	1		
TT	1/11	15.78 (2.03–122.91)		
<i>CYP19A1</i> (rs4646)			0.0401	0.2167
CC	71/1770	1		
CA/AA	79/1483	1.5 (1.02–2.22)		

Hazard Rate Ratios (HRR) adjusted for age, sex, center, tumor marker, and AJCC stage