

Common Single-Nucleotide Polymorphisms in the Estrogen Receptor β Promoter Are Associated with Colorectal Cancer Survival in Postmenopausal Women

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Abstract

Loss of estrogen receptor β (ER β) expression in the gut is associated with colorectal cancer (CRC) initiation and progression. Germline single-nucleotide polymorphisms (SNP) in genes for the sex-steroid hormone receptors are not strongly associated with CRC risk; however, these SNPs have not previously been evaluated in relation to survival after diagnosis. We enrolled 729 women, ages 50 to 74, diagnosed with invasive CRC between 1997 and 2002 in 13 counties covered by the Seattle-Puget Sound Surveillance Epidemiology and End Results cancer registry. Participants provided germline DNA. We selected 99 tag-SNPs for the androgen receptor (*AR*), ER α (*ESR1*), ER β (*ESR2*), and progesterone receptor (*PGR*) genes. Mortality outcomes were ascertained from the National Death Index. During a median of 6.6 years of follow-up, 244 deaths occurred (161 from CRC). We identified 20 SNPs (12 of *ESR2* and 8 of *PGR*) for replication in 1,729 women diagnosed with incident invasive CRC (555 deaths; 405 from CRC) from three prospective cohort studies that participate in the Genetics and Epidemiology of Colorectal Cancer Consortium. Three correlated SNPs in the promoter of *ESR2* (rs2987983, rs3020443, and rs2978381) were statistically significant predictors of CRC-specific and overall survival. Minor alleles of each were associated with improved survival [for rs2987983, CRC-specific HR, 0.77; 95% confidence interval (CI), 0.60–0.99 in the initial study, and HR, 0.79; CI, 0.64–0.98 in replication]. No associations were noted for SNPs of *AR*, *ESR1*, or *PGR*. SNPs in the promoter of *ESR2* may be important to pathways related to the association between ER β and tumor progression and metastasis. *Cancer Res*; 73(2); 767–75. ©2012 AACR.

Introduction

Endogenous and exogenous sex-steroid hormones play an important role in colorectal carcinogenesis (1). Women are less likely to develop colorectal cancer (CRC) than men, and are also less likely to develop fatal CRC (2). Use of postmenopausal hormone therapy reduces the risk of CRC (3), but the role of hormones on disease progression and survival remains unclear. Some studies have found that prediagnostic hormone

use is associated with improved CRC survival (4), whereas others have found no association (5), or associations for use only near the time of diagnosis (6). In the Women's Health Initiative (WHI) clinical trial, estrogen plus progestin therapy reduced the risk of developing CRC (7), but estrogen only therapy did not (8). Colorectal tumors in women taking estrogen plus progestin were generally discovered at a more advanced stage with no reduction in mortality (9).

The influence of hormones on colorectal carcinogenesis is facilitated by the expression of sex-steroid receptors in the colonic epithelium. These receptors belong to a superfamily of ligand-inducible transcription factors, including androgen and progesterone receptors (AR and PR), and 2 subtypes of estrogen receptors (ER α and ER β). AR, PR, ER α , and ER β are encoded by the genes *AR* on Xq12, *PGR* on 11q22-23, *ESR1* on 6q25, and *ESR2* on 14q23-24, respectively. All 4 receptors have been found to be expressed at some level in the gut, with ER β being the most abundantly expressed (10, 11). Loss of ER expression is commonly observed in neoplastic colon tissue (12, 13) and the degree of expression loss is correlated with characteristics of poorer CRC prognosis (14, 15).

Recent studies have evaluated whether the risk of developing CRC depends on inherited variation in genes that encode for the hormone receptors. A case-control study in Germany

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identified a single-nucleotide polymorphism (SNP) in the 3' untranslated region (UTR) of *ESR2* associated with CRC risk (16), but nested case-control studies from the Women's Health Study and WHI did not find SNPs in hormone-receptor genes to be related to CRC incidence (17, 18). Less is known about whether germline variants in hormone-receptor genes are associated with disease prognosis. A cytosine and adenine (CA)_n dinucleotide repeat polymorphism of *ESR2* has been linked to survival after diagnosis in patients with metastatic CRC (19, 20), but, to date, mortality outcomes have not been considered in a large-scale SNP-based study. Accordingly, we genotyped postmenopausal women with incident CRC to assess associations between tag-SNPs in *AR*, *PGR*, *ESR1*, and *ESR2* and CRC-specific and overall survival after diagnosis. We replicated our findings using survival time and genotype data from women with incident invasive CRC in 3 prospective cohort studies that participate in the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO): (i) Nurses' Health Study (NHS); (ii) VITamins And Lifestyle (VITAL) Study; and (iii) WHI.

Materials and Methods

Discovery study population

We identified postmenopausal women, ages 50 to 74, diagnosed with incident invasive colorectal adenocarcinoma between 1997 and 2002 among residents of the 13 counties in Washington State that participate in the Seattle-Puget Sound Surveillance Epidemiology and End Results (SEER) cancer registry. These women were recruited to serve as cases in a population-based case-control study of hormone therapy and CRC incidence (Postmenopausal Hormones Supplementary Study to the Colon Cancer Family Registry; PMH-CCFR). Recruitment and data collection procedures have been previously described (21). Age at diagnosis, race, tumor subsite, and stage at diagnosis were obtained from SEER records. Primary tumors were located proximal to and including the splenic flexure (proximal); in the descending or sigmoid colon (distal); or in the rectum according to ICD-O3 codes. Approximately 73% of eligible women with CRC invited to participate agreed to complete the interview and 70% of these women provided a blood or buccal sample to be genotyped for tag-SNPs in *AR*, *ESR1*, *ESR2*, and *PGR*. All women were followed prospectively for death from CRC or any cause. Vital status and cause of death information was obtained through December 31, 2009 from the National Death Index (NDI). Of 738 genotyped cases in PMH-CCFR, we excluded 7 (1%) missing survival time, and 2 (1%) missing stage. This study was approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center (FHCRC).

SNP selection

Tag-SNPs of *AR*, *ESR1*, *ESR2*, and *PGR* with minor allele frequency (MAF) $\geq 5\%$ were selected using the Genome Variation Server (GVS) based on CEU (Utah residents with northern and western European ancestry) populations genotyped by HapMap, Perlegen Sciences, Inc., and the National Institute of Environmental Health Sciences Environmental

Genome Project (22). We used a linkage-disequilibrium (LD) threshold of $r^2 \leq 0.8$. Coverage extended into the intragenic regions 2,000 bases upstream and 1,000 bases downstream of each gene. Monomorphic SNPs were excluded. When selecting SNPs from LD blocks, preference was given to functional variants. To ensure adequate representation of blocks in the event of genotyping failure, 2 SNPs were genotyped in each block that consisted of more than 5 SNPs. After genotyping was completed, blocks were pruned so that no 2 SNPs had pairwise $r^2 \geq 0.9$. A total of 125 tag-SNPs were selected from GVS (5 in *AR*, 69 in *ESR1*, 34 in *ESR2*, and 17 in *PGR*). Six SNPs were excluded for not meeting our genotyping quality-control (QC) criteria (described below; 4 in *ESR1*, 1 in *ESR2*, 1 in *PGR*). Pruning resulted in the exclusion of 20 SNPs (7 in *ESR1* and 13 in *ESR2*), leaving 99 total SNPs available for analyses (5 in *AR*, 58 in *ESR1*, 20 in *ESR2*, and 16 in *PGR*).

Genotyping procedures

DNA was extracted from stored blood or buccal samples using the QIAamp extraction kit (Qiagen) with PicoGreen Quantitation Reagent (Invitrogen). Genotyping was conducted by the Genomics Shared Resource at the FHCRC using the Illumina GoldenGate platform and BeadStudio software (Illumina). All SNPs were required to meet the following QC criteria for inclusion in our analysis: GenTrain Score > 0.4 ; 10% GC Score > 0.25 ; call frequency $> 85\%$; replicate errors < 2 ; duplicate concordance $> 85\%$; and Hardy-Weinberg P value > 0.0001 . External control samples from the HapMap project were included on each plate (NA17116, NA07034; Coriell Cell Repository), along with intraplate and interplate replicates. Replicates were more than 99% concordant.

Statistical analyses

Overall survival was calculated from the date of diagnosis until death from any cause or the end of available follow-up on December 31, 2009. In the CRC-specific survival analyses, survival time was censored at the date of death from causes other than CRC. Five-year survival proportions were estimated using the Kaplan-Meier method. Hazard ratios (HR) and 95% confidence intervals (CI) were estimated from proportional hazards regression models. Each of the 99 SNPs was evaluated in a separate model that adjusted for age at diagnosis (50–59, 60–69, and 70–74 years), stage (localized, regional, and distant), and race (Caucasian and non-Caucasian). P values for a linear trend in the genotype association with survival were calculated for each SNP using a variable coded 0, 1, or 2, based on the number of copies of the minor allele. We calculated corrected P values that accounted for multiple statistical comparisons using a gene-specific linear step-up false discovery rate (FDR; ref. 23). All SNPs with $P_{\text{FDR}} \leq 0.2$ for associations with CRC-specific or overall survival were eligible for further consideration in the replication stage.

Replication procedures

We conducted replication analyses using data from a subset of CRC studies participating in GECCO, a National Cancer Institute Epidemiology and Genomics Research Program-supported consortium. As of 2012, 3 GECCO studies that

included female participants had data on mortality outcomes available for this analysis (NHS, VITAL, and WHI).

WHI samples were genotyped on different platforms (described below) and have been split into 2 groups (WHI Set 1; WHI1 and WHI Set 2; WHI2). WHI enrolled study participants from 1993 to 1998; WHI1 includes women diagnosed with incident colon cancer from 1993 to 2005 and WHI2 includes those diagnosed with incident CRC from 1993 to 2009. WHI reviewed medical records and death certificates regularly as part of study follow-up, and sought mortality data from NDI for deaths that could not be adjudicated from available study records through August 31, 2009. NHS enrolled women in 1976 and 1989, with incident cases of CRC diagnosed between 1976 and 2008. NHS ascertained mortality outcomes from NDI, complete through June 1, 2008 and adjudicated cause of death from review of medical records and death certificates. VITAL, which recruited healthy individuals from the 13 counties covered by the Seattle-Puget Sound SEER cancer registry between 2000 and 2002, obtained mortality information for incident cases occurring between 2000 and 2009 from WA state death certificates through December 31, 2009 and censored follow-up time when women moved out of the state. Study-specific eligibility, data collection, and harmonization procedures have been published (24–26).

Tumor site was classified according to ICD-9/10 codes provided from each study. Stage at diagnosis was harmonized to reflect SEER summary stage categorizations. Germline genotype data from these GECCO studies have been previously used to evaluate the association between genome-wide association study-identified CRC susceptibility loci and survival after diagnosis (27). Genotyping procedures differed for each study. NHS used the Illumina OmniExpress platform and VITAL used the Illumina CytoSNP BeadChip platform. WHI1 included women from the WHI observational study and was genotyped on the Illumina 550 and 550-Duo platforms. WHI2 included women in the WHI observational study and clinical trials and was genotyped on the Illumina CytoSNP BeadChip platform. SNPs were imputed for each GECCO study using MaCH with the HapMap2 CEU population (release 24) as the reference (28). Additional details of the genotyping procedures and QC checks for studies that participate in GECCO are described in Peters and colleagues (29). Of 460, 1,006, 394, and 135 genotyped cases in WHI1, WHI2, NHS, VITAL, respectively, we excluded 25 (5%), 43 (4%), 97 (25%), and 5 (4%) missing survival time, and 5 (1%), 53 (6%), 34 (12%), and 1 (< 1%) missing stage, respectively. Three women younger than age 50 at diagnosis were also excluded from NHS.

SNPs that were found to have $P_{\text{FDR}} \leq 0.2$ in the discovery stage were evaluated in separate proportional hazards regression models that adjusted for age at diagnosis (50–59, 60–69, and ≥ 70 years), stage (localized, regional, and distant), and the first 3 principal components (to account for population substructure). SNP variables were coded 0, 1, or 2, based on the minor-allele count. Replication analyses were conducted separately for each of the 4 sets (NHS, VITAL, WHI1, and WHI2), and HR estimates were pooled using inverse variance-weighted random-effects meta-analysis.

Between-study heterogeneity was quantified with the I^2 statistic. SNPs were considered statistically significant if the P value from the pooled estimate for NHS, VITAL, WHI1, and WHI2 was 0.05 or less. For SNPs that met this replication threshold, we calculated 2 other HR estimates using the best call for imputed genotypes, in addition to the allele-dosage HR, based on: (i) a dominant model that compared those with 1 or 2 minor alleles to those who were homozygous for the major allele and (ii) a recessive model that compared those with 2 minor alleles to those with 1 or 2 major alleles. Estimates that use all available data by pooling the HRs from the discovery and replication stages are also provided.

We evaluated the proportional hazards assumption by testing the statistical significance of an interaction term with log-transformed survival-time using a gene-wise FDR correction based on $P_{\text{FDR}} \leq 0.05$. For SNPs included in replication analyses, the proportional hazards assumption was tested separately for each study. Analyses were conducted using SAS 9.2 (SAS Institute Inc.). All statistical tests were 2-sided.

Results

PMH-CCFR included 729 women with incident CRC, of whom 244 had died (161 from CRC) at the end of study follow-up. The GECCO replication cohort included 1,729 women with incident CRC, of whom 555 had died (405 from CRC). Selected characteristics of each study population are summarized in Table 1. Participants of PMH-CCFR were younger, on average, than women in the replication populations. In general, case-control studies may have difficulty enrolling rapidly fatal cases; however, PMH-CCFR had a similar stage distribution as the prospective cohort studies used for replication, suggesting that this was not a substantial problem. Tumor subsite distributions were also similar, with the exception of WHI1. By design of the WHI1 genotyping effort, investigators chose to include mostly women with cancers of the colon. Women in PMH-CCFR were predominantly Caucasian. The genotyping for the studies included in GECCO was restricted to Caucasians due to small numbers of other races. In PMH-CCFR, cases that contributed DNA for genotyping were more likely to be Caucasian than those who did not (92% vs. 88%), but age, subsite, and stage distributions were similar (data not shown). The median duration of follow-up after diagnosis was 6.6, 7.1, 5.0, 9.1, and 5.0 years for PMH-CCFR, WHI1, WHI2, NHS, and VITAL, respectively.

In the discovery stage, 15 SNPs (9 of *ESR2* and 6 of *PGR*) had $P_{\text{FDR}} \leq 0.2$ for both CRC-specific and overall survival. One SNP of *ESR2* had $P_{\text{FDR}} \leq 0.2$ for only CRC-specific survival, and 4 SNPs (2 of *ESR2* and 2 of *PGR*) had $P_{\text{FDR}} \leq 0.2$ for only overall survival. No SNPs of *ESR1* or *AR* reached this threshold. A total of 20 SNPs were subsequently considered for replication (Supplementary Table S1). Imputed or directly genotyped data were available for 19 of the 20 SNPs for each of the 4 replication sets (rs613120 was unavailable and no suitable proxy could be identified). Imputation quality was good (r^2 across the 4 replication sets was between 0.91 and 1.02 for these 19 SNPs). The

Table 1. Characteristics of women in the discovery and replication stages

	Discovery stage		Replication stage				Combined replication
	PMH-CCFR	WHI1	WHI2	NHS	VITAL		
Number of women with CRC	729	430	910	260	129	1,729	
Number of deaths	244	157	257	104	37	555	
Number of deaths from CRC	161	113	192	77	23	405	
Age at diagnosis (years), <i>N</i> (%)							
Range	50–74	52–86	50–91	52–85	55–83	50–91	
Mean (SD)	63.9 (7.2)	70.9 (7.0)	71.9 (7.2)	68.5 (7.3)	70.7 (6.1)	71.1 (7.2)	
50–59	220 (30)	25 (6)	33 (4)	28 (11)	9 (7)	95 (5)	
60–69	302 (41)	141 (33)	309 (34)	118 (45)	41 (32)	609 (35)	
≥ 70	207 (28)	264 (62)	568 (62)	114 (44)	79 (61)	1,025 (59)	
Caucasian race, <i>N</i> (%)	674 (92)	430 (100)	910 (100)	260 (100)	129 (100)	1,729 (100)	
Postmenopausal hormone therapy ^a , <i>N</i> (%)							
No	309 (44)	—	328 (56)	122 (52)	62 (53)	512 (54)	
Yes	401 (56)	—	261 (44)	114 (48)	56 (47)	431 (46)	
Not available	19	—	321	24	11	356	
Tumor subsite, <i>N</i> (%)							
Proximal colon	352 (48)	283 (66)	462 (51)	132 (51)	71 (56)	948 (55)	
Distal colon	251 (34)	135 (32)	216 (24)	72 (28)	31 (25)	454 (27)	
Rectum	126 (17)	9 (2)	227 (25)	54 (21)	24 (19)	314 (18)	
Not available	0	3	5	2	3	13	
Stage at diagnosis, <i>N</i> (%)							
Localized	309 (42)	172 (40)	392 (43)	60 (23)	58 (45)	682 (39)	
Regional	344 (47)	196 (46)	397 (44)	150 (58)	48 (37)	791 (46)	
Distant	76 (10)	62 (14)	121 (13)	50 (19)	23 (18)	256 (15)	
5-year CRC-specific survival, % (95% CI)	78 (74, 81)	73 (68, 77)	76 (72, 79)	72 (66, 77)	80 (71, 87)	75 (72, 77)	
5-year overall survival, % (95% CI)	75 (71, 78)	67 (62, 71)	70 (66, 73)	70 (64, 76)	73 (63, 80)	69 (67, 72)	

Abbreviation: OS, observational study.

^aPrediagnostic use of estrogen-alone or estrogen+progestin. Use one year prior to diagnosis for PMH-CCFR, at baseline questionnaire for WHI2 and VITAL, in 1990 for NHS. Not available for WHI-OS.

assumption of proportional hazards was not violated for any SNP.

Three of the 19 SNPs had HR estimates pooled across WHI1, WHI2, NHS, and VITAL with $P \leq 0.05$ (rs2987983, rs3020443, and rs2978381; Supplementary Table S2). All 3 are in the 5' intronic region of *ESR2*, and are moderately correlated with each other (in the HapMap CEU population, pairwise $r^2 = 0.67$ for rs2987983 and rs3020443; $r^2 = 0.51$ for rs2978381 and rs2987983; and $r^2 = 0.33$ for rs2978381 and rs3020443). rs3020443 was directly genotyped in all sets, rs2978381 was imputed in WHI2 and VITAL, and rs2987983 was imputed in WHI1, WHI2, and VITAL. For all 3 SNPs, the minor allele was associated with a decreased risk of death from CRC and death from any cause in adjusted regression models (Table 2). Kaplan–Meier CRC-specific survival estimates, stratified by genotype for each study, are displayed in Supplementary Figures S1–S3.

Using all available data from the discovery and replication stages, adjusted HRs per minor allele for rs2987983, rs3020443, and rs2978381 had $P = 0.002$, 0.006 , and 0.004 for

CRC-specific survival, respectively, and $P = 0.001$, 0.006 , and 0.01 for overall survival, respectively. The log-additive, dominant, and recessive models provided similar results. None of these 3 SNPs had statistically significant associations with stage at diagnosis in bivariate analyses, and survival estimates were similar in regression models that did not adjust for stage at diagnosis. Models including all 3 SNPs simultaneously did not inform whether one particular variant was an independent predictor of survival because of the high degree of multicollinearity. None of the associations for the 6 SNPs in *PGR* identified in the discovery stage replicated in the GECCO studies.

Inverse associations between survival and carriage of the minor allele were of similar magnitudes in each replication cohort, with the exception of WHI1. Heterogeneity across all replication cohorts was low ($I^2 = 36\%$; CI, 0%–78% for rs2987983; $I^2 = 0\%$; 95% CI, 0%–66% for rs3020443; and $I^2 = 0\%$; CI, 0%–65% for rs2978381 based on CRC-specific HRs). For these 3 SNPs of *ESR2*, we explored whether associations with survival differed by stage at diagnosis, prediagnostic use of

Table 2. Associations with CRC-specific and overall survival for rs2987983, rs3020443, and rs2978381 (ESR2)

	Genotype ^a			CRC-specific survival HR ^b (95% CI)			Overall survival HR ^b (95% CI)		
	AA	Aa	aa	Per a Allele	AA vs. Aa/aa	AA/Aa vs. aa	Per a Allele	AA vs. Aa/aa	AA/Aa vs. aa
rs2987983 (A>G)									
Discovery	353	307	67	0.77 (0.60, 0.99)	0.71 (0.52, 0.98)	0.74 (0.42, 1.30)	0.75 (0.61, 0.93)	0.69 (0.53, 0.89)	0.76 (0.48, 1.22)
PMH-CCFR ^c	182	195	53	1.01 (0.76, 1.34)	1.09 (0.74, 1.59)	0.86 (0.46, 1.59)	1.02 (0.80, 1.28)	1.11 (0.80, 1.54)	0.83 (0.49, 1.39)
Replication	424	392	94	0.75 (0.60, 0.94)	0.67 (0.50, 0.90)	0.81 (0.49, 1.35)	0.76 (0.63, 0.93)	0.71 (0.56, 0.92)	0.69 (0.44, 1.07)
WH1	138	100	22	0.66 (0.44, 0.99)	0.70 (0.42, 1.16)	0.21 (0.05, 0.88)	0.83 (0.61, 1.12)	0.73 (0.48, 1.11)	0.89 (0.46, 1.71)
NHS	69	49	11	0.54 (0.23, 1.26)	0.46 (0.18, 1.18)	0.93 (0.12, 7.32)	0.78 (0.43, 1.42)	0.56 (0.28, 1.12)	2.19 (0.74, 6.52)
VITAL	813	736	180	0.79 (0.64, 0.98)	0.76 (0.56, 1.03)	0.75 (0.49, 1.14)	0.85 (0.74, 0.98)	0.79 (0.61, 1.04)	0.85 (0.61, 1.15)
Pooled ^d	1,166	1,043	247	0.79 (0.68, 0.92)	0.75 (0.64, 0.93)	0.75 (0.55, 1.03)	0.82 (0.73, 0.92)	0.76 (0.63, 0.93)	0.81 (0.63, 1.04)
Discovery + replication ^e									
rs3020443 (A>C)									
Discovery	439	247	41	0.78 (0.59, 1.03)	0.71 (0.51, 1.00)	0.88 (0.43, 1.79)	0.78 (0.62, 0.98)	0.73 (0.56, 0.95)	0.87 (0.48, 1.60)
PMH-CCFR ^c	224	168	35	0.95 (0.71, 1.27)	0.92 (0.63, 1.33)	1.02 (0.52, 2.00)	0.96 (0.75, 1.23)	0.97 (0.70, 1.33)	0.90 (0.50, 1.61)
Replication	487	366	57	0.81 (0.63, 1.03)	0.74 (0.56, 0.99)	0.92 (0.51, 1.67)	0.81 (0.66, 1.00)	0.78 (0.61, 1.00)	0.77 (0.46, 1.32)
WH1	157	89	14	0.72 (0.48, 1.08)	0.76 (0.46, 1.24)	0.27 (0.06, 1.16)	0.87 (0.63, 1.19)	0.80 (0.53, 1.21)	0.90 (0.42, 1.91)
NHS	76	42	10	0.75 (0.33, 1.74)	0.68 (0.25, 1.86)	0.84 (0.11, 6.73)	0.94 (0.52, 1.68)	0.79 (0.39, 1.64)	1.59 (0.46, 5.57)
VITAL	944	665	116	0.83 (0.71, 0.98)	0.79 (0.65, 0.97)	0.86 (0.57, 1.31)	0.87 (0.76, 1.00)	0.84 (0.70, 0.99)	0.88 (0.63, 1.24)
Pooled ^d	1,383	912	157	0.82 (0.71, 0.94)	0.77 (0.65, 0.92)	0.87 (0.60, 1.24)	0.85 (0.75, 0.95)	0.80 (0.70, 0.93)	0.88 (0.65, 1.18)
Discovery + replication ^e									
rs2978381 (T>C)									
Discovery	256	350	123	0.83 (0.66, 1.05)	0.83 (0.60, 1.15)	0.72 (0.45, 1.13)	0.85 (0.71, 1.03)	0.76 (0.58, 0.99)	0.91 (0.64, 1.28)
PMH-CCFR	132	213	85	0.92 (0.70, 1.20)	1.00 (0.66, 1.58)	0.76 (0.47, 1.24)	0.93 (0.74, 1.16)	0.99 (0.70, 1.41)	0.81 (0.55, 1.21)
Replication	297	440	173	0.85 (0.69, 1.04)	0.66 (0.49, 0.89)	1.09 (0.77, 1.56)	0.87 (0.73, 1.04)	0.75 (0.58, 0.97)	0.98 (0.72, 1.34)
WH1	108	110	42	0.71 (0.51, 1.00)	0.77 (0.48, 1.25)	0.36 (0.15, 0.83)	0.83 (0.63, 1.08)	0.84 (0.56, 1.27)	0.63 (0.36, 1.10)
NHS	47	59	23	0.83 (0.41, 1.69)	0.63 (0.25, 1.58)	1.39 (0.36, 5.42)	0.96 (0.57, 1.62)	0.69 (0.35, 1.36)	1.81 (0.73, 4.48)
VITAL	584	822	323	0.84 (0.73, 0.97)	0.75 (0.60, 0.92)	0.80 (0.50, 1.28)	0.88 (0.78, 1.00)	0.82 (0.68, 0.98)	0.90 (0.67, 1.19)
Pooled ^d	840	1,172	446	0.84 (0.74, 0.95)	0.77 (0.64, 0.92)	0.79 (0.56, 1.12)	0.87 (0.79, 0.97)	0.80 (0.69, 0.93)	0.90 (0.74, 1.10)
Discovery + replication ^e									

^aA, major allele; a, minor allele. rs3020443 was directly genotyped in all sets, rs2978381 was imputed in WH12 and VITAL, and rs2987983 was imputed in WH11, WH12, and VITAL.
^bHR adjusted for age, race, stage at diagnosis for discovery and HR adjusted for age, first 3 principal components, stage at diagnosis for replication.
^cTwo women in PMH-CCFR missing genotypes for rs2987983 and rs3020443 have been excluded.
^dHR from random-effects meta-analysis of replication studies.
^eHR from random-effects meta-analysis of discovery and replication studies.

postmenopausal hormone therapy (any type), or anatomic tumor location by conducting a meta-analysis of interaction parameters across all study cohorts with available harmonized data and sufficient number of deaths per category (all 5 studies for stage; all studies except WHI1 for hormone therapy; all studies except WHI1 and VITAL for location). No evidence of statistically significant heterogeneity was detected for CRC-specific or overall survival.

Discussion

We found evidence that minor alleles of common intronic SNPs located in the 5' regulatory region of the ER β gene were associated with improved survival after a diagnosis of CRC. None of the SNPs of genes for ER α , PR, or AR were related to CRC-specific or overall survival. The fact that we detected a signal from variants of ER β , and not from those of ER α , PR, or AR, is consistent with the evidence from experimental and clinical studies that suggests a dominant role for ER β in CRC initiation and progression. ER β , which activates transcription of various targets upon binding to 17 β -estradiol or related ligands and can be distinguished from ER α in its response to estradiol at AP-1 sites (30), is the most abundantly expressed sex-steroid hormone receptor in the gut (11).

ER β expression is diminished in neoplastic colonic epithelium (13, 14), and is suspected to play a role in disease progression through pathways that influence invasion and metastasis. ER β appears to help maintain cellular organization in the colon through interactions with cellular adhesion and migration factors including α -catenin (31) and p38/MAPK (32). On the basis of studies in ApcMin/+ mice and CRC cell-lines, ER β has been shown to modulate growth-factor pathways (33), induce apoptosis (34), suppress inflammation (35), and reduce activity of factors leading to cell-cycle arrest including Myc, and cyclins D1 and E (36).

In clinical studies, loss of ER β expression is correlated with characteristics of poorer CRC prognosis including more poorly differentiated tissue, advanced stage, vascular invasion, and decreased apoptotic nuclei in neoplastic cells (14, 15, 37). At least 1 study has directly linked ER β -expression loss to poorer overall survival in patients with colon cancer (38). Moreover, expression of factors that interact with ER β -mediated transcription and transactivation in the gut, including ER coactivators AIB1, steroid sulfatase, and estrogen sulfotransferase have been found to be predictive of survival in CRC patients (39, 40).

Gender-specific associations have been reported between a (CA)_n dinucleotide repeat polymorphism of intron 5 of *ESR2* and survival outcomes in participants with metastatic CRC from chemotherapy clinical trials (19, 20). Gordon and colleagues observed that men with both long repeat alleles had poorer overall and progression-free survival than men with the short repeat alleles (19). Press and colleagues reported the same association for men, but further found evidence for the opposite relation among women (20). Studies that have evaluated this repeat polymorphism for CRC risk have been inconsistent, making it challenging to

compare the findings for survival with those for incidence. Two studies found associations only among women, but in opposite directions: Honma and colleagues reported that short repeat alleles were associated with increased risk (41), and Slattery and colleagues reported that long repeat alleles were associated with increased risk (42). Our study did not specifically evaluate this repeat polymorphism, but our tag-SNP-based approach identified variants associated with survival not near intron 5 of *ESR2*, but on the 5' end.

The relatively large 5' UTR of *ESR2* permits several ER β transcript variants, and so the SNPs we identified may be markers of differential action of various receptor isoforms. There is some evidence that the influence of ER β on CRC progression may be isoform dependent, as reduced expression of ER β 1 and ER β 2 appear to be more strongly correlated with cellular differentiation and advanced stage than the ER β 5 isoform (43). The 5' end of *ESR2* is also known to be rich in CpG islands (44). Hypermethylation of CpG islands near untranslated exon 0N of *ESR2* has been linked to transcriptional inactivation of ER β in breast (45), prostate (46), and ovarian (47) cancers. Our findings suggest that this locus of *ESR2* warrants further evaluation in genetic and epigenetic studies of CRC development and progression.

Evaluations of SNPs of hormone-receptor genes are lacking in the literature for CRC survival, but are available for CRC incidence (16–18). These studies have been mostly null, and may have been too small to identify the modest effect sizes expected from such SNPs. Lin and colleagues (18) specifically considered rs2978381 for risk, but no OR was reported as it did not reach statistical significance. rs2987983 and rs3020443 were not included in the analysis of Lin and colleagues or the other studies. Although not previously implicated in studies of colorectal neoplasia, rs2987983 has been identified as a putative susceptibility SNP for breast (48) and prostate (49) cancer. This SNP is located among binding sites for a number of transcription factors (including GATA2, c-Fos, and c-Jun) and is also within an H3K27Ac histone mark. rs2978381 and rs3020443 are about 3 and 29 kb upstream from rs2987983, respectively. The functionality of these correlated SNPs has not been well characterized. Fine mapping of polymorphic variation in the 5' regulatory region of *ESR2* identified rare SNPs, including 1 in the TATA box (rs35036378; not evaluated in our study), that appeared to diminish ER β expression (50). However, rs35036378 is more common in those of African descent, and is uncorrelated with the 3 SNPs we identified.

Our results should be considered in the context of several limitations. Unlike case-control studies of disease incidence, which would usually have sufficient statistical power to detect modest associations with more than 2,000 CRC cases, in our analyses of survival outcomes, where power depends on the number of deaths that occur, only more pronounced effect sizes could be detected. We evaluated CRC-specific and overall survival, but caution that less than half of the women in these survival studies were observed to die. Median follow-up ranged from 5 and 9 years after diagnosis, but additional follow-up time could be needed to identify

SNPs related to longer-term survival and other causes of death. In particular, only about one third of the combined cohort that died did so from a cause other than CRC; thus, we did not have a sufficient number of deaths from other causes to support a detailed cause-specific survival analysis. It is possible that variation in hormone-receptor genes may influence the incidence and progression of multiple diseases in women with CRC. Our study provides only limited, indirect evidence of SNP associations with deaths from other causes inasmuch as the CRC-specific and overall survival effects differ.

Another limitation of our study is the lack of information on patient treatment. At least 1 study has noted that ER β expression in colon cancer cells does not correlate with response to fluorouracil therapy (43). Although we were not able to evaluate heterogeneity in response to treatment by hormone-receptor genotype, we suspect that such genotype is unlikely to influence the selection and course of treatment beyond any possible effects on stage at diagnosis. Because treatment regimens for CRC tend to be uniform according to stage at diagnosis, our analyses adjusted for stage rather than treatment. Finally, it is unclear whether our results can be generalized to men. Replication in other populations should be conducted.

These specific variants of *ESR2* will likely contribute very little to the ability to predict individual survival outcomes. Comparing the area under the curve (AUC) for receiver operating characteristic curves generated from an age, race, and stage-adjusted model trained in the PMH-CCFR population and validated in the combined GECCO studies, inclusion of these SNPs of *ESR2* yielded essentially identical AUCs as a model without them. Our findings may have clinical implications in the future, however, given the possibility of CRC treatments that selectively activate ER β , as the effectiveness of such therapy could depend on observed ER β tumor expression levels, germline mutations in hormone-receptor genes, or other tumor characteristics.

The effects of estrogens on CRC progression are not fully understood, and survival studies of postmenopausal hormone therapy have been inconsistent (4–6). Our study investigated inherited variation in 4 hormone-receptor genes, *AR*, *PGR*, *ESR1*, and *ESR2* in a large group of postmenopausal women with incident CRC. Only SNPs of the promoter region of *ESR2* were associated with survival after a CRC diagnosis. One of the SNPs, rs2987983, has been linked to the risk of developing breast and prostate cancer, but not specifically CRC incidence. Our findings support the role of ER β as a marker of prognosis in CRC patients, although further research is needed to more fully understand the functionality of germline SNPs in this region of *ESR2* with respect to their involvement in genetic or epigenetic mechanisms.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.N. Passarelli, A.I. Phipps, K.W. Makar, A.T. Chan, C.M. Hutter, P.A. Newcomb

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VITAL

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WHI

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