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Postmenopausal hormone replacement therapy and colorectal cancer risk by molecular subtypes and pathways

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Cancer Epidemiology

Postmenopausal hormone replacement therapy (HRT) was found to be associated with lower risk of colorectal cancer (CRC). However, little is known regarding associations with molecular subtypes of CRC. The current study includes female participants of a large German population-based case–control study (922 CRC cases and 1,183 controls). Tumor tissue samples were analyzed for microsatellite instability (MSI), CpG island methylator phenotype (CIMP), BRAF and KRAS mutation status. Multivariable logistic regression models were used to assess the association of HRT use with molecular subtypes and pathways. Postmenopausal HRT use was overall associated with reduced risk of CRC (adjusted odds ratio (aOR) 0.62, 95% confidence interval (CI) 0.50–0.76) and no major differences were observed for molecular subtypes or for tumor marker combinations representing molecular pathways. When stratified by median age (≤/›71 years) potentially stronger risk reductions were observed in the older group for subtypes showing MSI (OR = 0.36, 95% CI 0.17–0.76), BRAF mutation (OR = 0.40, 95% CI 0.30–0.83) and CIMP-high (OR = 0.40, 95% CI 0.21–0.73) and for CRC suggestive of the sessile serrated pathway (OR = 0.45, 95% CI 0.20–1.01). In conclusion, postmenopausal use of HRT was similarly associated with risk reduction of major molecular tumor subtypes and pathways of CRC. Potentially stronger risk reductions with CRC subtypes diagnosed at higher ages require confirmation and clarification from other studies. The current study extends the limited understanding of the mechanisms of HRT in CRC prevention.

Introduction

Colorectal cancer (CRC), a leading cause of cancer morbidity and mortality, shows sex-specific differences between men and women, with women having around 30% lower CRC incidence.[1] CRC is a heterogeneous disease with underlying molecular pathways and features, such as microsatellite instability (MSI), CpG island methylator phenotype (CIMP) and mutations in the B-Raf proto-oncogene serine/threonine kinase (*BRAF*) gene or the Kirsten rat sarcoma viral oncogene homolog (KRAS) gene.[2–4] BRAF mutations, MSI and CIMP-

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Abbreviations: BMI: body mass index; BRAF: B-Raf proto-oncogene serine/threonine kinase gene; CI: confidence interval; CIMP: CpG island methylator phenotype; CRC: colorectal cancer; HRT: hormone replacement therapy; KRAS: Kirsten rat sarcoma viral oncogene homolog gene; MSI: microsatellite instability; MSS: microsatellite stable; NSAIDs: nonsteroidal anti-inflammatory drugs; OR: odds ratio

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What's new?

Evidence suggests that among women, risk of colorectal cancer (CRC) may be reduced by hormone replacement therapy (HRT). Little is known, however, about the impact of HRT on the risk of specific CRC molecular subtypes. In this analysis of female study participants in Germany, postmenopausal use of HRT was associated with a reduction in risk of major molecular subtypes of CRC, namely those characterized by microsatellite instability, CpG island methylator phenotype, or *BRAF* or *KRAS* mutations. Risk reductions were strongest for CRC subtypes diagnosed at older ages. The findings expand upon current knowledge of HRT mechanisms in CRC prevention.

high are more prevalent in proximal tumors which are found more often in women.[5–7]

A meta-analysis including 20 studies, published in 2012,[8] found that hormone replacement therapy (HRT) was associated with a reduced risk of CRC. Another meta-analysis published in 2013 found that the association between HRT and CRC risk reduction was stronger among current than past users.[9] With regards to HRT regimen, both estrogen-alone and estrogen and progestin were found to be associated with risk reduction for CRC though the association with estrogen-only therapy was heterogeneous in the meta-analysis by Lin *et al.*[8] Nonetheless, very few studies so far have investigated the association between HRT use and risk of CRC according to molecular subtypes defined by MSI, CIMP, BRAF and KRAS.

Five original studies, four of which conducted in the USA and one in Sweden, and one meta-analysis, have investigated the association between HRT use and CRC risk by MSI/MSS (microsatellite stable) status with conflicting results.[10–15] For CIMP, KRAS and BRAF mutation, only one study each looked at their association of HRT with CRC risk.[13,16] Better knowledge of the association between HRT use and CRC risk by molecular subtypes may lead to better understanding of the etiologic mechanisms by which HRT lowers CRC risk and help shed more light on the various carcinogenic processes. Therefore, the aim of this study on primary prevention was to investigate whether the associations between HRT use and CRC risk vary by the molecular tumor subtypes of CRC.

Methods

Study design and population

The current analyses were conducted within the DACHS study, an epidemiological case–control study of the German Cancer Research Center (DKFZ) in Heidelberg, Germany, designed to assess the potential of endoscopic screening for the prevention of CRC. Detailed description of the study can be found elsewhere.[17,18] In short, since 2003, patients with a first histologically confirmed diagnosis of CRC and randomly selected population-based control participants with no history of CRC are recruited in the Rhine-Neckar-Odenwald region in south-western Germany. Control participants and CRC patients are frequency-matched by 5-year age group, sex and county of residence. The DACHS study was approved by the ethics committees of the University of Heidelberg and the state medical boards of Baden-Württemberg and Rhineland-Palatinate. Written informed consent was given by all participants.

The current analyses included only female DACHS participants recruited until 2010 for whom molecular tumor analyses of MSI, CIMP, BRAF and KRAS were performed in full. DACHS participants who reported suffering from Crohn's disease or ulcerative colitis (n = 11), were premenopausal or peri-menopausal at the time of joining the study (n = 168) or did not report on HRT use (n = 17) were also excluded (Fig. 1).

Data collection

Patients were identified and informed about the study in participating hospitals and interviewed by trained interviewers

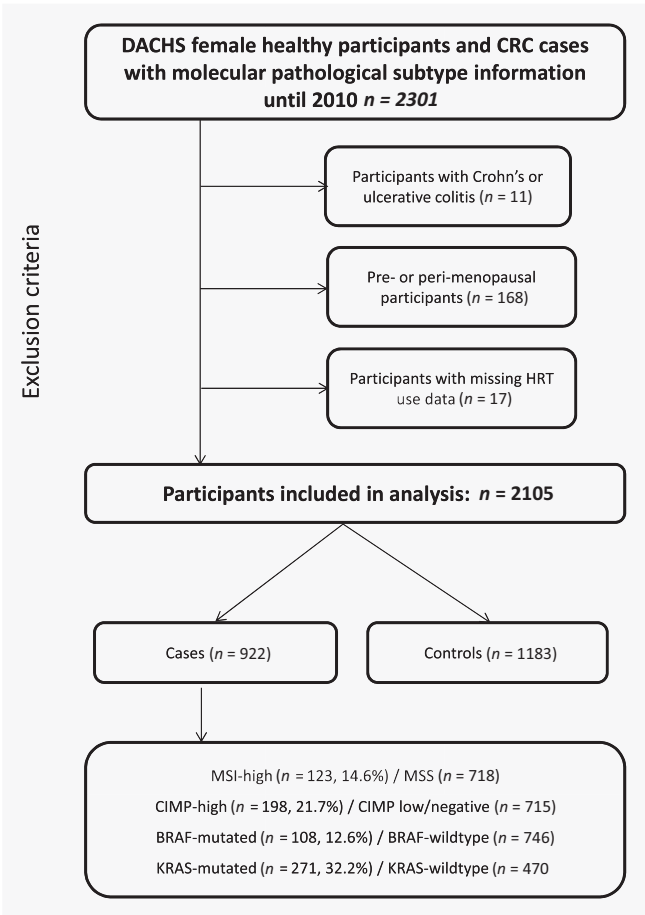

Figure 1. Study population flow diagram.

who collected sociodemographic, medical and lifestyle information using a standardized questionnaire. Patients who could not be recruited in the hospital were contacted after discharge and interviewed at home. The median time between diagnosis and interview was 24 days (IQR: 10–224 days). The controls were randomly selected from population registries and contacted through the study center by mail and by follow-up calls to schedule home interviews. Controls with a history of CRC were excluded. Controls opting out of the interview were offered a self-administered short questionnaire.

Assessment of menopausal status and regular use of HRT

Menopausal status was defined based on information collected in the interviews. Participants were classified as postmenopausal if at the time of diagnosis (for cases) or interview/filling of questionnaires (for controls) their menstrual bleeding has stopped naturally or due to bilateral oophorectomy, radiation therapy or chemotherapy, or if they were over 55 years old. The questionnaires were also the source of information on HRT use by study participants including start and end date, duration of use and hormone preparation for each period of use. The information was collected retrospectively up to the time of the index date (diagnosis/interview).

Tumor tissue analyses

Formalin-fixed paraffin-embedded surgical specimens of CRC patients were collected from cooperating pathology institutes and transferred to the tissue bank of the National Center for Tumor Diseases (NCT) in Heidelberg. MSI analysis was performed using a mononucleotide marker panel (BAT25, BAT26 and CAT25) which differentiates MSI-high from non-MSI-high tumors with a sensitivity of 98.2% and a specificity of 100%, and with 100% concordance of MSI-high tumors compared to the National Cancer Institute/International Collaborative Group on HNPCC (NCI/ICG-HNPCC) marker panel (BAT25, BAT26, D17S250, D2S123 and D5S346) for the evaluation of MSI in CRC.[19–21]

For KRAS, in about half of the tumor samples, mutation status was determined by a single-stranded conformational polymorphism technique using the same DNA sample, and expression of BRAF V600E was determined by immunohistochemical analyses and evaluated by two pathologists independently (91% concordance, Kappa 0.59). Discordant cases were discussed to obtain a final evaluation.[22] In the other half of the tumor samples, KRAS mutation status and BRAF mutation status were determined by Sanger sequencing as reported previously.[22] CIMP was determined after DNA bisulfite conversion as previously described.[23] CIMP high and CIMP low/negative were classified when 3–5 and 0–2 of the investigated loci (MGMT, MLH1, MINT1, MINT2 and MINT31) had a positive methylation status, respectively.

Statistical analyses

Descriptive statistics include participants' information from the questionnaire such as demographic data, education, smoking, known history of CRC in a first-degree relative, previous endoscopy, diabetes and rheumatic disease. HRT use was categorized as: (*i*) Current: regular use (at least 3 consecutive months) up to the time of diagnosis or beginning of symptoms that led to diagnosis (cases) or interview (controls); (*ii*) Ever: current or past regular use at any time. (*iii*) Never: no period of regular use. HRT use was classified as use of estrogen monotherapy or combined therapy (estrogen and progestin) for more specific analyses if the respective type of therapy was used exclusively by the women.

Multinomial logistic regression models were used to estimate adjusted odds ratios (OR) and 95% confidence intervals (CIs) for the association of HRT use with the risk of CRC for each of the different molecular subtypes (e.g., 0 = controls, 1 = MSS, 2 = MSI). Covariate selection for the final model was based on a list of known risk factors of CRC and included age and any covariate that showed a statistically significant association with the CRC molecular subtypes in individual unconditional bivariable logistic regression models (p-value < 0.05). All covariates in the final model, apart from BMI, have also shown an association with HRT (p-value < 0.05) in our study population. One unified list of covariates was used for all multivariable analyses and included: age at diagnosis/interview, body mass index (BMI), a history of CRC in a first-degree family member, previous bowel endoscopy, smoking, diabetes and regular nonsteroidal anti-inflammatory drugs (NSAIDs) use.

In case–control analyses, each molecular subtype was investigated compared to no CRC. In addition, combinations of single tumor markers suggestive of the traditional (MSS, CIMP-low/negative, BRAF-wild-type, KRAS-wild-type), the sessile serrated (CIMP-high, BRAF-mutated) and the alternate (MSS, CIMP low/negative, KRAS-mutated) pathways to the development of CRC were examined.[24] To assess heterogeneity in CRC risk by molecular subtypes, associations with regular HRT use were investigated in case–case analyses, using the same covariates as in the case–control analyses.

Participants with missing values in the variables included in the final model (between null for age to 2.7% for timing of HRT use) were excluded from the analyses (3.6% overall). All statistical tests were two-sided and the significance level (α) was set at $p < 0.05$. All analyses were conducted using R version 3.4.4.[25]

Data availability

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Results

The current analyses included 2,105 female participants of a large German population-based case–control study of which 922 (43.8%) women were diagnosed with CRC in 2003–2010 and 1,183 were healthy controls recruited during the same

years. Of the 922 included cases, MSI status was available for 91% of cases, BRAF for 93%, KRAS for 91% and CIMP for 99% of cases. Of those, 14.6% were MSI-high (n = 123), 21.7% were CIMP-high (n = 198), 12.6% had BRAF-mutation (n = 108) and 32.2% had KRAS-mutation (n = 271; Fig. 1). Cases and control participants did not differ with regard to their age. Cases tended to be less educated, consume less alcohol and smoke more. Among cases there were more participants with a first-degree family member diagnosed with CRC, only 23% of the cases had previous endoscopy compared to 53% of the controls, more cases were diagnosed with diabetes and less used NSAIDs regularly (Table 1).

Table 1. Characteristics of study population

Variables	Cases (n = 922)	Controls (n = 1,183)	p-value[1]
Age (years)			
‹71	418 (45)	575 (49)	0.147
≥71	504 (55)	608 (51)	
BMI (kg/m^2)			
‹25	399 (44)	490 (42)	0.050
25–30	338 (37)	494 (42)	
›30	177 (19)	195 (17)	
School education (years)			
1–8	660 (72)	791 (67)	0.049
9–10	173 (19)	243 (21)	
›10	85 (9)	141 (12)	
Smoking			
Never	640 (69)	870 (74)	0.085
Past	181 (20)	207 (18)	
Current	100 (11)	103 (9)	
First degree family history of CRC			
No	768 (84)	1,032 (88)	0.008
Yes	151 (16)	145 (12)	
Previous endoscopy			
No	712 (77)	554 (47)	‹0.001
Yes	209 (23)	629 (53)	
Diabetes			
No	756 (82)	1,009 (87)	0.001
Yes	164 (18)	146 (13)	
Ever regular use of NSAIDs			
Never	684 (75)	793 (68)	0.002
Yes	231 (25)	367 (32)	
Current alcohol consumption (g ethanol/day)			
None/low	493 (55)	476 (43)	‹0.001
High (≥3.1)	410 (45)	630 (57)	
Hormone replacement therapy (HRT)			
Never	653 (71)	657 (56)	‹0.001
Yes	263 (29)	515 (44)	
Current use of HRT			
Never	653 (73)	657 (57)	‹0.001
Past	174 (19)	328 (28)	
Current	71 (8)	167 (14)	
Mode of therapy			
None	653 (71)	657 (56)	‹0.001
Other	112 (12)	160 (14)	
Estrogen only	58 (6)	133 (11)	
Combination	93 (10)	224 (19)	

[1]Fisher exact test; numbers in table may not amount to n = 2,105 due to missing values.

Regular HRT use and CRC risk

Median duration of HRT use among study participants was 9.8 years (intra-quartile range [IQR] = 3–15 years). Ever HRT use in postmenopausal women was associated with a 38% decreased risk of CRC compared to never using HRT regularly (OR = 0.62, 95% CI 0.50, 0.76; Table 2). Current HRT use was associated with a 54% decreased risk of CRC (OR = 0.46, 95% CI 0.33–0.64). Using estrogen monotherapy was associated with a 43% lower risk of CRC (OR = 0.57, 95% CI 0.40–0.82) while combination therapy of estrogen and progestin was associated with a 48% lower risk (OR = 0.52, 95% CI 0.37–0.74) of CRC. There was no interaction between regular use of HRT and participation in screening endoscopy in this study (p-interaction = 0.50).

Regular HRT use and CRC risk by molecular subtypes

In the analyses of CRC risk by molecular subtypes, there were no major differences in their associations with HRT use and ever regular HRT use showed only slightly lower ORs for MSS (OR = 0.61, 95% CI 0.48, 0.76) compared to MSI-high CRC (OR = 0.66, 95% CI 0.43, 1.02, p-het = 0.913), for BRAF-wild-type (OR = 0.62, 95% CI 0.49, 0.77) compared to BRAF-mutated CRC (OR = 0.75, 95% CI 0.47, 1.20, p-het = 0.399), for KRAS mutated (OR = 0.56, 95% CI 0.40, 0.77) compared to KRAS-wild-type CRC (OR = 0.66, 95% CI 0.52, 0.83, p-het = 0.382) and for CIMP high CRC (OR = 0.58, 95% CI 0.41, 0.83) compared to CIMP low/negative CRC (OR = 0.63, 95% CI 0.51, 0.79, p-het = 0.512; Table 2). Similarly, no significant differences were found when looking into the association of ever use estrogen monotherapy or combination therapy and the separate molecular subtypes.

Regular HRT use and CRC risk for combined subtypes and pathways

Looking at the combination of single markers according to the different pathways, for the traditional pathway, ever regular use of HRT was associated in our study with a 26% lower risk of CRC (OR = 0.74, 95% CI 0.55, 1.00). For the sessile serrated pathway, ever regular use of HRT was associated with a 21% lower risk of CRC (OR = 0.79, 95% CI 0.45, 1.37). For the alternate pathway, ever regular use of HRT was associated with a 48% lower risk of CRC (OR = 0.52, 95% CI 0.35, 0.76). Risk reduction was not significantly different when comparing the sessile serrated pathway with the traditional or the alternate pathways.

Table 2. Association of postmenopausal HRT use and CRC risk by molecular tumor markers

		Regular use of HRT							
	Never	Ever		Current		Estrogen monotherapy		Combination therapy	
	n (%)	*n* (%)	OR (95% CI)[1]	*n* (%)	OR (95% CI)[1]	*n* (%)	OR (95% CI)[1]	*n* (%)	OR (95% CI)[1]
Controls	623 (56)	495 (44)	Ref	166 (15)	Ref	149 (13)	Ref	221 (20)	Ref
Cases	638 (71)	259 (29)	0.62 (0.50, 0.76)	72 (8)	0.46 (0.33, 0.64)	58 (6)	0.57 (0.40, 0.82)	93 (10)	0.52 (0.37, 0.74)
MSI	85 (70)	37 (30)	0.66 (0.43, 1.02)	10 (8)	0.53 (0.26, 1.08)	12 (10)	0.82 (0.42, 1.59)	6 (5)	0.44 (0.18, 1.07)
MSS	503 (72)	195 (28)	0.61 (0.48, 0.76)	55 (8)	0.44 (0.31, 0.64)	40 (6)	0.52 (0.35, 0.78)	55 (8)	0.55 (0.38, 0.80)
p-heterogeneity			0.913		0.852		0.289		0.459
BRAF-mut	74 (69)	33 (31)	0.75 (0.47, 1.20)	8 (8)	0.58 (0.26, 1.28)	8 (7)	0.72 (0.33, 1.57)	6 (6)	0.63 (0.26, 1.55)
BRAF-wt	512 (71)	213 (29)	0.62 (0.49, 0.77)	62 (9)	0.47 (0.33, 0.67)	48 (7)	0.58 (0.39, 0.85)	59 (8)	0.54 (0.37, 0.78)
p-heterogeneity			0.399		0.998		0.605		0.798
KRAS-mut	195 (74)	69 (26)	0.56 (0.40, 0.77)	26 (10)	0.58 (0.36, 0.94)	18 (7)	0.60 (0.35, 1.03)	15 (6)	0.39 (0.22, 0.71)
KRAS-wt	386 (69)	171 (31)	0.66 (0.52, 0.83)	43 (8)	0.44 (0.30, 0.65)	35 (6)	0.54 (0.36, 0.83)	48 (9)	0.61 (0.41, 0.90)
p-heterogeneity			0.382		0.194		0.729		0.231
CIMP-high	139 (72)	55 (28)	0.58 (0.41, 0.83)	20 (10)	0.63 (0.37, 1.07)	16 (8)	0.66 (0.37, 1.17)	12 (6)	0.48 (0.25, 0.92)
CIMP-low/negative	495 (71)	201 (29)	0.63 (0.51, 0.79)	52 (8)	0.42 (0.29, 0.61)	41 (6)	0.54 (0.37, 0.81)	54 (8)	0.54 (0.37, 0.78)
p-heterogeneity			0.512		0.426		0.606		0.531
Traditional pathway[2]	213 (67)	104 (33)	0.74 (0.55, 1.00)	27 (9)	0.46 (0.29, 0.75)	20 (6)	0.63 (0.37, 1.07)	41 (13)	0.55 (0.36, 0.83)
Sessile serrated pathway	56 (71)	23 (29)	0.79 (0.45, 1.37)	6 (8)	0.66 (0.27, 1.64)	7 (9)	0.93 (0.40, 2.18)	8 (10)	0.75 (0.33, 1.71)
p-heterogeneity (traditional vs serrated)			0.829		0.525		0.370		0.853
Alternate pathway	146 (76)	46 (24)	0.52 (0.35, 0.76)	17 (9)	0.50 (0.28, 0.88)	11 (6)	0.53 (0.27, 1.05)	12 (6)	0.26 (0.13, 0.49)
p-heterogeneity (alternate vs serrated)			0.435		0.621		0.478		0.283

[1]Multinomial logistic regression model adjusted for Age, BMI, history of colorectal cancer in first-degree relative, previous endoscopy, smoking, diabetes and regular use of NSAIDs. Ever/current/estrogen/combination compared to never use.
[2]Traditional pathway: MSS, BRAF-wt, KRAS-wt, CIMP-low/negative; Serrated pathway: CIMP-high & BRAF-mut; Alternate pathway: MSS, CIMP-low/negative, KRAS-mut.

Regular HRT use and CRC risk stratified by age group

Additionally, the association between ever regular use of HRT CRC risk by molecular subtypes and pathway was analyzed in two age groups divided by the median: ≤71 years and >71 (Table 3). Among participants aged 71 years or younger, ever use of HRT was not associated with lower risk of MSI, BRAF-mutated, CIMP-high CRC, and the sessile serrated pathway. For participants older than 71 years, HRT use was associated with lower risk of CRC for all molecular subtypes although some comparison groups were too small for meaningful statistical inference. Examination of the interaction between age group and regular HRT use revealed interaction for BRAF-mutated CRC (p-interaction = 0.021).

Discussion

The results of this large German population-based case–control study indicate that postmenopausal HRT was similarly associated with a reduced risk of CRC for the investigated major molecular subtypes. In exploratory analyses, there were suggestive differences in the associations when the study population was divided by age, but this finding requires confirmation from other studies. The current study adds to the limited knowledge available on the association of HRT use with molecular subtypes of CRC.

Only a few original studies, all but one conducted in the US, have looked thus far into the relationship between HRT use and CRC risk by molecular subtypes.[10–14,16] Of those, five studies examined the association of HRT and CRC risk by MSI status but only one study each looked into the association of HRT use with CRC risk by BRAF-mutation, KRAS-mutation or CIMP status.

In previous studies on the association between HRT use and CRC risk by MSI status, Brandstedt *et al.*[14] and Slattery *et al.*[10] did not find a statistically significant reduced risk of MSI or MSS CRC. Limsui *et al.*,[13] Newcomb *et al.*[11] and Lin *et al.*[12] found that HRT use was associated with lower risk of MSS CRC but not with MSI CRC. The results were differential between the groups in the study by Lin *et al.*[12] but no heterogeneity testing was done in the other two studies. In a meta-analysis by Carr *et al.*,[15] summarizing the association between HRT use and CRC risk by MSI status, risk reduction was found for MSS CRC (relative risk [RR] = 0.80, 95% CI 0.75–0.89) but not for MSI CRC (RR = 1.02, 95% CI 0.85–1.21). In the current study, ever and current use of HRT were both associated with reduced risk of both MSI and MSS CRC, though the association with MSI CRC risk was not statistically significant. Heterogeneity testing showed no statistically significant difference in the association with risk of MSI and MSS CRC. The differences in results between studies may be due to different timing of HRT use, different hormonal regimens, various age groups,[13] very small comparison groups[14] and differences arising from a cohort[12–14] *versus* case–control[10,11] study design though no between-study heterogeneity (I^2 = 0) was observed in the published meta-analysis from 2018.[15]

For BRAF-mutation and CIMP status, Limsui *et al.*[13] found that HRT use was associated with lower risk of BRAF-wild-type CRC and CIMP-negative CRC. In our study, we observed no major differences in CRC risk by BRAF mutation or CIMP status in the entire study population, but a notable difference was found between BRAF-wild-type and BRAF-mutated CRC when looking exclusively at women 71 years old or younger. Limburg *et al.*[16] found that ever use of HRT was inversely associated with risk of both KRAS-wild-type and KRAS-mutated CRC, though the observed risk estimates were not statistically significant. In our study, the risk reduction of both KRAS-wild-type and KRAS-mutated CRC associated with HRT use was stronger and statistically significant, but overall also very similar.

It has been previously suggested that HRT use may have more pronounced inhibitory effects on the traditional pathway compared to the alternate or serrated pathways.[13,14] Our analyses did not show a significant differential association between the pathways to support this hypothesis. In an exploratory analysis, we did find that ever regular use of HRT was associated with risk reduction of 59% in the alternate pathway in women 71 years old or younger. In addition, two former studies[11,26] suggested that the association of regular HRT use and CRC risk might be different for different age groups, with stronger association in the older age groups. The results of our study support this hypothesis especially with regards to risk of CRC with MSI, BRAF-mutated or the sessile serrated pathway, although some of our comparison groups in the >71 years population were too small for robust statistical inference.

Several studies have looked into the different modes of HRT, mainly estrogen-only treatment and combined estrogen–progestin. Newcomb *et al.*[11] found that combined hormonal treatment was associated with a statistically significant CRC risk reduction but not estrogen alone. On the other hand, Lin *et al.*[12] found that CRC risk reduction was mostly present in estrogen-only therapy. In our study, no major difference in CRC risk reduction effect was found between estrogen monotherapy and combined hormonal therapy.

The mechanisms by which HRT use affects CRC risk have not yet been identified. A meta-analysis by Lin *et al.* published in 2012, including four randomized clinical trials (RCTs), eight cohort studies and eight case–control studies,[8] concluded that there was consistent evidence to support an association between combined hormonal therapy and CRC risk reduction. For estrogen-only therapy, the association was seen only for recent regular use. Slattery *et al.*[10] suggested that estrogen metabolism affects CRC risk in women through a mechanism involving MSI, as estrogen protects against instability. Newcomb *et al.*[11] suggested that estrogen and perhaps progestin may be a key factor in the pathway leading to hyper-methylation. It was also hypothesized that estrogen binds to estrogen receptor beta in the large bowel, suggesting that estrogen induces MLH1 expression. Thus, the protective effect of estrogen may be restricted only to tumors with normally expressed DNA mismatch repair system.[15]

Table 3. Association of postmenopausal HRT use and CRC risk by molecular tumor markers stratified by age group

	≤71 years				>71 years				
	Never	Ever			Never	Ever			*p*-interaction Age group × HRT use[1]
Controls	*n* (%) 245 (44)	*n* (%) 323 (56)	OR (95% CI)[2] Ref	*p*-heterogeneity	*n* (%) 412 (69)	*n* (%) 192 (31)	OR (95% CI)[2] Ref	*p*-heterogeneity	
Cases	266 (60)	181 (40)	0.66 (0.50, 0.86)	–	372 (83)	78 (17)	0.62 (0.45, 0.86)	–	0.572
MSI	28 (50)	28 (50)	0.96 (0.54, 1.70)	0.417	57 (86)	9 (14)	0.36 (0.17, 0.76)	0.196	0.255
MSS	215 (62)	134 (38)	0.62 (0.46, 0.83)		288 (83)	61 (17)	0.67 (0.47, 0.96)		0.779
BRAF-mut	16 (41)	23 (59)	1.24 (0.63, 2.44)	0.094	58 (85)	10 (15)	0.40 (0.20, 0.83)	0.387	0.021
BRAF-wt	227 (60)	150 (40)	0.64 (0.48, 0.85)		285 (82)	63 (18)	0.67 (0.47, 0.95)		0.760
KRAS-mut	81 (64)	45 (36)	0.54 (0.35, 0.82)	0.217	114 (83)	24 (17)	0.65 (0.39, 1.07)	0.854	0.500
KRAS-wt	163 (57)	121 (43)	0.71 (0.53, 0.97)		223 (82)	50 (18)	0.64 (0.44, 0.94)		0.835
CIMP-high	48 (54)	41 (46)	0.77 (0.48, 1.24)	0.701	91 (87)	14 (13)	0.40 (0.21, 0.73)	0.111	0.089
CIMP-low/negative	216 (61)	137 (39)	0.63 (0.47, 0.85)		279 (81)	64 (19)	0.72 (0.50, 1.02)		0.414
Traditional pathway[3]	88 (56)	68 (44)	0.72 (0.49, 1.06)	0.966[4]	124 (78)	35 (22)	0.89 (0.57, 1.39)	0.171[4]	0.417
Sessile serrated pathway	12 (44)	15 (56)	1.01 (0.49, 2.47)		44 (85)	8 (15)	0.45 (0.20, 1.01)		0.077
Alternate pathway	56 (68)	26 (32)	0.41 (0.25, 0.70)	0.129[4]	90 (82)	20 (18)	0.78 (0.45, 1.35)	0.495[4]	0.143

[1]Test for interaction was conducted by adding a multiplicative term (age group * HRT use) to the model.
[2]Multinomial logistic regression model adjusted for BMI, history of colorectal cancer in first-degree relative, previous endoscopy, smoking, diabetes and ever regular use of NSAIDS. Ever compared to never use.
[3]Traditional pathway: MSS, BRAF-wt, KRAS-wt, CIMP-low/negative; Sessile serrated pathway: CIMP-high & BRAF-mut; Alternate pathway: MSS, CIMP-low/negative, KRAS-mut.
[4]*p*-heterogeneity compares the serrated to the traditional or the alternative pathway.

The notable strengths of the current study are its large size, population-based design, comprehensive assessment of HRT use and adjustment for other lifestyle, medical and family history exposures and the analyses of multiple molecular tumor tissue markers. To the best of our knowledge, this is the first study looking at postmenopausal HRT use and its effect on CRC risk by all four major multiple molecular tumor features, including testing for subtype heterogeneity.

The study also has limitations. As this is an observational case–control study, based on self-reports during standardized interviews, HRT use and other relevant factors may be subject to recall bias and participants may have been subject to selection bias. Another limitation is that some subgroups of cases were too small for detecting statistically significant results, particularly when multiple tumor features were combined and when the different hormone regimens were considered separately. Also, some analyses were exploratory and need confirmation from other studies.[27] Since a tumor tissue analysis rate of 100% is nearly impossible in molecular pathological epidemiology studies, not all patients with available tumor tissue samples could be included in the analyses. However, we observed no differences by HRT status ($p = 0.696$) between the cases with and without tumor marker data. Finally, we are able to assess associations with major tumor markers of CRC, but other tumor markers or pathways not yet analyzed or identified may be more relevant when trying to disentangle the way how HRT is associated with reduced risk of CRC.

Conclusion

In summary, postmenopausal HRT use was associated with CRC risk reduction and no major differences were observed between the subtypes defined by MSI, CIMP, BRAF and KRAS. Among women older than the median age of 71 years, potentially stronger associations were seen for molecular subtypes and pathways that are predominantly linked to CRC of the proximal colon (MSI, CIMP-high, BRAF mutated and sessile serrated pathway). These results require a confirmation from other large studies. In light of the Women's Health Initiative randomized controlled trial (WHI RCT) results,[28] according to which overall health risks exceeded benefits from the use of combined estrogen and progestin among healthy postmenopausal women, HRT may not be recommended as a public health measure to lower CRC risk among women. Nonetheless, further studies looking into the mechanisms by which HRT influences CRC risk by the different molecular pathways will help shed light on the way HRT has a protective effect against CRC.

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Conflict of interest

The authors declare no potential conflict of interests.

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