Published in final edited form as:
Gut. 2012 September ; 61(9): 1299–1305. doi:10.1136/gutjnl-2011-300719.

Postmenopausal hormone therapy and colorectal cancer risk by molecularly defined subtypes among older women

David Limsui[1], **Robert A Vierkant**[2], **Lori S Tillmans**[3], **Alice H Wang**[2], **Daniel J Weisenberger**[4], **Peter W Laird**[4], **Charles F Lynch**[5], **Kristin E Anderson**[6], **Amy J French**[3], **Robert W Haile**[7], **Lisa J Harnack**[8], **John D Potter**[9], **Susan L Slager**[2], **Thomas C Smyrk**[3], **Stephen N Thibodeau**[3], **James R Cerhan**[10], and **Paul J Limburg**[1]

[1]Division of Gastroenterology & Hepatology, Mayo Clinic, Rochester, Minnesota, USA

[2]Division of Biomedical Statistics & Informatics, Mayo Clinic, Rochester, Minnesota, USA

[3]Department of Laboratory Medicine & Pathology, Mayo Clinic, Rochester, Minnesota, USA

[4]USC Epigenome Center, Norris Comprehensive Cancer Center, Los Angeles, California, USA

[5]Department of Epidemiology, University of Iowa, Iowa City, Iowa, USA

[6]Department of Epidemiology, University of Minnesota, Minneapolis, Minnesota, USA

[7]Department of Preventive Medicine, Keck School of Medicine of USC, Los Angeles, California, USA

[8]Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota, USA

[9]Fred Hutchinson Cancer Research Center, Seattle, Washington, USA

[10]Division of Epidemiology, Mayo Clinic, Rochester, Minnesota, USA

Abstract

Background—Postmenopausal hormone (PMH) therapy may reduce colorectal cancer (CRC) risk, but existing data are inconclusive.

Correspondence to Dr Paul J Limburg, 200 First Street SW, Rochester, MN 55905, USA; limburg.paul@mayo.edu.

Competing interests Dr Weisenberger holds patents and receives royalties from the University of Southern California (outside of the submitted work). Dr Laird serves on the Scientific Advisory Board and as a Consultant to Epigenomics, AG., and also receives financial support from patents and royalties related to methylation technology from this group (outside of the submitted work). Dr Limburg also receives support for his research from Olympus America, BENEO-Orafti Group, Bayer Healthcare, Fujinon, Boston Scientific, and Astra Zeneca (outside of the submitted work). He is listed as a co-inventor on US patent 5891651, served as a consultant for Genomic Health, Inc. from 8/12/08-4/19/10, and Mayo Clinic has licensed Dr Limburg's intellectual property to Exact Sciences, for which he and Mayo Clinic have contractual rights to receive royalties(outside of the submitted work).

Ethics approval This study was reviewed and approved by the institutional review boards for human research of the University of Iowa, University of Minnesota and Mayo Clinic.

Contributors PJL had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analyses. Study concept and design (RV, KA, JC, PL); acquisition of data (LT, DW, PL, CL, KA, AF, TS, ST, JC, PL); analysis and interpretation of data (DL, RV, AW, KA, RH, LH, JP, SS, JC, PL); drafting of the manuscript (DL, RV, AW, PL); critical revision of the manuscript for important intellectual content (DW, PL, CL, KA, RH, LH, JP, SS, TS, ST, JC); statistical analysis (RV, AW, SS, PL); obtained funding (KA, JC, PL).

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Data from this study will be made available upon request to the Corresponding Author (Dr Limburg), in accordance with relevant guidelines from the funding agency.

Objectives—To evaluate associations between PMH therapy and incident CRC, overall and by molecularly defined subtypes, in the population-based Iowa Women's Health Study of older women.

Methods—Exposure data were collected from Iowa Women's Health Study participants (55–69 years) at baseline (1986). Archived, paraffin-embedded tissue specimens for 553 CRC cases were collected and analysed to determine microsatellite instability (MSI-L/MSS or MSI-H), CpG island methylator phenotype (CIMP-negative or CIMP-positive) and *BRAF* mutation (*BRAF*-wildtype or *BRAF*-mutated) status. Multivariable Cox regression models were fit to estimate RRs and 95% CIs.

Results—PMH therapy (ever vs never use) was inversely associated with incident CRC overall (RR=0.82; 95% CI 0.72 to 0.93), with a significantly lower risk for MSI-L/MSS tumours (RR=0.75; 95% CI 0.60 to 0.94), and borderline significantly lower risks for CIMP-negative (RR=0.79; 95% CI 0.63 to 1.01) and *BRAF*-wildtype (RR=0.83; 95% CI 0.66 to 1.04) tumours. For PMH therapy >5 years, the subtype-specific risk estimates for MSI-L/MSS, CIMP-negative and *BRAF*-wildtype tumours were: RR=0.60, 95% CI 0.40 to 0.91; RR=0.68, 95% CI 0.45 to 1.03; and RR=0.70, 95% CI 0.47 to 1.05, respectively. PMH therapy was not significantly associated with the MSI-H, CIMP-positive or *BRAF*-mutated CRC subtypes.

Conclusions—In this prospective cohort study, PMH therapy was inversely associated with distinct molecularly defined CRC subtypes, which may be related to differential effects from oestrogen and/or progestin on heterogeneous pathways of colorectal carcinogenesis.

INTRODUCTION

Colorectal cancer (CRC) is the third most common malignancy among women in the USA, with 69 360 new cases projected in 2011.[1] Encouragingly, emerging trends show that CRC incidence rates are declining for women (and men), with computer simulation models estimating essentially equal benefits from CRC screening and risk factor modification.[2] Among older women, postmenopausal hormone (PMH) therapy could be used to complement CRC prevention strategies focused on diet, lifestyle and screening behaviours. Based on an early meta-analysis of 18 observational studies, Grodstein *et al* found that PMH therapy was associated with a 20% decrease in colon cancer risk, particularly among women who described themselves as current PMH users (RR=0.66; 95% CI 0.59 to 0.74).[3] Subsequent epidemiological studies have observed generally similar, although not entirely consistent, inverse associations between PMH therapy and CRC risk (as recently reviewed),[4] indicating that exogenous oestrogen and/or progestin compounds may inhibit the development of colorectal neoplasia.

To date, a limited number of randomised, controlled trials have examined PMHs as candidate CRC chemopreventive agents, with mixed results. In the Heart and Estrogen/Progestin Replacement Study (HERS), women with coronary disease were randomly assigned to receive oestrogen plus progestin versus placebo for an average follow-up period of 4.1 years. Incident CRC was analysed as a secondary outcome, with an observed HR of 0.69 (95% CI 0.32 to 1.49), suggesting a potentially protective effect.[5,6] In the Women's Health Initiative clinical trial, intervention with oestrogen plus progestin yielded an even more striking 44% reduction in incident CRC (HR=0.56; 95% CI 0.38 to 0.81; mean follow-up period 5.6 years), while oestrogen alone did not appear to affect CRC risk (HR=1.12; 95% CI 0.77 to 1.63; mean follow-up period 7.1 years).[7,8] However, further analyses of the Women's Health Initiative data revealed that women assigned to the oestrogen plus progestin arm were more likely to be diagnosed with advanced stage CRC, raising the possibility of differential effects from PMH therapy on heterogeneous pathways[9–11] of colorectal carcinogenesis.

At present, data referent to associations between PMH therapy and molecularly defined CRC subtypes are limited and inconsistent.[12–14] Thus, we sought to evaluate associations between PMH therapy and incident CRC, overall and by microsatellite instability (MSI), CpG island methylator phenotype (CIMP) and *BRAF* mutation status, among women enrolled in the prospective, population-based Iowa Women's Health Study (IWHS). The current report updates and extends previously described associations between PMH exposure and CRC risks based on IWHS data[315] by including additional follow-up time and molecularly defined, subtype-specific analyses.

MATERIALS AND METHODS

This study was reviewed and approved by the institutional review boards for human research of the University of Iowa, University of Minnesota and Mayo Clinic.

Study population

The methods used for recruiting IWHS subjects have been previously described.[16] In brief, a 16-page questionnaire was mailed in January 1986 to randomly selected women, age 55–69 years, who resided in Iowa and held a valid driver's licence at baseline. Of the 98 029 women who received questionnaires, 41 836 (43%) responded. CRC rates have been shown to be similar between the baseline questionnaire responders and non-responders.[17] For the present study, exclusion criteria (not mutually exclusive) were history of malignancy other than skin cancer (n=3830), unable to be followed longitudinally for ≥1 day (n=10), not postmenopausal (n=569) or incomplete oestrogen data (n=200), leaving 37 285 women in the final analytic cohort.

Exposure assessment

Demographic, dietary, lifestyle, medication and other CRC risk factor data were collected by self-administered questionnaire during the IWHS baseline evaluation (1986). PMH therapy was determined by the following question: "Have you ever used pills other than birth control pills which contain oestrogen or other female hormones (eg, at the change of life or menopause, after surgery, or at any other time)?" with response levels of yes, currently; yes, but not currently; and never. Subjects who responded yes (currently or not currently) were then asked: "How long did you take oestrogens or other female hormone pills (other than birth control pills)?" with response levels of 1 month or less; 2–6 months; 7–12 months; 13 months–2 years; 3–5 years; and more than 5 years. Further information regarding specific type and dose of PMH therapy was not obtained.

Ascertainment of incident CRC cases

Follow-up questionnaires were mailed in 1987, 1989, 1992, 1997 and 2004 to update vital status. Participants who did not respond to the follow-up questionnaires were checked against the National Death Index to identify decedents. Incident CRC cases were identified through the Iowa Cancer Registry, which participates in the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) programme.[18] Annual matching between a computer-generated list of all cohort members and the records of Iowans with incident cancer in the SEER programme registry was performed using combinations of first, last and maiden names; zip code; birth date; and social security number. CRC cases were identified using International Classification for Diseases in Oncology (ICD-O) codes of 18.0, 18.2–18.9, 19.9 and 20.9. Stage was categorised according to SEER definitions for localised, regional or distant disease. Cancers located in the caecum, ascending colon, hepatic flexure, transverse colon and splenic flexure were categorised as proximal CRC. Cancers located in the descending colon, sigmoid colon, rectosigmoid junction and rectum were categorised as distal CRC.

Tissue collection and processing

Beginning in 2006, archived, paraffin-embedded tissue specimens were requested from incident CRC cases diagnosed through 31 December 2002, as previously described.[19] Tissue specimens were retrieved for 732/1255 (58%) subjects with incident CRC, with all cases confirmed by a single gastrointestinal pathologist (TCS). Comparisons of general demographics and tumour characteristics (size and stage) between incident CRC cases with retrieved versus non-retrieved tissue specimens were not statistically significant ($p>0.05$ for each comparison; data not shown). Paraffin blocks were serially cut into 5- or 10-μ thick sections. One slide was stained with H&E and areas of neoplastic (>50%) and juxtaposed normal tissue were identified. Tumour and normal tissues were scraped from unstained slides and placed into separate tubes for DNA extraction using the QIAamp Tissue Kit (QIAgen, Valencia, California, USA), according to the manufacturer's instructions. A total of 179 retrieved CRC cases were subsequently excluded from the present study due to inadequate/unusable tissue from the first primary CRC, multiple primary CRCs at initial diagnosis, or incomplete PMH therapy data, leaving 553 incident CRC cases for the defined molecular analyses.

Microsatellite instability

MSI testing was performed on paired tumour and normal DNA samples for each subject, using 10 established markers: four mononucleotide repeats (BAT25, BAT26, BAT40 and BAT34C4), five dinucleotide repeats (ACTC, D5S346, D18S55, D17S250 and D10197), and one complex marker (MYCL).[20] Incident CRCs were classified as MSI-H if ≥30% of the markers demonstrated instability, and as MSI-L or microsatellite stable (MSS) if <30% of the markers demonstrated instability.[2122] MSI status could be determined for 539/553 incident CRC cases (98%).

CpG island methylation

Tumour DNA was treated with sodium bisulphite and subsequently analysed using automated real-time PCR-based MethyLight to amplify methylated CpG sites in the promoter regions of an established five-gene panel (*CACNA1G, IGF2, NEUROG1, RUNX3* and *SOCS1*).[23] CIMP status was reported as CIMP-positive or CIMP-negative, as defined by promoter hypermethylation in ≥3 or 0–2 genes in the five gene panel, respectively. CIMP status could be determined for 525/553 incident CRC cases (95%).

BRAF mutation

Tumour DNA was analysed using fluorescent allele specific PCR to detect the V600E point mutation in exon 15 of the *BRAF* gene. *BRAF*-mutation and *BRAF*-wildtype cases were defined by the presence or absence of the V600E point mutation, respectively. *BRAF* mutation status could be determined for 536/553 incident CRC cases (97%).

Statistical analyses

Data were descriptively summarised using frequencies and percentages for categorical variables, and means and SDs for continuous variables. Among cases, we assessed pairwise agreement between the various biomarker values using κ coefficients. Follow-up was calculated as the time from completion of the baseline questionnaire until either incident CRC diagnosis or censorship, as defined by a move from Iowa or death. Otherwise, study participants were assumed to be alive, cancer-free and living in Iowa through 31 December 2002. Cox proportional hazards regression analysis was used to estimate RRs and 95% CIs for associations between the PMH exposures of interest and incident CRC. All eligible IWHS subjects were included in these Cox regression analyses, regardless of eventual cancer status. Incidence was modelled as a function of age.[24] PMH therapy at baseline was

analysed with respect to exposure status (ever, never), with ever users further categorised with respect to recency of exposure (current, former) and duration of exposure (>5 years, ≤5 years). Never users were defined as the reference group for all risk associations. Tests for trend were carried out by ordering the categorised values from lowest to highest and including the resulting variable as a one degree-of-freedom linear term in a Cox proportional hazards model.

We first assessed associations of PMH therapy with any incident CRC. Subsequent analyses examined CRC risks defined by subtypes according to presenting stage (localised, regional or distant), anatomic subsite (proximal or distal), microsatellite instability phenotype (MSI-H or MSS/MSI-L), CIMP status (CIMP-positive or CIMP-negative) and *BRAF* mutation status (*BRAF*-mutated or *BRAF*-wildtype). For the subtype-specific analyses, the outcome variable was incident CRC with the molecular marker of interest; all other incident CRCs (including those with missing or unknown values for the molecular marker of interest) were considered censored observations at the date of diagnosis. We determined if risk ratios for the oestrogen-related exposures differed according to these cancer subtypes using a competing risk form of Cox proportional hazards analysis.[25] This approach allowed us to model and test the interaction between oestrogen exposure (modelled as a covariate) and molecular/tumour subtype (modelled as a stratum variable). We also examined associations of PMH therapy with CRC risk based on subsets defined by tissue availability (available vs not available) to determine if incomplete tissue access introduced any biases. Two sets of Cox regression models were fit: one accounting for age only and one additionally adjusting for other potential CRC risk factors, including body mass index (quartiles), waist to hip ratio (quartiles), smoking status (current, former, never), age at menopause (≤44, 45–49, 50–54, ≥55 years), age at menarche (8–12, 13–14, ≥15 years), oral contraceptive use (ever, never), physical activity level (low, moderate, high), alcohol consumption (0, 0–3.4, >3.4 g/day based on cohort median split), history of diabetes (ever, never), and daily intake (quartiles) of total energy (kcal/day), total fat (g/day), sucrose (g/day), red meat (g/day), calcium (mg/day), folate (mg/day), methionine (g/day) and vitamin E (mg/day). Family history of CRC, non-steroidal anti-inflammatory drug use and exposure to CRC screening were not systematically recorded at baseline and were not included in the current analyses. All statistical tests were two-sided, and all analyses were carried out using the SAS (SAS version 9.2.) and R software systems.

RESULTS

According to data from the baseline questionnaire, PMH therapy was categorised as ever use for 14 299 (38%) women and never use for 22 986 (62%) women. Age at baseline (mean ±SD) was similar between ever users (62.3±4.1 years) and never users (62.2±4.3 years). Other baseline characteristics are shown in table 1, by PMH therapy status. Among women who reported using PMH therapy, 4062 (11%) and 10 237 (28%) were further categorised as current users and former users, respectively. With respect to duration of use, 10 017 (27%) women reported ≤5 years and 4017 (11%) women reported >5 years of PMH therapy at baseline. For the 553 incident CRC cases with complete molecular marker data, the mean (±SD) age at diagnosis was 74.0 (±5.9) years. By SEER stage, cases were distributed as 181 (33%) localised, 236 (43%) regional, and 71 (13%) distant disease (stage data were not available for the remaining 65 cases). The molecular subtype distributions were: 394 (73%) MSI-L/MSS and 145 (27%) MSI-H; 361 (69%) CIMP-negative and 164 (31%) CIMP-positive; and 383 (72%) *BRAF*-wildtype and 153 (28%) *BRAF*-mutated. Relatively strong agreement was observed between the paired biomarker values (MSI and CIMP, κ=0.70; MSI and *BRAF*, κ=0.66; CIMP and *BRAF*, κ=0.82).

In age-adjusted risk models, PMH ever users were at lower risk for any incident CRC compared to PMH never users (RR=0.78, 95% CI 0.69 to 0.88). Multivariate adjustment resulted in a similar, though slightly attenuated, risk association (RR=0.82; 95% CI 0.72 to 0.93) (table 2). Therefore, further analyses are reported based on the multivariate risk model. Risk estimates for PMH ever versus never use by presenting SEER stage were RR=0.91 (95% CI 0.66 to 1.27) for localised, RR=0.78 (95% CI 0.58 to 1.04) for regional, and RR=0.72 (95% CI 0.42 to 1.23) for distant disease (p=0.13 for comparison across presenting stages). Risk estimates for PMH ever users and CRC overall were not statistically different between women 55–59 and ≥60 years of age at baseline (RR=0.85; 95% CI 0.65 to 1.10 and RR=0.80; 95% CI 0.69 to 0.93, respectively; test for interaction p=0.99), or for women who reported previous hysterectomy (RR=0.85; 95% CI 0.68 to 1.07) or no previous hysterectomy (RR 0.82; 95% CI 0.69 to 0.98, test for interaction p=0.65) at baseline. CRC risk estimated based on recency of exposure were RR=0.85 (95% CI 0.74 to 0.98) for former PMH users and RR=0.73 (95% CI 0.58 to 0.92) for current PMH users, with a statistically significant trend across categories (p=0.001). Duration of exposure was also inversely associated with CRC risk, with estimates of RR=0.86 (95% CI 0.75 to 0.99) and RR=0.72 (95 CI 0.57 to 0.90) for ever users reporting ≤5 years and >5 years of PMH therapy at baseline, respectively (p trend <0.001). Similar associations were observed when PMH duration at baseline was evaluated within the current user and former user subgroups.

Analyses based on anatomic subsite demonstrated that the inverse association between PMH use and incident CRC was more pronounced for distal than for proximal tumours (table 2). Further separation of distal colon and rectal cancer cases did not appreciably alter the subsite-specific risk estimates (data not shown). When the CRC outcome was defined as only those cases for which adequate tissue samples were obtained for molecular testing, the multivariate risk estimate for PMH therapy ever users versus never users (RR=0.80; 95% CI 0.67 to 0.97) was not statistically different from the estimate based on all incident CRC cases, supporting a low likelihood of selection bias introduced by tissue availability status.

With respect to the molecularly defined CRC subtypes, baseline PMH therapy status was associated with a statistically significant reduction in risk for MSI-L/MSS tumours, but not MSI-H tumours (RR=0.75; 95% CI 0.60 to 0.94 and RR=0.98; 95% CI 0.69 to 1.39 for comparison of ever vs never users, respectively) (table 3). Further, PMH therapy of >5 years duration was associated with a 40% lower risk for MSI-L/MSS tumours with no appreciable influence on MSI-H tumour risk. Longer duration of PMH therapy was also associated with borderline statistically significant risk reductions for CIMP-negative (RR=0.68; 95% CI 0.45 to 1.03) or *BRAF*-wildtype (RR=0.70; 95% CI 0.47 to 1.05) tumours, while associations with the CIMP-positive or *BRAF*-mutated subtypes were less apparent. Due to sparse case numbers in some cells, we were not able to meaningfully estimate molecularly defined CRC risks stratified by proximal versus distal subsites.

DISCUSSION

In this large cohort study of older women, we found that baseline-reported PMH therapy was inversely associated with incident CRC, with statistically significant trends by recency and duration of exposure, as well as by CRC anatomic subsite. Further analyses based on molecularly defined CRC subtypes demonstrated that PMH therapy was associated with a statistically significantly lower risk for MSS/MSI-L tumours, as well as borderline statistically significant risk reductions for CIMP-negative and *BRAF*-wildtype tumours among women with prolonged exposure to PMH therapy (ie, >5 years duration). Conversely, PMH therapy-related risk estimates for the MSI-H, CIMP-positive and *BRAF*-mutated CRC subtypes were not statistically significant. To our knowledge, we report the first prospective evaluation of PMH therapy and CRC risks based on MSI, CIMP and *BRAF*

mutation status. Based on emerging data, several molecular models have been described to account for the clinicopathological heterogeneity in colorectal carcinogenesis.[1126–29] Using the framework proposed by Issa[29] and later expanded by Leggett and Whitehall,[11] our data suggest that PMH therapy may have more pronounced inhibitory effects on the 'traditional' pathway, as compared to the serrated or alternate pathways, of colorectal carcinogenesis. Further molecular marker analyses (ie, CIMP-low marker panel, *KRAS* mutation status), as well as confirmation by other studies, would strengthen this impression.

Our overall observations are similar to a previous case–control study of older women (ages 50–74 years) reported by Newcomb *et al*.[13] Among 1004 cases and 1062 controls recruited from the Seattle, Washington area, exposure to PMH therapy was non-significantly associated with decreased risk for any CRC risk (OR=0.9; 95 CI 0.7 to 1.1 for comparison of ever use vs never use). Stronger associations were observed when recency and duration of exposure were factored into the analyses, with current PMH use for ≥5 years resulting in a 30% CRC risk reduction (OR=0.7; 95% CI 0.6 to 0.9). Further consideration of CRC subtypes defined by a panel of nine MSI markers demonstrated a statistically significant association between oestrogen plus progestin use and MSI-L/MSS tumours (OR=0.6; 95% CI 0.4 to 0.9), but not MSI-H tumours (OR=0.7; 95% CI 0.4 to 1.4). Of note, PMH therapy with oestrogen alone did not appear to favourably affect CRC risk, overall or by MSI status. Since IWHS participants were not asked to provide detailed information regarding the type of PMH therapy used, we were unable to estimate CRC risks stratified by exposure to oestrogen plus progestin versus oestrogen alone in our study. However, based on data from pharmaceutical marketing surveys conducted near the time of IWHS cohort inception, it has been estimated that only ~20% of study participants were using combination PMH therapy.[3031] Further, based on results from the Women's Health Initiative observational study[32] and other recently reported cohort studies,[33–35] the degree to which specific hormone regimens modify the association between PMH therapy and CRC risk remains unresolved.

Additional data referent to MSI-defined CRC risks associated with PMH therapy are limited to a multicentre case–control study from Slattery *et al*.[12] Using a previously published panel of 12 MSI markers, CRC cases among postmenopausal women were categorised as MSI-positive (n=129) or MSI-negative (n=487). Compared to controls (n=982), neither MSI-positive (OR=1.0; 95% CI 0.7 to 1.5) nor MSI-negative (OR=0.9; 95% CI 0.7 to 1.1) tumours were significantly associated with ever versus never use of PMH therapy. Nonetheless, based on risk patterns observed for other hormonal exposures and reproductive factors, the authors proposed that oestrogen may interrupt colorectal carcinogenesis by preventing DNA methylation-induced silencing of oestrogen receptor expression, with additional speculation that one or more DNA mismatch repair genes may be oestrogen responsive as well (resulting in oestrogen-mediated protection for MSI-positive, rather than MSI-negative, tumours). Subsequently, Miyamoto *et al* showed that oestradiol can induce MLH1 and MLH2 protein expression in an endometrial cell culture system.[36] However, data from a more recent case–control study[14] suggest that PMH therapy may actually increase the methylation of *ESR* and related genes, emphasising the need for further evaluation of the mechanism(s) through which oestrogen and/or progestin might protect against colorectal neoplasia formation.

Following publication of the Women's Health Initiative (WHI) clinical trial results in 2002, the prevalence of PMH therapy declined by approximately 80%.[37] More recently, usage rates appear to be rebounding, based in part on updated information about the potential health benefits of PMH therapy in the early postmenopausal period.[37] According to one recent review,[38] the composite risk:benefit ratio may be more favourable for women who are 50–59 years of age or <10 years post-menopause. Among IWHS participants, the

association between PMH therapy and incident CRC was nearly identical for women aged 55–59 or >60 years at baseline. Observations from our study are also in keeping with secondary analyses of CRC endpoints from the WHI clinical trial and observational study,[3239] although one recent report from the WHI oestrogen-alone trial[40] suggested greater potential harm associated with conjugated equine oestrogen therapy among women aged 70–79 versus 50–59 or 60–69 years. Thus, based on limited existing data, the possibility of differing effects on CRC risk from PMH therapy when used in the earlier versus later postmenopausal periods remains inconclusive.

General strengths of our study include the population-based, prospective design; ability to control for multiple potential confounding factors in multivariate risk models; prolonged follow-up time with comprehensive ascertainment of incident CRC cases; and large sample size, which permitted relevant subgroup analyses. One acknowledged limitation of our study is that exposure to PMH therapy was assessed at a single time point (baseline), without enquiring about the dose, formulation, start date or stop date (for former users), which could have provided deeper insights for defining potential CRC chemoprevention strategies. Also, data regarding family history of CRC and exposure to CRC screening were not routinely available from the baseline or follow-up questionnaires. Because PMH therapy users may be more willing to undergo CRC screening than non-users, at least based on utilisation rates for faecal occult blood testing,[41] it is conceivable that differential screening might have influenced our risk estimates. However, as noted by others,[42] this effect is difficult to predict, since CRC incidence rates may be higher (due to increased detection) or lower (due to preventive polypectomies) among screen-adherent women. As with any observational study, our findings may have been influenced by the demographics of our subject population (ie, older and primarily Caucasian women) and should only be extrapolated to younger, more racially/ethnically diverse groups with appropriate caution. In addition, our molecularly defined risk estimates were based on a representative, but not complete, subset of CRC cases. Nonetheless, as noted above, biases related to tissue collection did not appear to influence the observed associations between PMH therapy and incident CRC.

In summary, data from this prospective, population-based cohort study provide support for an inverse association between PMH therapy and specific, molecularly defined CRC subtypes. Our results are consistent with the possibility of differential chemopreventive effects from PMH therapy on CRCs that arise through the traditional pathway of carcinogenesis.[1129] Analyses of additional molecular markers using data and tissue resources from the IWHS cohort are planned, to reinforce the currently reported observations. If confirmed by other studies, our findings may have direct implications for clinical practice, for example with respect to future application of PMH-based chemoprevention based on clinicopathological characteristics that predict the highest anticipated risk:benefit ratio, as well as for further translational research regarding the effects of PMH dose, duration and formulation on molecular mechanisms of colorectal carcinogenesis.

Acknowledgments

Funding This work was supported by grant funding from the National Institutes of Health (USA), including R01 CA107333 (Limburg, PI). Dr Weisenberger's participation was also partially supported by seed funding from the American Cancer Society.

REFERENCES

1. Siegel R, Ward E, Brawley O, et al. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA Cancer J Clin. 2011; 61:212–36. [PubMed: 21685461]

2. Edwards BK, Ward E, Kohler BA, et al. Annual report to the nation on the status of cancer, 1975-2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. Cancer. 2010; 116:544–73. [PubMed: 19998273]
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; 106:574–82. [PubMed: 10335731]
4. Chan AT, Giovannucci EL. Primary prevention of colorectal cancer. Gastroenterology. 2010; 138:2029–43. e10. [PubMed: 20420944]
5. Hulley S, Furberg C, Barrett-Connor E, et al. Noncardiovascular disease outcomes during 6.8 years of hormone therapy: Heart and Estrogen/progestin Replacement Study follow-up (HERS II). JAMA. 2002; 288:58–66. [PubMed: 12090863]
6. Hulley S, Grady D, Bush T, et al. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. JAMA. 1998; 280:605–13. [PubMed: 9718051]
7. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA. 2002; 288:321–33. [PubMed: 12117397]
8. Anderson GL, Limacher M, Assaf AR, et al. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. JAMA. 2004; 291:1701–12. [PubMed: 15082697]
9. Pino MS, Chung DC. The chromosomal instability pathway in colon cancer. Gastroenterology. 2010; 138:2059–72. [PubMed: 20420946]
10. Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology. 2010; 138:2073–87. e3. [PubMed: 20420947]
11. Leggett B, Whitehall V. Role of the serrated pathway in colorectal cancer pathogenesis. Gastroenterology. 2010; 138:2088–100. [PubMed: 20420948]
12. Slattery ML, Potter JD, Curtin K, et al. Estrogens reduce and withdrawal of estrogens increase risk of microsatellite instability-positive colon cancer. Cancer Res. 2001; 61:126–30. [PubMed: 11196149]
13. Newcomb PA, Zheng Y, Chia VM, et al. Estrogen plus progestin use, microsatellite instability, and the risk of colorectal cancer in women. Cancer Res. 2007; 67:7534–9. [PubMed: 17671225]
14. Wu AH, Siegmund KD, Long TI, et al. Hormone therapy, DNA methylation and colon cancer. Carcinogenesis. 2010; 31:1060–7. [PubMed: 20064828]
15. Folsom AR, Mink PJ, Sellers TA, et al. Hormonal replacement therapy and morbidity and mortality in a prospective study of postmenopausal women. Am J Public Health. 1995; 85:1128–32. [PubMed: 7625511]
16. Folsom AR, Kaye SA, Prineas RJ, et al. Increased incidence of carcinoma of the breast associated with abdominal adiposity in postmenopausal women. Am J Epidemiol. 1990; 131:794–803. [PubMed: 2138863]
17. Bisgard KM, Folsom AR, Hong CP, et al. Mortality and cancer rates in nonrespondents to a prospective study of older women: 5-year follow-up. Am J Epidemiol. 1994; 139:990–1000. [PubMed: 8178787]
18. Hankey BF, Ries LA, Edwards BK. The surveillance, epidemiology, and end results program: a national resource. Cancer Epidemiol Biomarkers Prev. 1999; 8:1117–21. [PubMed: 10613347]
19. Limsui D, Vierkant RA, Tillmans LS, et al. Cigarette smoking and colorectal cancer risk by molecularly defined subtypes. J Natl Cancer Inst. 2010; 102:1012–22. [PubMed: 20587792]
20. Cunningham JM, Kim CY, Christensen ER, et al. The frequency of hereditary defective mismatch repair in a prospective series of unselected colorectal carcinomas. Am J Hum Genet. 2001; 69:780–90. [PubMed: 11524701]
21. Boland CR, Thibodeau SN, Hamilton SR, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res. 1998; 58:5248–57. [PubMed: 9823339]

22. Thibodeau SN, French AJ, Cunningham JM, et al. Microsatellite instability in colorectal cancer: different mutator phenotypes and the principal involvement of hMLH1. Cancer Res. 1998; 58:1713–18. [PubMed: 9563488]
23. Weisenberger DJ, Siegmund KD, Campan M, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with *BRAF* mutation in colorectal cancer. Nat Genet. 2006; 38:787–93. [PubMed: 16804544]
24. Korn EL, Graubard BI, Midthune D. Time-to-event analysis of longitudinal follow-up of a survey: choice of the time-scale. Am J Epidemiol. 1997; 145:72–80. [PubMed: 8982025]
25. Lunn M, McNeil D. Applying Cox regression to competing risks. Biometrics. 1995; 51:524–32. [PubMed: 7662841]
26. Shen L, Catalano PJ, Benson AB 3rd, et al. Association between DNA methylation and shortened survival in patients with advanced colorectal cancer treated with 5-fluorouracil based chemotherapy. Clin Cancer Res. 2007; 13:6093–8. [PubMed: 17947473]
27. Jass JR. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. Histopathology. 2007; 50:113–30. [PubMed: 17204026]
28. Sweeney C, Boucher KM, Samowitz WS, et al. Oncogenetic tree model of somatic mutations and DNA methylation in colon tumors. Genes Chromosomes Cancer. 2009; 48:1–9. [PubMed: 18767147]
29. Issa JP. Colon cancer: it's CIN or CIMP. Clin Cancer Res. 2008; 14:5939–40. [PubMed: 18829469]
30. Hemminki E, Kennedy DL, Baum C, et al. Prescribing of noncontraceptive estrogens and progestins in the United States, 1974-86. Am J Public Health. 1988; 78:1479–81. [PubMed: 3177727]
31. Sellers TA, Mink PJ, Cerhan JR, et al. The role of hormone replacement therapy in the risk for breast cancer and total mortality in women with a family history of breast cancer. Ann Intern Med. 1997; 127:973–80. [PubMed: 9412302]
32. Prentice RL, Pettinger M, Beresford SA, 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; 18:1531–7. [PubMed: 19423530]
33. Tannen RL, Weiner MG, Xie D, et al. Estrogen affects post-menopausal women differently than estrogen plus progestin replacement therapy. Hum Reprod. 2007; 22:1769–77. [PubMed: 17347166]
34. Johnson JR, Lacey JV Jr, Lazovich D, et al. Menopausal hormone therapy and risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev. 2009; 18:196–203. [PubMed: 19124498]
35. Hildebrand JS, Jacobs EJ, Campbell PT, et al. Colorectal cancer incidence and postmenopausal hormone use by type, recency, and duration in cancer prevention study II. Cancer Epidemiol Biomarkers Prev. 2009; 18:2835–41. [PubMed: 19843681]
36. Miyamoto T, Shiozawa T, Kashima H, et al. Estrogen up-regulates mismatch repair activity in normal and malignant endometrial glandular cells. Endocrinology. 2006; 147:4863–70. [PubMed: 16825317]
37. Santen RJ, Allred DC, Ardoin SP, et al. Postmenopausal hormone therapy: an Endocrine Society scientific statement. J Clin Endocrinol Metab. 2010; 95:s1–s66. [PubMed: 20566620]
38. Pines A, Sturdee DW, Birkhauser MH, et al. HRT in the early menopause: scientific evidence and common perceptions. Climacteric. 2008; 11:267–72. [PubMed: 18645691]
39. Prentice RL, Manson JE, Langer RD, et al. Benefits and risks of postmenopausal hormone therapy when it is initiated soon after menopause. Am J Epidemiol. 2009; 170:12–23. [PubMed: 19468079]
40. LaCroix AZ, Chlebowski RT, Manson JE, et al. Health outcomes after stopping conjugated equine estrogens among postmenopausal women with prior hysterectomy: a randomized controlled trial. JAMA. 2011; 305:1305–14. [PubMed: 21467283]
41. Barrett-Connor E. Postmenopausal estrogen and prevention bias. Ann Intern Med. 1991; 115:455–6. [PubMed: 1872493]
42. Troisi R, Schairer C, Chow WH, et al. A prospective study of menopausal hormones and risk of colorectal cancer (United States). Cancer Causes Control. 1997; 8:130–8. [PubMed: 9134236]

Significance of this study

What is already known on this subject?

▶ Postmenopausal hormone (PMH) therapy could be used to complement colorectal cancer (CRC) prevention strategies focused on diet, lifestyle and screening behaviours.

▶ Existing data regarding the association between PMH and CRC risk are inconclusive.

▶ Data referent to associations between PMH therapy and molecularly defined CRC subtypes are limited and inconsistent.

What are the new findings?

▶ PMH therapy was inversely associated with incident CRC, with statistically significant trends by recency and duration of exposure, in this large prospective, population-based cohort study of older women.

▶ PMH therapy was associated with a statistically significant lower risk for MSS/MSI-L tumours, as well as borderline statistically significant risk reductions for CIMP-negative and *BRAF*-wildtype tumours among women with prolonged exposure to PMH therapy (ie, >5 years duration); conversely, PMH therapy-related risk estimates for the MSI-H, CIMP-positive and *BRAF*-mutated CRC subtypes were not statistically significant.

▶ These data suggest that PMH therapy may have more pronounced inhibitory effects on the ‘traditional’ pathway, as compared to the serrated or alternate pathways, of colorectal carcinogenesis.

How might it impact on clinical practice in the foreseeable future?

▶ These findings may have direct implications for clinical practice, for example with respect to future application of PMH-based chemoprevention based on clinicopathological characteristics that predict the highest anticipated risk:benefit ratio, as well as for further translational research regarding the effects of PMH dose, duration and formulation on molecular mechanisms of colorectal carcinogenesis.

Table 1

Baseline characteristics of study subjects, by postmenopausal hormone (PMH) therapy status

Characteristic	PMH therapy	
	Never use (n = 22986)	Ever use (n = 14299)
Age, years	62.2 (4.3)	62.3 (4.1)
Body mass index, kg/m^2	27.3 (5.3)	26.6 (4.8)
Waist:hip ratio	0.8 (0.09)	0.8 (0.08)
Smoking status, N (%)		
Never	15291 (68%)	8849 (63%)
Former	4063 (18%)	3012 (21%)
Current	3261 (14%)	2221 (16%)
Age at menopause, y	48.5 (5.9)	46.2 (7.0)
Age at menarche, y	12.9 (1.5)	12.8 (1.5)
Oral contraceptive use, N (%)		
Ever	18858 (82%)	11413 (80%)
Never	4067 (18%)	2815 (20%)
Physical activity index, N (%)		
Low	10869 (48%)	6453 (46%)
Moderate	6059 (27%)	4009 (29%)
High	5569 (25%)	3586 (26%)
Alcohol consumption, g/day	3.4 (8.7)	3.9 (9.1)
Total energy, kcal/day	1802.2 (762.1)	1776.1 (685.0)
Total fat, g/day	68.7 (33.2)	67.1 (30.3)
Sucrose, g/day	41.7 (25.3)	40.9 (23.6)
Red meat, g/day	92.0 (78.2)	86.9 (69.4)
Calcium, mg/day *	1051.6 (561.3)	1150.9 (581.9)
Folate, μg/day *	417.2 (259.7)	445.5 (269.8)
Methionine, g/day	1.9 (0.9)	1.8 (0.8)
Vitamin E, mg/day *	59.3 (139.2)	79.2 (164.3)
PMH therapy		
Current user, N (%)	–	4062 (28%)
Former user, N (%)	–	10237 (72%)
Duration ≤5 years	–	10017 (71%)
Duration >5 years	–	4017 (29%)

Results presented as mean (SD) unless otherwise indicated.

* Including supplements.

Table 2

Associations between postmenopausal hormone (PMH) therapy at baseline and incident colorectal cancer (CRC), overall and by anatomic subsite

		Any CRC (n = 1234)*		Proximal CRC (n = 618)		Distal CRC (n = 588)	
PMH therapy	**Person-years**	**Events, N**	**RR (95% CI)†**	**Events, N**	**RR (95% CI)†**	**Events, N**	**RR (95% CI)†**
Never user	341 377	827	1.00 (ref.)	402	1.00 (ref.)	409	1.00 (ref.)
Ever user	212 696	407	0.82 (0.72 to 0.93)	216	0.90 (0.75 to 1.08)	179	0.73 (0.60 to 0.88)
Former user	151 535	312	0.85 (0.74 to 0.98)	169	0.93 (0.77 to 1.12)	135	0.76 (0.62 to 0.94)
Current user	61 161	95	0.73 (0.58 to 0.92)	47	0.81 (0.59 to 1.11)	44	0.64 (0.45 to 0.89)
Duration ≤5 years	148704	300	0.86 (0.75 to 0.99)	163	0.95 (0.78 to 1.15)	127	0.75 (0.61 to 0.93)
Former user	127 499	270	0.88 (0.76 to 1.01)	145	0.95 (0.77 to 1.16)	118	0.79 (0.64 to 0.99)
Current user	21 205	30	0.74 (0.51 to 1.08)	18	0.99 (0.61 to 1.61)	9	0.45 (0.23 to 0.88)
Duration >5 years	60064	99	0.72 (0.57 to 0.90)	49	0.75 (0.55 to 1.03)	48	0.68 (0.49 to 0.94)
Former user	21 795	37	0.72 (0.51 to 1.01)	22	0.82 (0.52 to 1.30)	14	0.60 (0.35 to 1.03)
Current user	38 269	62	0.72 (0.54 to 0.95)	27	0.71 (0.47 to 1.06)	34	0.73 (0.49 to 1.08)

*Includes n=28 CRC cases for which anatomic subsite was not specified.

†Adjusted for age, body mass index, waist:hip ratio, smoking status, age at menopause, age at menarche, oral contraceptive use, physical activity level, alcohol consumption, self-reported diabetes mellitus, and daily intake of total energy, total fat, sucrose, red meat, calcium, folate, methionine and vitamin E (mg/day).

Table 3

Associations between postmenopausal hormone (PMH) therapy at baseline and molecularly defined colorectal cancer (CRC) subtypes

		Microsatellite instability (MSI)				CpG island methylator phenotype (CIMR)				BRAF mutation			
		MSI-1/MSS		MSI-H		CIMP-negative		CIMR-positive		BRAF-wildtype		BRAF-mutated	
RMH therapy	Rerson-years	Events, N	RR (95% CI)*	Events, N	RR (95% CI)*	Events, N	RR (95% CI)*	Events, N	RR (95% CI)*	Events, N	RR (95% CI)*	Events, N	RR (95% CI)*
Never user	341 377	269	1.00 (ref.)	90	1.00 (ref.)	242	1.00 (ref.)	106	1.00 (ref.)	254	1.00 (ref.)	102	1.00 (ref.)
Ever user	212 696	125	0.75 (0.60 to 0.94)	55	0.98 (0.69 to 1.39)	119	0.79 (0.63 to 1.01)	58	0.86 (0.61 to 1.20)	129	0.83 (0.66 to 1.04)	51	0.78 (0.55 to 1.11)
Former user	151 535	91	0.73 (0.57 to 0.94)	42	0.99 (0.67 to 1.45)	85	0.76 (0.59 to 0.99)	43	0.84 (0.58 to 1.22)	92	0.80 (0.62 to 1.03)	39	0.78 (0.53 to 1.15)
Current user	61 161	34	0.82 (0.56 to 1.19)	13	0.95 (0.51 to 1.76)	34	0.89 (0.61 to 1.31)	15	0.91 (0.51 to 1.61)	37	0.91 (0.63 to 1.33)	12	0.79 (0.43 to 1.46)
Duration <5 years	148704	94	0.81 (0.63 to 1.04)	39	0.98 (0.66 to 1.45)	87	0.84 (0.65 to 1.08)	41	0.86 (0.59 to 1.25)	95	0.88 (0.69 to 1.13)	36	0.79 (0.54 to 1.17)
Former user	127 499	81	0.78 (0.60 to 1.01)	35	1.00 (0.67 to 1.50)	75	0.80 (0.61 to 1.06)	36	0.85 (0.58 to 1.27)	81	0.84 (0.65 to 1.10)	33	0.81 (0.54 to 1.21)
Current user	21 205	13	1.09 (0.62 to 1.92)	4	0.80 (0.25 to 2.53)	12	1.12 (0.62 to 2.01)	5	0.89 (0.33 to 2.44)	14	1.17 (0.67 to 2.06)	3	0.68 (0.21 to 2.15)
Duration >5 years	60 064	28	0.60 (0.40 to 0.91)	16	1.03 (0.59 to 1.81)	29	0.68 (0.45 to 1.03)	17	0.90 (0.52 to 1.55)	31	0.70 (0.47 to 1.05)	15	0.80 (0.45 to 1.43)
Former user	21 795	9	0.53 (0.27 to 1.04)	7	1.00 (0.43 to 2.30)	9	0.60 (0.31 to 1.18)	7	0.84 (0.36 to 1.92)	10	0.64 (0.34 to 1.22)	6	0.70 (0.28 to 1.75)
Current user	38 269	19	0.64 (0.39 to 1.06)	9	1.05 (0.52 to 2.12)	20	0.73 (0.44 to 1.20)	10	0.95 (0.49 to 1.84)	21	0.74 (0.46 to 1.21)	9	0.87 (0.44 to 1.75)

*Adjusted for age, body mass index, waist:hip ratio, smoking status, age at menopause, age at menarche, oral contraceptive use, physical activity level, alcohol consumption, self-reported diabetes mellitus, and daily intake of total energy, total fat, sucrose, red meat, calcium, folate, methionine and vitamin E (mg/day).