Associations between *ERα*, *ERβ*, and *AR* Genotypes and Colon and Rectal Cancer

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Abstract

Estrogen and androgens are thought to be involved in the etiology of colorectal cancer. We evaluate genetic variants of the estrogen receptor genes (*ERα* and *ERβ*) and the androgen receptor gene (*AR*). We use data from two large case-control studies of colon (n = 1,580 cases and 1,968 controls) and rectal (n = 797 cases and 1,016 controls) cancer. We evaluated the 351A>G *Xba*I polymorphism of *ERα*, the 1,082 G>A and CA repeat polymorphisms of *ERβ*, and the CAG repeat of *AR*. Having two 25 or more CA repeats in *ERβ* was associated with an increased relative risk of colon cancer in women [odds ratio (OR), 2.13; 95% confidence interval (95% CI), 1.24-3.64] but not in men ($P_{interaction}$ relative excess risk from interaction < 0.01; multiplicative = 0.03). Increasing number of *AR* CAG repeats was directly associated with colon cancer among men (OR, 1.28; 95% CI, 1.06-1.54), but not women (OR, 0.83; 95% CI, 0.68-1.02); the interaction *P* value for *AR* gene × sex was <0.01. Taking hormone replacement therapy (HRT) was associated with a reduced risk of colon cancer in the presence of the *R* allele of the *ERβ* gene, whereas an *R* allele was associated with increased risk among postmenopausal women who did not take HRT. Postmenopausal women not using HRT who had ≥25 CA repeats of the *ERβ* gene had over a 6-fold increased risk of colon cancer (OR, 6.71; 95% CI, 2.89-15.6). Our results suggest that the *ERβ* gene is more important than ERα in the etiology of colorectal cancer. (Cancer Epidemiol Biomarkers Prev 2005;14(12):2936–42)

Introduction

The importance of estrogens and androgens to the etiology of colon and rectal cancer is supported by many avenues of research. Observational epidemiologic studies and controlled clinical trials show that women who use hormone replacement therapy (HRT) after menopause are at lower risk of developing both colon and rectal cancer (1-3). Furthermore, HRT use has been associated with a reduced likelihood of having a microsatellite unstable tumor (4) and gender-specific associations have been observed for *p53* tumor mutations (5). Studies have shown that among premenopausal women, postmenopausal women taking HRT, and men, high body mass index (BMI) is directly associated with colon cancer (6); however, high BMI does not increase risk among postmenopausal women not taking HRT. Because of the similarities in BMI associations for women exposed to estrogen and for men, it has been hypothesized that androgen, as well as estrogen, might be related to colon cancer risk. Both estrogen and androgen receptors are present in colorectal tissue (7-12). Most of the actions of estrogens and androgens seem to be exerted through the respective receptors (13, 14), suggesting that the estrogen receptor and androgen receptor may be important in regulating colorectal cancer risk associated with these hormones.

There are two forms of the estrogen receptor, α and β (14). They are part of the nuclear hormone receptor family and are ligand-inducible transcription factors. Studies suggest tissue specificity for expression of the two estrogen-receptor genes, with *ERβ* expressed more in colon tissue than the *ERα* (11, 15, 16). The highly polymorphic *ERα* gene, also called *ER1*, is located on human chromosome 6q25. The most widely studied polymorphisms of the *ERα*, which seem to be in linkage disequilibrium, are the *Pvu*II 397T>C, the *Xba*I (351A>G) restriction fragment length polymorphisms in intron I, and the TA repeat within the promoter region of the gene. The *P* and *X* alleles designate the absence of the restriction sites, whereas the *p* and *x* alleles designate the presence of the restriction site. The *Xba*I polymorphism has been associated with upper body obesity, BMI, and circulating levels of androstenedione with the *xx* genotype having higher levels (17).

The *ERβ* gene, also called *ER2*, is located on chromosome human 14q23-24.1 (17). Polymorphic variants of *ERβ* also have been identified although their functional association is less well understood than polymorphisms of ERα. Three silent transitions (1,421T>C in exon 7, 1,082G>A within the ligand-binding domain in exon 5, and the 1,730A>G in the 3′ untranslated region of exon 8) have been reported (17). A CA dinucleotide repeat in intron 5 initially characterized in a Japanese population (13) is also present in other racial groups, and five additional polymorphisms have been identified in African American populations. The number of CA repeats has been shown to be associated with bone mineral density and androgen and sex hormone–binding globulin levels in premenopausal women. Women with short repeats had higher hormone levels and lower sex hormone–binding globulin levels than women with many CA repeats (18). Having an *R* allele (absence of the *Rsa*I site) at 1,082G>A has been reported as being associated with increased ovulatory dysfunction (19) and body weight (20).

The gene encoding the AR is located on the X chromosome. The *AR* gene contains two polymorphic trinucleotide repeat segments that encode polyglutamine (CAG) and polyglycine (GGC). The CAG repeat has been studied most extensively and has been shown to range from 6 to 39 repeats, with some studies showing that the number of repeats is inversely related to prostate cancer risk. Fewer CAG repeats result in higher transcription of AR mRNA (21). One study showed that in the presence of relatively long CAG repeats, decline in age-related serum androgen levels did not occur in men (22). Westberg

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et al. (18) found that women with fewer CAG repeats in the *AR* gene had higher serum androgens levels and lower leutenizing hormone levels than women with more repeats. There is evidence that there is an acquired reduction in the number of CAG repeats in the *AR* gene in colon tumors (23).

In this study, we examine associations between genetic variants of the *ERα, ERβ*, and *AR* genes and risk of colon and rectal cancer. We hypothesize that colon and rectal cancer risk will vary by polymorphisms that influence hormone levels. Additionally, we evaluate associations between polymorphisms in the *ERα, ERβ*, and *AR* genes to determine differences in association by specific types of tumor mutations, given our previous work that indicate gender-specific associations with tumors (5) and associations between estrogen and unstable tumors (4). We examine associations by age at diagnosis and by sex. We also evaluate interactions between *ERα, ERβ*, and *AR* with estrogen status and use of HRT.

Materials and Methods

Study Populations. All aspects of this study were approved by the University of Utah Institutional Review Board as well as Institutional Review Boards at the Kaiser Permanente Medical Care Program of Northern California (KPMCP) and the University of Minnesota.

Participants were from the KPMCP, the state of Utah, and the Twin City Metropolitan area of Minnesota (colon cancer study only). All eligible cases within these defined areas were identified and recruited for a study of colon cancer and a study of rectal cancer. The colon study included cases of first primary colon cancer (International Classification of Diseases-O second edition codes 18.0 and 18.2 to 18.9) diagnosed between October 1, 1991, and September 30, 1994, conducted in all three target areas. Cases from the second study were diagnosed with a first primary tumor in the rectosigmoid junction or rectum and were identified between May 1997 and May 2001 and restricted to cases and controls from Utah and KPMCP. Case eligibility was determined by the Surveillance Epidemiology and End Results Cancer Registries in Northern California and in Utah and the Minnesota Cancer Surveillance System (colon cancer cases only). Registries identified all cases living in the geographic area of the study who met study criteria. These cases were asked to participate in the study. Eligibility included being between 30 and 79 years of age at time of diagnosis, English speaking, mentally competent to complete the interview, no previous history of colorectal cancer (24), and no known (as indicated on the pathology report) familial adenomatous polyposis, ulcerative colitis, or Crohn's disease. Of cases contacted, 83% participated at KPMCP, 76% in Utah, and 67% in Minnesota. For the rectal cancer study, the cooperation rates were 75.4% of cases from KPCMP and 69.7% of cases from Utah.

Controls were frequency matched to cases by sex and by 5-year age groups. At the KPMCP, controls were randomly selected from membership lists. In Utah, controls ≥65 years were randomly selected from lists provided by the Centers for Medicare and Medicaid Services (formerly Health Care Financing Administration) and controls <65 years were randomly selected from driver's license lists. In Minnesota, controls were randomly selected from driver's license lists. Of controls contacted for the colon cancer study, 73% participated at KPMCP, 53% participated from Minnesota, and 69% participated from Utah. For the rectal cancer study, cooperation rates were 69.9% for KPMCP and 67.2% for Utah.

Data Collection. Trained and certified interviewers collected diet and lifestyle data (25, 26). The reference period for the study was the calendar year ~2 years before date of diagnosis (cases) or selection (controls). Information was collected on demographic factors, such as age, sex, and study center; diet and lifestyle, including medical and reproductive history. HRT was ascertained including dates first used, length of time used, and dates stopped using. Women were asked to report menopausal status.

Genotyping. DNA was extracted from peripheral blood leukocytes. For quality control, controls representing all known polymorphic variants and blanks were included in each 96-well tray. All genotypes were scored by two individuals with any discrepancies being scored by a third reader.

The 351A>G *Xba*I polymorphism of the ERα was evaluated by PCR amplifying 20 ng of genomic DNA with primers ESRα *Xba*I-F-5′-GATATCCAGGGTTATGTGGCA-3′ and ESRα-*Xba*I-R-5′-AGGTGTTGCCTATTATATTAACCTTGA-3′ in the presence of 0.2 mmol/L deoxynucleotide triphosphates, 1.5 mmol/L Mg^{2+}, and 0.4 units of Taq polymerase (Perkin-Elmer, Foster City, CA) in a 12 μL volume. The PCR amplification reactions consisted of 94°C for 2 minutes, followed by 30 cycles of 15 seconds at 94°C, 15 seconds at 60°C, and 20 seconds at 72°C. A final extension of 5 minutes at 72°C was done. The entire 12 μL PCR product was restriction digested with 5 units of *Xba*I (NEB, Ipswich, MA) at 37°C overnight. The restriction-digested products were size fractionated in a 2% Nusieve agarose gel containing ethidium bromide and visualized with UV light. The *A* allele is cut, yielding bands of 148 and 198 bp, whereas the *G* allele remains uncut and results in a band of 346 bp (modified from ref. 27). The cut allele is defined as *x* (*A*) and the uncut allele is defined as *X* (*G*).

ERβ CA repeat genotyping was PCR amplified from 20 ng of genomic DNA using oligonucleotide primers *ERβ* CA-F-5′-HEX-GGTAAACCATGGTCTGTACC-3′ and *ESRβ* CA-R-5′-AACAAAATGTTGAATGAGTGGG-3′ (19). The 14 μL PCR reaction contained 0.2 mmol/L deoxynucleotide triphosphates, 1.5 mmol/L Mg^{2+}, and 0.4 units of AmpliTaq Gold polymerase (Perkin-Elmer). The PCR reactions were denatured at 95°C for 5 minutes, amplified for 35 cycles of 10 seconds at 95°C, 10 seconds at 60°C, and 10 seconds at 72°C. A final extension of 7 minutes at 72°C was done. The Hex-labeled products were then analyzed on an ABI 3700 automated sequencer. Allele sizes were converted into CA repeat copy number for analysis by sequencing specific alleles in the population.

The *Rsa*I polymorphism of *ERβ* was determined using primers *ERβ-Rsa*I-F-5′-TCTTGCTTTCCCCAGGCTTT-3′ and *ESRβ-Rsa*I-R-5′-ACCTGTCCAGAACAAGATCT-3′ in the presence of 0.2 mmol/L deoxynucleotide triphosphates, 1.5 mmol/L Mg^{2+}, and 0.4 units of Taq polymerase (Perkin-Elmer) in a 12 μL volume. The PCR amplification reactions consisted of 94°C for 2 minutes, followed by 35 cycles of 15 seconds at 94°C, 15 seconds at 53°C, and 20 seconds at 72°C. A final extension of 5 minutes at 72°C was done. The entire 12 μL PCR product was restriction digested with 5 units of *Rsa*I (NEB) at 37°C overnight. The restriction-digested products were size fractionated in a 2% Nusieve gel containing ethidium bromide and visualized with UV light. The *G* allele remains uncut and results in a band of 156 bp; the *A* allele is cut and yields bands of 125 and 31 bp (modified from ref. 19). The cut allele is defined as *r* (*A*) and the uncut allele is defined as *R* (*G*).

AR CAG repeat genotyping was done according the methods of Westberg et al. (18) with minor modifications. Briefly, 20 ng of genomic DNA was PCR amplified using oligonucleotide primers *AR*-F2-5′-GTTTCTGTGGGGCCTCTACGATGG-3′ and *AR*-R2-5′-GTTTCTGCGCGAAGTGATCCAGAA-3′ (HEX labeled). The 14 μL PCR reaction contained 0.2 mmol/L deoxynucleotide triphosphates, 1.5 mmol/L Mg^{2+}, and 0.4 units of Taq polymerase (Perkin-Elmer). The PCR reactions were initially denatured at 94°C for 1 minute then subjected to 35 cycles consisting of 15 seconds at 94°C, 15 seconds at 57°C, followed by 30 seconds at 72°C. A final

extension of 5 minutes at 72°C was done. The Hex-labeled products were then analyzed on an ABI 3700 automated sequencer. Allele sizes were converted into CAG repeat copy number for analysis by sizing alleles that had been sequenced.

Methods for obtaining tumor tissue have been described (28). Mutational analysis for microsatellite instability and mutations in Ki-*ras* and *p53* genes in 1,572 colon cancer cases were determined in previous studies and results have been reported previously (29-31).

Statistical Analysis. SAS statistical package, version 8.2, was used to conduct the analyses; SAS genetics was used to test for Hardy-Weinberg equilibrium and determine allele frequencies. Analyses included evaluating the distribution of the alleles and genotypes in the population studied the independent associations of genetic polymorphisms with colorectal cancer risk, and the joint effect of genotypes on colorectal cancer risk. Multiple logistic regression models, adjusting for age, race, and sex, were used to determine associations between genotypes and colorectal cancer risk and reported as odds ratio (OR) and 95% confidence intervals (95% CI). In these models, other potentially confounding factors were considered: BMI, physical activity, dietary composition, energy intake, and aspirin/nonsteroidal anti-inflammatory drug use. Both estrogen status and HRT use were examined in conjunction with genotype. For assessment of interaction of genotype and estrogen exposure in women, estrogen status was defined as being positive if during the past 2 years women were either premenopausal or using HRT if postmenopausal. All women are included in the analysis when using this variable, and results are similar if using either HRT or estrogen status. Accordingly, we present results from the more inclusive and robust estrogen status variable that includes all women. We explored several cut points for categorizing the *ERβ* and *AR* repeat variants based on what others have used; we present the categories that seemed to best describe associations in the data.

The majority of the population studied was non-Hispanic white and adjustment for racial group did not influence results; stratification by ethnic group was not meaningful, given small sample size for participants who were not White (see Table 1). No age-specific associations were detected; age was used as an adjustment variable. Site-specific analyses were done for colon and rectal (rectosigmoid junction and rectum) cancers.

Several tests for interaction were done to evaluate the joint effect of genotype with estrogen status on colorectal cancer risk. The relative excess risk from interaction (RERI) and the corresponding 95% CI were calculated to provide insight into differences that might be expected on an additive scale of interaction as described by Hosmer and Lemoshow (32); multiplicative interaction was tested using the cross product of the two variables of interest in the logistic model. Polytomous regression models were used to evaluate associations with specific mutations in colon tumors. In these regression models, those with any *p53* or any Ki-*ras* mutation or any microsattelite instability (MSI) were compared with controls. Associations between months survived and genotype were determined using Cox proportional hazards models, adjusting for age at time of diagnosis, sex, and tumor stage. Months of survival were determined using data of diagnosis and vital status as reported to tumor registries as of January 2004. We estimated the likelihood of dying from any cause as well as death from colorectal cancer.

Results

The majority of the population was non-Hispanic white, ~92% to 93% for colon cancer study and 83% to 86% of the rectal cancer study (Table 1). For both studies, the majority of cases were men.

Table 1. Description of study population

	Colon		Rectal	
	Cases, *n* (%)	Controls, *n* (%)	Cases, *n* (%)	Controls, *n* (%)
Site				
KPMCP	763 (48.2)	814 (41.2)	506 (63.5)	627 (61.7)
Utah	247 (15.6)	363 (18.4)	291 (36.5)	389 (38.3)
Minnesota	573 (36.2)	799 (40.4)	NA	NA
Age (y)				
<40	23 (1.5)	40 (2.0)	20 (2.5)	22 (2.2)
40-49	102 (6.5)	126 (6.4)	100 (12.6)	110 (10.8)
50-59	297 (18.8)	330 (16.8)	209 (26.2)	256 (25.2)
60-69	549 (34.8)	682 (34.7)	270 (33.9)	349 (34.4)
70-79	609 (38.5)	790 (40.1)	198 (24.8)	279 (27.5)
Ethnicity				
White Caucasian	1,446 (91.7)	1,835 (93.3)	653 (83.0)	861 (85.7)
Hispanic	61 (3.9)	78 (4.0)	54 (6.9)	71 (7.1)
African American	70 (4.4)	53 (2.7)	30 (3.8)	40 (4.0)
Asian	0	0	40 (5.1)	31 (3.1)
AIAN	0	0	10 (1.3)	2 (0.2)
Sex				
Male	887 (56.1)	1,051 (53.4)	469 (58.9)	572 (56.3)
Female	693 (43.9)	917 (46.6)	328 (41.1)	444 (43.7)
ERα				
xx	632 (42.8)	774 (42.6)	334 (43.3)	407 (41.4)
Xx	679 (45.9)	812 (44.7)	342 (44.3)	455 (46.2)
XX	167 (11.3)	230 (12.7)	96 (12.4)	122 (12.4)
ERβ				
rr	1,449 (93.1)	1,802 (93.4)	704 (89.9)	909 (91.6)
Rr	103 (6.6)	125 (6.5)	75 (9.6)	77 (7.8)
RR	4 (0.3)	2 (0.1)	4 (0.5)	6 (0.6)
*ERβ** (CA repeats)				
9-19	268 (8.8)	339 (8.9)	154 (10.5)	166 (8.8)
20	299 (9.8)	423 (11.1)	160 (10.9)	233 (12.4)
21	101 (3.3)	110 (2.9)	48 (3.3)	73 (3.9)
22	198 (6.5)	227 (5.9)	94 (6.4)	116 (6.2)
23	497 (16.2)	597 (15.6)	238 (16.2)	320 (17.0)
24	1,017 (33.2)	1,357 (35.5)	487 (33.1)	601 (32.0)
25	494 (16.1)	543 (14.2)	200 (13.6)	276 (14.7)
26-34	190 (6.2)	225 (5.9)	89 (6.1)	93 (5.0)
*AR** (CAG repeats)				
9-16	61 (2.7)	77 (2.7)	30 (2.7)	21 (1.5)
17	33 (1.5)	41 (1.5)	19 (1.7)	26 (1.8)
18	130 (5.8)	184 (6.5)	62 (5.6)	76 (5.3)
19	228 (10.2)	292 (10.3)	121 (11.0)	152 (10.6)
20	251 (11.3)	372 (13.1)	104 (9.5)	147 (10.2)
21	408 (18.3)	467 (16.5)	179 (16.3)	259 (18.0)
22	255 (11.4)	310 (11.0)	121 (11.0)	159 (11.1)
23	228 (10.2)	316 (11.2)	148 (13.5)	148 (10.3)
24	235 (10.5)	280 (9.9)	119 (10.8)	182 (12.7)
25	165 (7.4)	211 (7.5)	89 (8.1)	118 (8.2)
26	103 (4.6)	137 (4.8)	49 (4.5)	64 (4.5)
27	56 (2.5)	49 (1.7)	22 (2.0)	37 (2.6)
28	41 (1.8)	54 (1.9)	17 (1.6)	19 (1.3)
29-41	37 (1.7)	42 (1.5)	19 (1.7)	23 (2.0)

Abbreviations: AIAN, American Indian and Alaska native; NA, not applicable.
*Reports numbers of alleles, rather than individuals, with the CA and CAG repeat; therefore, numbers are greater than the population studied.

Genetic variants in the *ERα*, *ERβ*, and *AR* polymorphisms were in Hardy-Weinberg equilibrium. The *ERαX* allele frequency was 35% of the control population for all racial groups, except for Asians in whom it was 20%. The frequency of the *R* allele of the *ERβ* gene was ~3% in white and non-Hispanic white populations, slightly more frequent among African American (11% in colon study controls and 6% in rectal study controls), and considerably more frequent in Asian populations (29%). The most common CA repeat of the *ERβ* gene was 24 with the range from 9 to 34. The range in *AR* CAG repeats went from 9 to 41 repeats among controls, with most of the population having between 19 and 25 repeats. More women were classified as having any allele with 23 or more CAG repeats because 48%

were heterozygous for a short and a long allele. Roughly 82% of African American study participants had an *AR* allele with fewer than 23 CAG repeats.

There were no significant associations between *ERα* and *ERβ* genotypes and risk of colon and rectal cancer (Table 2). However, we observed a significant interaction between sex and number of CA repeats of the *ERβ* gene and risk of colon cancer (RERI $P_{interaction} < 0.01$; multiplicative $P = 0.03$). Over a 2-fold risk of colon cancer was observed among women with both alleles of ≥25 CA repeats, whereas for men no association was observed. Having any *R* allele of the *ERβ* gene was associated with a significant increased risk of rectal cancer among the total population if diagnosed before 60 years of age (OR, 1.68; 95% CI, 1.02-2.79; $P_{interaction}$ between age and genotype < 0.01; data not shown in table), whereas having more *ERβ* CA repeats increased risk of colon cancer among individuals ≥60 years of age irrespective of sex (OR, 1.82; 95% CI, 1.18-2.81; multiplicative $P_{interaction} = 0.09$). No significant associations were observed between the number of CA repeats in the *ERβ* gene and rectal cancer nor did we observe age or sex-specific associations. We did not detect any statistically significant association between *ERα* genotypes and either colon or rectal cancer.

Because the *AR* gene is sex-linked, heterozygosity is only observed for women. We observed a significant interaction between sex and number of CAG repeats in the *AR* gene (Table 3). Among men, having ≥23 CAG repeats resulted in a 30% increased risk of colon cancer (95% CI, 1.06-1.53), whereas among women 23 or more CAG repeats was associated with reduced risk of colon cancer (OR, 0.8; 95% CI, 0.7-1.0). The $P_{interaction}$ was significant on both the additive and multiplicative scale. *AR* genotype was not associated with rectal cancer risk.

There was no significant association between survival after diagnosis with colon or rectal cancer and *ERα, ERβ*, or *AR* genotypes (data not shown in table). There was no evidence of interaction between the *ER* and *AR* genotypes (data not shown in table). However, the group at greatest risk was women who had both long alleles of the *ERβ* gene and the *AR* gene (OR, 2.85; 95% CI, 1.27-6.39) when compared with women with the 24/24 *ERβ* gene and short *AR* CAG repeats.

Polytomous regression analysis of *ERα, ERβ*, and *AR* genotypes with specific tumor mutations compared with controls showed that *ERα* was not associated with any tumor mutations (data not shown in table). However, having the Rr or RR *ERβ* genotype significantly increased the odds of having a Ki-*ras* mutation among women (OR, 1.8; 95% CI, 1.1-3.2; Table 4), and produced a nonsignificant increased likelihood of having MSI in tumors (OR, 1.6; 95% CI, 0.8-3.0). Among women, having a 24/24 CA repeat genotype of the *ERβ* gene decreased the risk of MSI and Ki-*ras* mutated tumors and did not influence risk of *p53* mutations. The *AR* genotype was only associated with *p53* mutations and MSI tumors in men, where having 23 or more CAG repeats was associated with significantly higher risk (p53: OR, 1.4; 95% CI, 1.1-1.8; MSI: OR, 1.4; 95% CI, 1.0-2.2).

Evaluation of the interaction between estrogen status and *ERα, ERβ*, and *AR* genotypes showed a significant interaction between *ERβ* and estrogen status and risk of colon cancer (Table 5). Women who were estrogen positive were at reduced risk of colon cancer if they had the *Rr* or *RR* genotype; whereas among women who were estrogen negative, having this genotype had the strongest association with increased colon cancer risk. The risk was most pronounced among women with two alleles of 25 or more CA repeats (multiplicative $P_{interaction} = 0.01$). Women with two long alleles and who were estrogen negative were almost seven times (OR, 6.71; 95% CI, 2.89-15.6) more likely to develop colon cancer than women who were estrogen positive and had 24/24 CA repeats. No significant interaction was observed between (19) α and *AR* genes and estrogen status.

Table 2. Association between *ERα* and *ERβ* and colon and rectal cancer

	Everyone		Men		Women	
	ERα	OR (95% CI)	*ERα*	OR (95% CI)	*ERα*	OR (95% CI)
Colon	*xx*	1.0	*xx*	1.0	*xx*	1.0
	Xx or *XX*	1.0 (0.9-1.1)	*Xx* or *XX*	1.0 (0.9-1.2)	*Xx* or *XX*	1.0 (0.8-1.2)
$P_{interaction}$ for genotype and gender, RERI: 0.66; m: 0.65						
Rectal	*xx*	1.0	*xx*	1.0	*xx*	1.0
	Xx or *XX*	0.9 (0.8-1.1)	*Xx* or *XX*	1.0 (0.8-1.3)	*Xx* or *XX*	0.9 (0.6-1.1)
$P_{interaction}$ for genotype and gender, RERI: 0.54; m: 0.50						
	ERβ	OR (95% CI)	*ERβ*	OR (95% CI)	*ERβ*	OR (95% CI)
Colon	*rr*	1.0	*rr*	1.0	*rr*	1.0
	RR or *Rr*	1.1 (0.8-1.4)	*RR* or *Rr*	1.1 (0.8-1.6)	*RR* or *Rr*	1.0 (0.6-1.4)
$P_{interaction}$ for genotype and gender, RERI: 0.57; m: 0.58						
Rectal	*rr*	1.0	*rr*	1.0	*rr*	1.0
	RR or *Rr*	1.2 (0.9-1.7)	*RR* or *Rr*	1.4 (0.9-2.1)	*RR* or *Rr*	1.0 (0.6-1.7)
$P_{interaction}$ for genotype and gender, RERI: 0.36; m: 0.39						
	CA repeats	OR (95% CI)	CA repeats	OR (95% CI)	CA repeats	OR (95% CI)
Colon	<24/<24	1.2 (0.9-1.5)	<24/<24	1.1 (0.8-1.5)	<24/<24	1.2 (0.9-1.8)
	24/24	1.0	24/24	1.0	24/24	1.0
	≥25/≥25	1.4 (0.9-2.0)	≥25/≥25	1.0 (0.6-1.6)	≥25/≥25	2.1 (1.2-3.6)
	H	1.1 (0.9-1.4)	H	0.9 (0.6-1.2)	H	1.4 (1.0-2.0)
$P_{interaction}$ for genotype and gender, RERI: <0.01; m: 0.03						
Rectal	<24/<24	0.9 (0.6-1.3)	<24/<24	0.7 (0.4-1.2)	<24/<24	1.1 (0.7-2.0)
	24/24	1.0	24/24	1.0	24/24	1.0
	≥25/≥25	0.9 (0.5-1.6)	≥25/≥25	0.8 (0.4-1.8)	≥25/≥25	1.1 (0.5-2.4)
	H	0.9 (0.6-1.2)	H	0.8 (0.5-1.3)	H	0.9 (0.6-1.5)
$P_{interaction}$ for genotype and gender, RERI: 0.69; m: 0.43						

NOTE: Data were adjusted for age, sex, and race.
Abbreviations: m, multiplicative interaction; H, heterozygote defined as any combination of <24, 24, or >25 CA repeats.

Table 3. Association between *AR* CAG repeats and colon and rectal cancer

	Everyone		Men		Women	
	AR (CAG)	OR (95% CI)	*AR* (CAG)	OR (95% CI)	*AR* (CAG)	OR (95% CI)
Colon	<23/<23	1.0	<23	1.0	<23/<23	1.0
	Any ≥23	1.1 (0.9-1.2)	≥23	1.3 (1.1-1.5)	Any ≥23	0.8 (0.7-1.0)
$P_{interaction}$ for genotype and gender, RERI: <0.01; m: <0.01						
Rectal	<23/<23	1.0	<23/<23	1.0	<23/<23	1.0
	Any ≥23	1.1 (0.9-1.3)	Any ≥23	1.0 (0.8-1.3)	Any ≥23	1.1 (0.8-1.5)
$P_{interaction}$ for genotype and gender, RERI: 0.76; m: 0.73						

NOTE: Data were adjusted for age, sex, and race.

Discussion

Results from this study provide support for involvement of estrogen and androgen signaling in the etiology of colorectal cancer. We observed a stronger and more consistent association between polymorphisms of the *ERβ* than the *ERα* gene and colorectal cancer. Among younger people, having an *R* allele of the *ERβ* gene increased risk of rectal cancer. *ERβ* interacted with estrogen status to alter risk of colon cancer, with those having an *R* allele having significantly lower risk of colon cancer if estrogen positive but higher risk of colon cancer if estrogen negative than those who had the rr genotype; those at greatest risk were women with two or more 25 CA repeat alleles, the group of women thought to have the lowest hormone levels based on data from Westberg et al. (18). We also observed a significant interaction between gender and the *ERβ* CA repeat and the *AR* CAG repeat, with number of repeats being directly associated with risk of colon cancer in men and inversely associated with risk among women for the CAG repeat; the opposite was true for the CA repeat.

Estrogens and androgens regulate growth, differentiation, and functioning of a variety of tissues, including that found in the gastrointestinal tract (33-35). Most of the action of estrogens and androgens seems to be exerted through the respective receptors of target cells (13, 14). The estrogen receptor and androgen receptor function as ligand-activated transcription factors that regulate the synthesis of specific RNAs and proteins among different cellular signaling pathways in the regulation of gene expression and cell proliferation (36). The two distinct *ER* genes (*ERα* and *ERβ*) seem to react differently to estrogen, with *ERα* decreasing as E2 levels increase while *ERβ* levels increase.

Studies have shown that ERβ is the predominate estrogen receptor expressed in colonic tissue (11, 37). In our study, we observed associations with acquired mutations in colon tumors only for *ERβ* genotype among women. The 24/24 CA repeat genotype was associated with a lower likelihood of having a Ki-*ras* mutation or a MSI tumor, although we had limited power to assess the association with MSI among women. Our previous work has shown that HRT is inversely associated with MSI (4), suggesting that women were less likely to have an unstable tumor in the presence of estrogen. Studies have shown that ER is involved in Ki-*ras*-mediated transcription (38). Although limited data exist on functionality of CA repeats, data suggest that few repeats are suggested with higher testosterone levels and lower sex hormone–binding globulin levels and many CA repeats are associated with lower testosterone and higher sex hormone–binding globulin levels. The 24/24 CA repeat was the most common repeat number in our data and may represent a level of hormones that are more balanced than either high or low number of repeats. Interestingly, this group was at the lowest risk of either MSI or Ki-*ras* mutations, suggesting that at either low or high hormone levels may influence increased risk of these tumor alterations. Overall, among women, those with more repeats were at increased risk of colon cancer. This would support that women with lower hormone levels are at increased risk of colorectal cancer, which is consistent with the protective effect observed for HRT and risk of colorectal cancer (1). Our data also suggest that with increasing CAG repeats, there is a greater likelihood of MSI or *p53* mutated tumors among men. These observed associations of estrogens and androgens with several different tumor types reinforce their importance in the etiology of colon cancer and their role in multiple signaling pathways.

ERβ has been shown to be involved in multiple functions that may importantly influence colorectal cancer risk. It has been shown to mediate the regulation of glutathione *S*-transferases and transcriptionally activate other major detoxifying enzymes. ERβ also has been shown to play an important role in cellular protection against oxidative stress (35). ERβ has been shown to interact with interleukin-6, suggesting involvement in

Table 4. Age-adjusted polytomous regression of *ERβ* and *AR* on types of colon tumor mutations compared with healthy controls

	Men				Women			
	p53, OR (95% CI)	MSI, OR (95% CI)	Ki-*ras*, OR (95% CI)	None detected, OR (95% CI)	*p53*, OR (95% CI)	MSI, OR (95% CI)	Ki-*ras*, OR (95% CI)	None detected, OR (95% CI)
ERβ								
rr	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Rr or RR	0.9 (0.5-1.6)	1.1 (0.4-2.4)	1.0 (0.6-1.9)	1.7 (0.9-1.6)	1.1 (0.6-2.0)	1.6 (0.8-3.0)	1.8 (1.1-3.2)	0.6 (0.2-1.8)
CA repeats								
<24/<24	1.0 (0.7-.17)	1.1 (0.5-2.3)	0.8 (0.5-1.5)	1.2 (0.6-2.5)	1.0 (0.6-1.8)	2.1 (0.9-4.8)	2.2 (1.1-4.6)	0.9 (0.4-2.1)
24/24	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
>25/>25	0.7 (0.3-1.5)	0.6 (0.2-2.1)	1.0 (0.5-2.2)	0.6 (0.2-2.1)	1.8 (0.9-3.8)	2.7 (0.9-8.3)	2.8 (1.1-7.4)	2.5 (0.9-7.0)
H	0.8 (0.5-1.1)	0.8 (0.4-1.5)	0.8 (0.5-1.2)	0.9 (0.5-1.8)	1.2 (0.7-1.8)	2.1 (1.0-4.5)	1.9 (1.0-3.7)	1.4 (0.7-2.7)
AR								
<23/<23	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Any ≥23	1.4 (1.1-1.8)	1.4 (1.0-2.2)	1.0 (0.8-1.4)	1.1 (0.7-1.6)	0.8 (0.6-1.1)	0.8 (0.5-1.1)	0.8 (0.5-1.1)	0.7 (0.5-1.1)

Table 5. Associations between *ERβ* genotypes and estrogen status and risk of colon cancer among women

ERβ	Estrogen status positive, OR (95% CI)	Estrogen status negative, OR (95% CI)
rr	1.0	1.4 (1.1-1.8)
Rr or RR	0.5 (0.2-1.0)	2.0 (1.1-3.4)
$P_{interaction}$ for estrogen status and genotype, RERI: 0.05; m: 0.02		
CA repeat		
<24/<24	1.0 (0.6-1.9)	1.7 (1.0-3.1)
24/24	1.0	1.3 (0.7-2.5)
≥25/≥25	0.8 (0.3-2.0)	6.7 (2.9-15.6)
H	1.4 (0.8-2.3)	1.8 (1.04-3.0)
$P_{interaction}$ for estrogen status and genotype, RERI: 0.78; m: 0.01		

NOTE: OR values were adjusted for age, BMI, physical activity, energy intake, cigarette smoking status, dietary calcium, and dietary fiber.

inflammation processes (39). Differences in ERβ expression have been observed in adipose tissue for men and women, with women having higher levels than men; no differences in ERα levels were detected between men and women (40). Studies have shown decreases in ERβ expression as colonic tumors progress, suggesting a silencing of the gene. Our results suggest that the *ERβ* genotype may be involved in risk of colorectal cancer and that it may alter colon cancer risk through interaction with estrogen status. Given the findings from Westberg et al. (18) that women with more CA repeats have lower hormone levels, our data suggest that these women are most susceptible to lack of estrogen. Our data suggest that women are at a ~7-fold increase of colon cancer if have two long alleles and are estrogen negative relative to women who have shorter alleles and are estrogen positive. It is of interest to note that colon cancer risk is reduced in response to HRT use, whereas HRT increases risk of breast cancer. Because ERα is the predominant form of the estrogen receptor in breast tissue and ERβ is the predominant form of estrogen receptor in colonic tissue, it is possible that the two forms of ER react differently to HRT.

Little is known about the role of androgens in the etiology of colorectal cancer, although studies in rats suggest that androgens work as promoters in the development of colon cancer (41). Androgen receptors are also expressed in colon tissue and acquired changes in the length of the CAG repeat of the *AR* in colon tumors have been detected (42). AR transcriptional activity is determined by the presence or absence of other cofactors that can alter AR activity (43). Like estrogens, androgens up-regulate IRS-1. IRS-1 has been associated with colon cancer. Clinical studies suggest the functional importance of the CAG repeat sequence of the *AR* gene, with fewer repeats being associated with higher transcriptional activity of the receptor; more repeats are associated with lower serum androgen levels (22). However, a study by Westburg et al. (18) suggests that serum androgen levels in women are regulated by both the *AR* and *ERβ* genes, and that fewer *AR* CAG repeats were associated with higher androgen levels in women. They suggested that this difference in association between number of CAG repeats and androgen levels would imply that in women, the influence of AR on androgen production is stimulatory rather than inhibitory. Results from our study suggest that lower androgen levels increase risk of colon cancer. We also observed that women at greatest risk of colon cancer were those who had long repeats of both genes. This is consistent with the finding that low estrogen increases risk of colon cancer and implies similar mechanisms of action for sex-steroid hormones on colon cancer risk.

There are several limitations to the current study, perhaps the major one being limited information as to the functionality of polymorphisms being studied. Haplotype analysis could be informative to identify important variants and better understand genetic variation of these genes in population. However, haplotype analysis of the *AR* gene in one study suggested that the CAG repeat may be the most informative variant; other data suggest functionality for that polymorphism in terms of regulation of androgen levels. We believe that associations seen with various polymorphisms examined provide support for the involvement of both androgen receptor and estrogen receptors in the etiology, and that more thorough analysis of the genes may provide additional support and may strengthen the observed associations. It is likely that the associations imply the importance of the gene rather than of the specific polymorphism examined. Additional studies examining other polymorphisms and functionality of these genes are needed.

Other study limits include response rates that are slightly below 70%. It is unclear if participants are different from those who do not participate on the key variables of interest for this study. We also have tested several associations, although we believe that our *a priori* hypothesis was strong. Our large sample of three populations reduced our likelihood of a false-positive finding as described by Wacholder et al. (44). However, it also is recognized that despite our large sample, several of the alleles being studied were relatively rare. We believe that it is important for our findings to be confirmed by other large studies because is it possible that these result may be the consequence of chance.

These results add to other data and support the involvement of estrogens and androgens in the etiology of colorectal cancer. Our results reinforce the importance of the *ERβ* gene rather than the *ERα* gene in colorectal cancer, and may therefore help better understand how estrogens work in the etiology of colorectal cancer. The associations with the *AR* gene seem to differ by sex and relate to specific types of tumor mutations. Efforts to better understand how estrogen and androgen receptors operate within multiple disease pathways are needed.

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Associations between *ERα*, *ERβ*, and *AR* Genotypes and Colon and Rectal Cancer

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