Published in final edited form as:
Cancer Res. 2013 January 15; 73(2): 767–775. doi:10.1158/0008-5472.CAN-12-2484.

Common single nucleotide polymorphisms in the estrogen receptor β promoter are associated with colorectal cancer survival in postmenopausal women

Michael N. Passarelli[1,2], **Amanda I. Phipps**[1], **John D. Potter**[1,2,3], **Karen W. Makar**[1], **Anna E. Coghill**[1,2], **Karen J. Wernli**[4], **Emily White**[1,2], **Andrew T. Chan**[5,6], **Carolyn M. Hutter**[1,2], **Ulrike Peters**[1,2], and **Polly A. Newcomb**[1,2]

[1]Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA [2]Department of Epidemiology, University of Washington School of Public Health, Seattle, WA [3]Centre for Public Health Research, Massey University, Wellington, New Zealand [4]Group Health Research Institute, Seattle, WA [5]Gastrointestinal Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA [6]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

Abstract

Loss of estrogen receptor β (ERβ) expression in the gut is associated with colorectal cancer (CRC) initiation and progression. Germline single nucleotide polymorphisms (SNPs) 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 50–74, diagnosed with invasive CRC between 1997–2002 in 13 counties covered by the Seattle-Puget Sound SEER cancer registry. Participants provided germline DNA. We selected 99 tag-SNPs for the androgen receptor (*AR*), ERα (*ESR1*), ERβ (*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 5' 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 hazard ratio (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 5' promoter of *ESR2* may be important to pathways related to the association between ERβ and tumor progression and metastasis.

Keywords

colorectal cancer; survival; genetic polymorphisms; estrogen receptor alpha; estrogen receptor beta; progesterone receptor; androgen receptor; postmenopausal women

Corresponding author: Polly A. Newcomb, PhD, MPH, Cancer Prevention Program, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, M4-B402, Seattle, WA 98109, Phone: 206-667-3476, Fax: 206-667-7850, pnewcomb@fhcrc.org.

Disclosure of Potential Conflicts of Interests: Andrew T. Chan declares a minor conflict of interest in his role as a consultant/ advisory board member of Bayer HealthCare, Pfizer, and Millennium Pharmaceuticals.

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 two subtypes of estrogen receptors (ERα and ERβ). AR, PR, ERα, and ERβ are encoded by the genes *AR* on Xq12, *PGR* on 11q22–23, *ESR1* on 6q25, and *ESR2* on 14q23–24, respectively. All four receptors have been found to be expressed at some level in the gut, with ERβ being the most abundantly expressed (10, 11). Loss of estrogen receptor 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 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 three prospective cohort studies that participate in the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO): 1) Nurses' Health Study (NHS); 2) VITamins And Lifestyle (VITAL) Study; and 3) Women's Health Initiative (WHI).

METHODS

Discovery study population

We identified postmenopausal women, ages 50–74, 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 (IRB) of the Fred Hutchinson Cancer Research Center (FHCRC).

SNP selection

Tag-SNPs of *AR, ESR1, ESR2*, and *PGR* with minor allele frequency (MAF) ≥ 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 (NIEHS) Environmental Genome Project (EGP) (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, two SNPs were genotyped in each block that consisted of more than five SNPs. After genotyping was completed, blocks were pruned so that no two 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 QIAmp extraction kit (Qiagen, Germantown, MD) with PicoGreen Quantitation Reagent (Invitrogen, Carlsbad, CA). Genotyping was performed by the Genomics Shared Resource at the FHCRC using the Illumina GoldenGate platform and BeadStudio software (Illumina, San Diego, CA). 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, Camden, NJ), along with intraplate and interplate replicates. Replicates were > 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 (HRs) and 95% CIs (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, 70–74 years), stage (localized, regional, distant), and race (Caucasian, 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) (23). All SNPs with $P_{FDR} \leq 0.2$ for

associations with CRC-specific or overall survival were eligible for further consideration in the replication stage.

Replication procedures

We performed replication analyses using data from a subset of CRC studies participating in GECCO, a National Cancer Institute Epidemiology and Genomics Research Program (EGRP)-supported consortium. As of 2012, three 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 two groups (WHI1 and WHI Set 2; WHI2). WHI enrolled study participants from 1993–1998; WHI1 includes women diagnosed with incident colon cancer from 1993–2005 and WHI2 includes those diagnosed with incident CRC from 1993–2009. 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 1976–2008. 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 2000–2002, obtained mortality information for incident cases occurring between 2000–2009 from WA state death certificates through December 31, 2009 and censored follow-up time at the time 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 GWAS-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 et al. (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 $P_{FDR} \leq 0.2$ in the discovery stage were evaluated in separate proportional hazards regression models that adjusted for age at diagnosis (50–59, 60–69, ≥ 70 years), stage (localized, regional, distant), and the first three 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 four sets (NHS, VITAL, WHI1, 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. For SNPs that met this replication threshold, we calculated two other HR estimates using the best call for imputed genotypes, in addition to the allele-dosage HR, based on: 1) a dominant model that compared those with 1 or 2 minor alleles to those who were homozygous for the major allele; and 2) 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 $P_{FDR} \leq 0.05$. For SNPs included in replication analyses, the proportional hazards assumption was tested separately for each study. Analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, NC). All statistical tests were two-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, 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 $P_{FDR} \leq 0.2$ for both CRC-specific and overall survival. One SNP of *ESR2* had $P_{FDR} \leq 0.2$ for only CRC-specific survival, and four SNPs (2 of *ESR2* and 2 of *PGR*) had $P_{FDR} \leq 0.2$ 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 1). Imputed or directly genotyped data were available for 19 of the 20 SNPs for each of the four replication sets (rs613120 was unavailable and no suitable proxy could be identified). Imputation quality was good (r^2 across the four replication sets was between 0.91 and 1.02 for these 19 SNPs). The 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 2). All three 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 three 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 1–3.

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 three 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 three 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 six 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; I^2 = 0%; CI: 0–65% for rs2978381 based on CRC-specific HRs). For these three SNPs of *ESR2*, we explored whether associations with survival differed by stage at diagnosis, prediagnostic use of postmenopausal hormone therapy (any type), or anatomic tumor location by performing a meta-analysis of interaction parameters across all study cohorts with available harmonized data and sufficient number of deaths per category (all five 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 ERβ gene were associated with improved survival after a diagnosis of CRC. None of the SNPs of genes for ERα, PR, or AR were related to CRC-specific or overall survival. The fact that we detected a signal from variants of ERβ, and not from those of ERα, PR, or AR, is consistent with the evidence from experimental and clinical studies that suggests a dominant role for ERβ in CRC initiation and progression. ERβ, which activates transcription of various targets upon binding to 17β-estradiol or related ligands and can be distinguished from ERα in its response to estradiol at AP-1 site (30), is the most abundantly expressed sex-steroid hormone receptor in the gut (11).

ERβ 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. ERβ 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). Based on studies in ApcMin/+ mice and CRC cell-lines, ERβ 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 ERβ 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 one study has directly linked ERβ-expression loss to poorer overall survival in patients with colon cancer (38). Moreover, expression of factors that interact with ERβ-mediated transcription and transactivation in the gut, including estrogen receptor 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 et al. observed that men with both long repeat alleles had poorer overall and progression-free survival than men with the short

repeat alleles (19). Press et al. 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 et al. reported that short repeat alleles were associated with increased risk (41), and Slattery et al. 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 ERβ 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 ERβ on CRC progression may be isoform-dependent, as reduced expression of ERβ1 and ERβ2 appear to be more strongly correlated with cellular differentiation and advanced stage than the ERβ5 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 ERβ 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 et al. (18) specifically considered rs2978381 for risk, but no odds ratio was reported as it did not reach statistical significance. rs2987983 and rs3020443 were not included in the analysis of Lin et al. 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 a H3K27Ac histone mark. rs2978381 and rs3020443 are about 3 kb 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 one in the TATA box (rs35036378; not evaluated in our study), that appeared to diminish ERβ expression (50). However, rs35036378 is more common in those of African descent, and is uncorrelated with the three 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 > 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 insomuch as the CRC-specific and overall survival effects differ.

Another limitation of our study is the lack of information on patient treatment. At least one study has noted that ERβ 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. Lastly, it is unclear whether our results can be generalized to men. Replication in other populations should be performed.

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 received operating characteristic (ROC) curves generated from an age, race, and stage-adjusted model trained in the PMH-CCFR population and validated in the combined GECCO studies including 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 ERβ, as the effectiveness of such therapy could depend on observed ERβ 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 four hormone-receptor genes, *AR, PGR, ESR1*, and *ESR2* in a large group of postmenopausal women with incident CRC. Only SNPs of the 5' 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 ERβ 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

PMH-CCFR: The authors thank the study participants and staff of the Seattle Colon Cancer Family Registry and Seattle-Puget Sound Cancer Surveillance System.

GECCO: The authors would like to thank all those at the GECCO Coordinating Center for helping bring together the data and the people that made this project possible.

NHS: We wish to acknowledge Patrice Soule and Hardeep Ranu for genotyping at the Dana-Farber Harvard Cancer Center High Throughput Polymorphism Core, under the supervision of Immaculata Devivo, as well as Carolyn Guo and Haiyan Zhang for programming assistance. We would like to thank the participants and staff of the Nurses' Health Study for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY.

VITAL: The authors thank the participants and study staff.

WHI: The authors thank the WHI investigators and staff for their dedication, and the study participants for making the program possible. A full listing of WHI investigators can be found on the program website (https://cleo.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Short%20List.pdf).

GRANT SUPPORT

PMH-CCFR is supported by NCI/NIH/HHS (U24-CA074794 and R01-CA076366 to P.A.N.). GECCO is supported by NCI/NIH/HHS (U01-CA137088 to U.P.). NHS is supported by NCI/NIH/HHS (P01-CA087969 and R01-CA137178 to A.T.C.). VITAL is supported in part by NCI/NIH/HHS (K05-CA154337 to E.W.). The WHI program is funded by NHLBI/NIH/HHS through contracts HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HHSN268201100003C, HHSN268201100004C, and HHSN271201100004C. Funding for the genome-wide scan of WHI was provided by NCI/NIH/HHS (R01-CA059045 to U.P.). Three authors were supported by training grants from NCI/NIH/HHS (T32-CA009168 to M.N.P. and A.E.C. and R25-CA094880 to A.I.P.)

REFERENCES

1. McMichael AJ, Potter JD. Reproduction, endogenous and exogenous sex hormones, and colon cancer: a review and hypothesis. J Natl Cancer Inst. 1980; 65:1201–1207. [PubMed: 7001123]
2. Thun MJ, Calle EE, Namboodiri MM, Flanders WD, Coates RJ, Byers T, et al. Risk factors for fatal colon cancer in a large prospective study. J Natl Cancer Inst. 1992; 84:1491–1500. [PubMed: 1433333]
3. Grodstein F, Newcomb PA, Stampfer MJ. Postmenopausal hormone therapy and the risk of colorectal cancer: a review and meta-analysis. Am J Med. 1999; 106:574–582. [PubMed: 10335731]
4. Slattery ML, Anderson K, Samowitz W, Edwards SL, Curtin K, Caan B, et al. Hormone replacement therapy and improved survival among postmenopausal women diagnosed with colon cancer (USA). Cancer Causes Control. 1999; 10:467–473. [PubMed: 10530618]
5. Coghill AE, Newcomb PA, Chia VM, Zheng Y, Wernli KJ, Passarelli MN, et al. Pre-diagnostic NSAID use but not hormone therapy is associated with improved colorectal cancer survival in women. British Journal of Cancer. 2011; 104:763–768. [PubMed: 21304527]
6. Chan JA, Meyerhardt JA, Chan AT, Giovannucci EL, Colditz GA, Fuchs CS. Hormone replacement therapy and survival after colorectal cancer diagnosis. J Clin Oncol. 2006; 24:5680–5686. [PubMed: 17179103]
7. Chlebowski RT, Wactawski-Wende J, Ritenbaugh C, Hubbell FA, Ascensao J, Rodabough RJ, et al. Estrogen plus progestin and colorectal cancer in postmenopausal women. N Engl J Med. 2004; 350:991–1004. [PubMed: 14999111]
8. Ritenbaugh C, Stanford JL, Wu L, Shikany JM, Schoen RE, Stefanick ML, et al. Conjugated equine estrogens and colorectal cancer incidence and survival: the Women's Health Initiative randomized clinical trial. Cancer Epidemiol Biomarkers Prev. 2008; 17:2609–2618. [PubMed: 18829444]
9. Prentice RL, Pettinger M, Beresford SA, Wactawski-Wende J, Hubbell FA, Stefanick ML, et al. Colorectal cancer in relation to postmenopausal estrogen and estrogen plus progestin in the Women's Health Initiative clinical trial and observational study. Cancer Epidemiol Biomarkers Prev. 2009; 18:1531–1537. [PubMed: 19423530]
10. Fiorelli G, Picariello L, Martineti V, Tonelli F, Brandi ML. Functional estrogen receptor beta in colon cancer cells. Biochem Biophys Res Commun. 1999; 261:521–527. [PubMed: 10425218]
11. Campbell-Thompson M, Lynch IJ, Bhardwaj B. Expression of estrogen receptor (ER) subtypes and ERbeta isoforms in colon cancer. Cancer Res. 2001; 61:632–640. [PubMed: 11212261]
12. Issa JP, Ottaviano YL, Celano P, Hamilton SR, Davidson NE, Baylin SB. Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon. Nat Genet. 1994; 7:536–540. [PubMed: 7951326]
13. Foley EF, Jazaeri AA, Shupnik MA, Jazaeri O, Rice LW. Selective loss of estrogen receptor beta in malignant human colon. Cancer Res. 2000; 60:245–248. [PubMed: 10667568]
14. Konstantinopoulos PA, Kominea A, Vandoros G, Sykiotis GP, Andricopoulos P, Varakis I, et al. Oestrogen receptor beta (ERbeta) is abundantly expressed in normal colonic mucosa, but declines in colon adenocarcinoma paralleling the tumour's dedifferentiation. Eur J Cancer. 2003; 39:1251–1258. [PubMed: 12763213]
15. Jassam N, Bell SM, Speirs V, Quirke P. Loss of expression of oestrogen receptor beta in colon cancer and its association with Dukes' staging. Oncol Rep. 2005; 14:17–21. [PubMed: 15944762]
16. Sainz J, Rudolph A, Hein R, Hoffmeister M, Buch S, von Schonfels W, et al. Association of genetic polymorphisms in ESR2, HSD17B1, ABCB1, and SHBG genes with colorectal cancer risk. Endocr Relat Cancer. 2011; 18:265–276. [PubMed: 21317201]

17. Lin J, Zee RY, Liu KY, Zhang SM, Lee IM, Manson JE, et al. Genetic variation in sex-steroid receptors and synthesizing enzymes and colorectal cancer risk in women. Cancer Causes Control. 2010; 21:897–908. [PubMed: 20148360]
18. Lin JH, Manson JE, Kraft P, Cochrane BB, Gunter MJ, Chlebowski RT, et al. Estrogen and progesterone-related gene variants and colorectal cancer risk in women. BMC Med Genet. 2011; 12:78. [PubMed: 21627810]
19. Gordon MA, Zhang W, Yang D, Iqbal S, El-Khouiery A, Nagashima F, et al. Gender-specific genomic profiling in metastatic colorectal cancer patients treated with 5-fluorouracil and oxaliplatin. Pharmacogenomics. 2011; 12:27–39. [PubMed: 21174620]
20. Press OA, Zhang W, Gordon MA, Yang D, Haiman CA, Azuma M, et al. Gender-related survival differences associated with polymorphic variants of estrogen receptor-beta (ERbeta) in patients with metastatic colon cancer. Pharmacogenomics J. 2011; 11:375–382. [PubMed: 20548329]
21. Newcomb PA, Zheng Y, Chia VM, Morimoto LM, Doria-Rose VP, Templeton A, et al. Estrogen plus progestin, use, microsatellite instability, and the risk of colorectal cancer in women. Cancer Res. 2007; 67:7534–7539. [PubMed: 17671225]
22. Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. Am J Hum Genet. 2004; 74:106–120. [PubMed: 14681826]
23. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B. 1995:289–300.
24. Design of the Women's Health Initiative clinical trial and observational study. The Women's Health Initiative Study Group. Control Clin Trials. 1998; 19:61–109. [PubMed: 9492970]
25. White E, Patterson RE, Kristal AR, Thornquist M, King I, Shattuck AL, et al. VITamins And Lifestyle cohort study: study design and characteristics of supplement users. Am J Epidemiol. 2004; 159:83–93. [PubMed: 14693663]
26. Colditz GA, Hankinson SE. The Nurses' Health Study: lifestyle and health among women. Nat Rev Cancer. 2005; 5:388–396. [PubMed: 15864280]
27. Phipps AI, Newcomb PA, Garcia-Albeniz X, Hutter CM, White E, Fuchs CS, et al. Association between colorectal cancer susceptibility loci and survival time following diagnosis with colorectal cancer. Gastroenterology. 2012
28. Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. Genet Epidemiol. 2010; 34:816–834. [PubMed: 21058334]
29. Peters U, Hutter CM, Hsu L, Schumacher FR, Conti DV, Carlson CS, et al. Meta-analysis of new genome-wide association studies of colorectal cancer risk. Hum Genet. 2012; 131:217–234. [PubMed: 21761138]
30. Paech K, Webb P, Kuiper GGJM, Nilsson S, Gustafsson JÅ, Kushner PJ, et al. Differential ligand activation of estrogen receptors ERα and ERβ at AP1 sites. Science. 1997; 277:1508–1510. [PubMed: 9278514]
31. Wada-Hiraike O, Imamov O, Hiraike H, Hultenby K, Schwend T, Omoto Y, et al. Role of estrogen receptor beta in colonic epithelium. Proc Natl Acad Sci U S A. 2006; 103:2959–2964. [PubMed: 16477031]
32. Caiazza F, Galluzzo P, Lorenzetti S, Marino M. 17Beta-estradiol induces ERbeta up-regulation via p38/MAPK activation in colon cancer cells. Biochem Biophys Res Commun. 2007; 359:102–107. [PubMed: 17524358]
33. Giroux V, Bernatchez G, Carrier JC. Chemopreventive effect of ERbeta-Selective agonist on intestinal tumorigenesis in Apc(Min/+) mice. Mol Carcinog. 2011; 50:359–369. [PubMed: 21480389]
34. Qiu Y, Waters CE, Lewis AE, Langman MJ, Eggo MC. Oestrogen-induced apoptosis in colonocytes expressing oestrogen receptor beta. J Endocrinol. 2002; 174:369–377. [PubMed: 12208656]
35. Edvardsson K, Strom A, Jonsson P, Gustafsson JA, Williams C. Estrogen receptor beta induces antiinflammatory and antitumorigenic networks in colon cancer cells. Mol Endocrinol. 2011; 25:969–979. [PubMed: 21493669]

36. Hartman J, Edvardsson K, Lindberg K, Zhao C, Williams C, Strom A, et al. Tumor repressive functions of estrogen receptor beta in SW480 colon cancer cells. Cancer Res. 2009; 69:6100–6106. [PubMed: 19602591]
37. Elbanna HG, Ebrahim MA, Abbas AM, Zalata K, Hashim MA. Potential value of estrogen receptor Beta expression in colorectal carcinoma: interaction with apoptotic index. J Gastrointest Cancer. 2012; 43:56–62. [PubMed: 20872292]
38. Fang YJ, Lu ZH, Wang F, Wu XJ, Li LR, Zhang LY, et al. Prognostic impact of ERbeta and MMP7 expression on overall survival in colon cancer. Tumour Biol. 2010; 31:651–658. [PubMed: 20680712]
39. Grivas PD, Tzelepi V, Sotiropoulou-Bonikou G, Kefalopoulou Z, Papavassiliou AG, Kalofonos H. Estrogen receptor alpha/beta, AIB1, and TIF2 in colorectal carcinogenesis: do coregulators have prognostic significance? Int J Colorectal Dis. 2009; 24:613–622. [PubMed: 19198856]
40. Sato R, Suzuki T, Katayose Y, Miura K, Shiiba K, Tateno H, et al. Steroid sulfatase and estrogen sulfotransferase in colon carcinoma: regulators of intratumoral estrogen concentrations and potent prognostic factors. Cancer Res. 2009; 69:914–922. [PubMed: 19141651]
41. Honma N, Arai T, Takubo K, Younes M, Tanaka N, Mieno MN, et al. Oestrogen receptor-beta CA repeat polymorphism is associated with incidence of colorectal cancer among females. Histopathology. 2011; 59:216–224. [PubMed: 21884200]
42. Slattery ML, Sweeney C, Murtaugh M, Ma KN, Wolff RK, Potter JD, et al. Associations between ERalpha, ERbeta, and AR genotypes and colon and rectal cancer. Cancer Epidemiol Biomarkers Prev. 2005; 14:2936–2942. [PubMed: 16365013]
43. Wong NA, Malcomson RD, Jodrell DI, Groome NP, Harrison DJ, Saunders PT. ERbeta isoform expression in colorectal carcinoma: an in vivo and in vitro study of clinicopathological and molecular correlates. J Pathol. 2005; 207:53–60. [PubMed: 15954165]
44. Li LC, Yeh CC, Nojima D, Dahiya R. Cloning and characterization of human estrogen receptor beta promoter. Biochem Biophys Res Commun. 2000; 275:682–689. [PubMed: 10964723]
45. Zhao C, Lam EW, Sunters A, Enmark E, De Bella MT, Coombes RC, et al. Expression of estrogen receptor beta isoforms in normal breast epithelial cells and breast cancer: regulation by methylation. Oncogene. 2003; 22:7600–7606. [PubMed: 14576822]
46. Li LC, Chui R, Nakajima K, Oh BR, Au HC, Dahiya R. Frequent methylation of estrogen receptor in prostate cancer: correlation with tumor progression. Cancer Res. 2000; 60:702–706. [PubMed: 10676656]
47. Suzuki F, Akahira J, Miura I, Suzuki T, Ito K, Hayashi S, et al. Loss of estrogen receptor beta isoform expression and its correlation with aberrant DNA methylation of the 5'-untranslated region in human epithelial ovarian carcinoma. Cancer Sci. 2008; 99:2365–2372. [PubMed: 19032364]
48. Treeck O, Elemenler E, Kriener C, Horn F, Springwald A, Hartmann A, et al. Polymorphisms in the promoter region of ESR2 gene and breast cancer susceptibility. J Steroid Biochem Mol Biol. 2009; 114:207–211. [PubMed: 19429453]
49. Thellenberg-Karlsson C, Lindstrom S, Malmer B, Wiklund F, Augustsson-Balter K, Adami HO, et al. Estrogen receptor beta polymorphism is associated with prostate cancer risk. Clin Cancer Res. 2006; 12:1936–1941. [PubMed: 16551880]
50. Philips S, Richter A, Oesterreich S, Rae JM, Flockhart DA, Perumal NB, et al. Functional characterization of a genetic polymorphism in the promoter of the ESR2 gene. Horm Cancer. 2012; 3:37–43. [PubMed: 21979797]

TABLE 1

Characteristics of women in the discovery and replication stages.

	Discovery Stage		Replication Stage									
	PMH-CCFR		WHI1		WHI2		NHS		VITAL		Combined Replication	
No. of women with CRC	729		430		910		260		129		1,729	
No. of deaths	244		157		257		104		37		555	
No. of deaths from CRC	161		113		192		77		23		405	
Age at diagnosis (years), N (%)												
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, N (%)	674	(92)	430	(100)	910	(100)	260	(100)	129	(100)	1,729	(100)
Postmenopausal hormone therapy[a], N (%)												
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, N (%)												
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, N (%)												
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)	

[a]Prediagnostic 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.

Abbreviations: CI, confidence interval; CRC, colorectal cancer; PMH-CCFR, Postmenopausal Hormones Supplementary Study to the Colon Cancer Family Registry; NHS, Nurses' Health Study; OS observational study; SD, standard deviation; VITAL, VITamins And Lifestyle Study; WHI1, Women's Health Initiative WHI2, Women's Health Initiative Set 2.

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	MAF (%)	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										
PMH-CCFR[c]	353	307	67	30	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)
Replication										
WHI1	182	195	53	35	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)
WHI2	424	392	94	32	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)
NHS	138	100	22	28	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)
VITAL	69	49	11	28	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)
Pooled[d]	813	736	180	32	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)
Discovery + Replication[e]	1,166	1,043	247	31	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)
rs3020443 (A>C)										
Discovery										
PMH-CCFR[c]	439	247	41	23	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)
Replication										
WHI1	224	168	35	28	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)
WHI2	487	366	57	26	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)
NHS	157	89	14	23	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)
VITAL	76	42	10	24	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)
Pooled[d]	944	665	116	26	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)
Discovery + Replication[e]	1,383	912	157	25	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)
rs2978381 (T>C)										
Discovery										
PMH-CCFR	256	350	123	41	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)
Replication										
WHI1	132	213	85	45	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)
WHI2	297	440	173	43	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)

	Genotype[a]				CRC-specific Survival HR[b] (95% CI)			Overall Survival HR[b] (95% CI)		
	AA	Aa	aa	MAF (%)	Per a Allele	AA vs Aa/aa	AA/Aa vs aa	Per a Allele	AA vs Aa/aa	AA/Aa vs aa
NHS	108	110	42	37	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)
VITAL	47	59	23	41	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)
Pooled[d]	584	822	323	42	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)
Discovery + Replication[e]	840	1,172	446	42	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)

[a] A represents major allele, a represents minor allele. rs3020443 was directly genotyped in all sets, rs2978381 was imputed in WHI2 and VITAL, and rs2987983 was imputed in WHI1, WHI2, and VITAL.

[b] HR adjusted for age, race, stage at diagnosis for discovery and HR adjusted for age, first three principal components, stage at diagnosis for replication.

[c] Two women in PMH-CCFR missing genotypes for rs2987983 and rs3020443 have been excluded.

[d] HR from random-effects meta-analysis of replication studies.

[e] HR from random-effects meta-analysis of discovery and replication studies.

Abbreviations: CI, confidence interval; CRC, colorectal cancer; HR, hazard ratio; PMH-CCFR, Postmenopausal Hormones Supplementary Study to the Colon Cancer Family Registry; MAF, minor allele frequency; NHS, Nurses' Health Study; VITAL, VITamins And Lifestyle Study; WHI1, Women's Health Initiative WHI2, Women's Health Initiative Set 2.