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Mean Leukocyte Telomere Length and Risk of Incident Colorectal Carcinoma in Women: A Prospective, Nested Case-Control Study

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Abstract

Background—To date, no prospective, epidemiological data are available, particularly in women, on mean leukocyte telomere length as a risk predictor.

Methods—Using leukocyte DNA samples collected at baseline in a prospective cohort of over 28,000 initially healthy women, we examined the relationship of mean leukocyte telomere repeat copy number to single gene copy number (TSR) amongst 134 incident CRC cases, and 357 matched controls; all were white.

Results—The observed $\log_e$-transformed TSRs were similar between cases and controls (p=0.79). In an adjusted analysis, we found no evidence for an association of the $\log_e$-TSRs with CRC risk (adjusted odds ratio=0.943, 95%CI=0.647–1.376, p=0.762). Further stratified analysis by median follow-up time, or postmenopausal status also showed similar null findings.

Conclusion—In concordance with our previous findings in white men, the present study in white women found no evidence for an association of mean leukocyte telomere length with risk of incident CRC, further suggesting that leukocyte telomere length may not be a useful indicator for risk assessment.

Keywords

mean leukocyte telomere length; colorectal carcinoma; risk predictor; women

INTRODUCTION

Telomeres are tandem repeats of DNA sequences —special chromatin structures— located at the ends of eukaryotic chromosomes, and are believed to protect the telomeric regions from recombination and degradation, thus avoiding chromosomal instability and cell senescence (1,2). Genomic instability is a hallmark of tumourogenesis, and is widely believed to play an important role in cancer development, including colorectal carcinoma (CRC) development (1,3). Recent studies have implicated telomere length shortening as an independent marker for the progression and/or prognosis of CRC (4–11) based on the comparison of paired cancerous and adjacent non-cancerous tissue specimens from the same individuals. Using a nested case-control approach, we recently examined the relationship of leukocyte telomere length with

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CONFLICT-OF-INTEREST DISCLOSURE
None

incident CRC in white men, and found no evidence for an association (12). Moreover, both experimental and human studies have shown longer telomeres in adult females than in adult males (13–17). The gender difference in relation to leukocyte telomere length may reflect true, differential biological-responses (and environmental/lifestyle factors) to oxidative stress (18–21). Owing to the gender-specific phenomenon along with the fact that, to date, no prospective epidemiological studies, particularly in women, on the relationship of leukocyte telomere length as a risk predictor with incident CRC are available, the present study was conducted to further examine the possible association of peripheral blood leukocyte (PBL) telomere length with risk of incident CRC using a nested, matched case-control approach among women from the Women's Health Study (WHS).

MATERIALS AND METHODS

Study Design

We conducted a case-control study nested within the WHS cohort, a completed randomized, double-blinded, placebo-controlled trial of aspirin and vitamin E in the primary prevention of cancer and cardiovascular disease (22–24). Beginning in 1993, 39,876 US female health professionals, predominantly white (>94%), aged ≥45 years and free of cancer or cardiovascular disease enrolled in the study and completed a baseline questionnaire about their medical history and potential risk factors for CRC. Blood samples were collected from 28,345 (71%) women before randomization. Baseline characteristics of women who provided blood were largely similar to those who did not (25).

The present study consisted of 134 confirmed incident CRC cases as of December 2005, and 357 healthy controls matched by age (±2 years) and length of follow-up since randomization; all cases and controls were white. To increase the statistical power, we attempted to match up to three controls for each case. Median length of follow-up since randomization for the cases was 5.80 years (interquartile range: 3.68–7.58). The present study was approved by the Brigham and Women's Hospital Institutional Review Board for Human Subjects Research.

Mean Telomere Length Determination

Unified Quantitative Polymerase Chain Reaction Assay—Genomic DNA was extracted from whole blood using the QIAmp Spin Column protocol (Qiagen, Chatsworth, CA). Telomere length was determined by a previously described, unified quantitative polymerase chain reaction (qPCR) protocol (26). In brief, two master mixes of PCR reagents were prepared, one for telomere reaction and one for single-copy gene reaction (*36B4* on chromosome 12). Telomere repeat copy number to single gene copy number ratio (TSR) was determined on an ABI 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) in a 384-well format using the following PCR protocol: 95°C for 15 minutes to activate Taq-polymerase; 40 cycles of denaturation at 95°C for 15 seconds, and annealing-extension at 54°C for 2 minutes. The primer sequences used were described elsewhere (26,27). All samples for both the telomere and single-copy gene amplifications were done in duplicate on the same 384-well plate. Ct-value assignment was carried out by two independent observers, and if necessary, a complete re-amplification was performed. Duplicates of a no-template control were included in each run. Melting (dissociation) curve analysis was performed on every run to verify specificity and identity of the PCR products. The Ct values generated were used to calculate the TSR for each sample using the equation: $T/S=2^{-\Delta Ct}$ (where $\Delta Ct=Ct_{\text{single-copy gene}}-Ct_{\text{telomere}}$). Results were scored blinded as to case-control status.

Statistical Analysis

The observed TSRs had a skewed distribution, and thus the data were $\log_e$-transformed. Spearman's correlation analysis was used to assess the associations of age, current smoking,

bodymass index (BMI), alcohol use, exercise, and current HRT use on TSRs amongst all participants. The $\log_e$-TSRs between cases and controls were compared using the non-paired t-test. Risk ratio of CRC associated with $\log_e$-TSRs were calculated by conditional logistic regression analysis, adjusting for age, and smoking status, and further controlling for randomized treatment assignment, BMI, alcohol use, exercise, presence of colorectal polyps, postmenopausal status and hormone therapy (HT) use. All analyses were carried out using SAS 9.1 package [SAS Institute Inc., Cary, NC]. A two-tailed *p*-value of <0.05 was considered a statistically significant result.

RESULTS

The baseline characteristics of the study participants are shown in Table 1. No significant correlation was found between the observed TSRs and the clinical/demographic parameters evaluated (Table 2). The observed TSRs were similar between cases and controls (p=0.790; Table 1). Furthermore, no association of mean $\log_e$-TSRs with risk of incident CRC was found in the regression analysis (adjusted odds ratio=0.943, 95%CI=0.647–1.376, p=0.762; Table 3). Regression analysis limited to postmenopausal female participants (adjusted odds ratio=1.013, 95%CI=0.668–1.534, p=0.952; Table 3), and stratified analysis by median follow-up time since randomization (data not shown) were also performed, and again showed similar null findings. Further regression analysis using a quartile comparison approach of $\log_e$-TSR again found similar null findings (data not shown). The coefficients of variation of the telomere, single-gene, and TSR duplicate assays were all <3%, respectively.

DISCUSSION

To the best of our knowledge, the present nested, matched case-control study is the first to examine the relationship of mean leukocyte telomere length with risk of incident CRC in women. We found no evidence for an association, and the present null findings in white women support our recent findings in white men using a similar study design (12).

Recent studies have shown telomere length shortening as an independent marker for the progression and/or prognosis of CRC (4–11). However, these studies used colonocyte-telomere length measurements from paired cancerous-noncancerous tissue specimens from the same individuals, as opposed to PBL. Furthermore, it has been shown that telomere dynamics in colonocytes differ from other tissues including PBL (10,28), due partly to the local dynamics of telomere-telomerase complex in cell proliferation (8), and exposure/responses to oxidative damage (29) in persons already had CRC. Moreover, a case-control study by Risques *et al*. (30) examining telomere length from various tissue types (including circulating leukocytes) in ulcerative colitis (UC), a chronic inflammatory condition that predisposes to CRC, a modest shortening in leukocytes from UC subjects (N=102) compared to the controls (N=45) was observed (p=0.046). The authors hypothesized that the moderate shortening of leukocyte telomeres due to its proliferative properties and frequent travel through the inflamed colon. Taking altogether, the potential involvement of leukocyte telomere biology in CRC risk requires further investigation in future large prospective studies.

The nature of the present investigation in which the determination of a case status was based solely on the subsequent development of disease rather than on any arbitrary selection criteria designed by the investigators, greatly reduce the possibility of bias and confounding. Nonetheless, our study population consists of white females only, so the data may not be applicable to other ethnic groups, non-white men, or populations with different socioeconomic background. The present null findings could be partly due to bias from different environmental/lifestyle factors obtained for the present female sample population compared to those in our previous male sample population (12), or play of chance. Furthermore, in contrast to our

previous observation in men (12), no correlation of leukocyte telomere length with age was observed. This may partly due to the present (age) matching selection criteria and the modest sample size of our control participants, from whom would be unlikely to capture enough variability of age to detect a relationship.

In our study, we had the ability to detect, based on the present sample size, assuming 80% power, at an alpha of 0.05, a difference in the $\log_e$-transformed TSR of <-0.218 or >0.218 between cases and controls. Thus, the present study may have limited power to detect a true, small-to-moderate difference of telomere length between cases and controls.

In conclusion, the present prospective, nested case-control study of white US women found no evidence for an association of mean leukocyte telomere length with risk of incident colorectal carcinoma. In concordance with our previous findings in a prospective, nested case-control study of middle-aged US white men, the present findings further suggest that leukocyte telomere length may not be a useful predictor for CRC risk assessment.

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Table 1

Baseline characteristics of study participants.

	Controls (N=357)	Cases (N=134)	p^{**}
Age (years)	60.66±8.63	60.10±8.68	0.53
Smoking Status (%)			0.24
Never	52.94	47.01	
Past	38.10	46.27	
Current	8.96	6.72	
Bodymass Index (kg/m^2)	25.87±4.94	26.18±5.56	0.55
Alcohol use (%)			0.30
never	41.18	45.52	
>0–<15 g/day	51.26	44.03	
≥15 g/day	7.56	10.45	
Exercise (%)			0.54
Daily	12.61	11.94	
Weekly	29.13	34.33	
Rarely	58.26	53.73	
Aspirin use (%)	52.38	46.27	0.23
Beta-carotene use (%)	48.74	51.49	0.59
Median length of follow-up since randomization (years)*	5.84 [4.04–5.00]	5.80[3.68–7.58]	0.33
Postmenopausal (%)	84.31	84.33	0.99
Current HT use (%)	42.58	35.07	0.28
Polyps (%)	1.96	3.73	0.26
Cancer site (%)			--
Colon	--	76.34	
Rectum	--	23.66	
Family history of CRC (%)	10.92	11.19	0.93
Log_e-transformed TSR	4.51±0.77	4.49±0.78	0.79

Mean±SD unless otherwise stated.

CRC, colorectal carcinoma; HT, hormone therapy; TSR, telomere repeat copy number to single gene copy number.

*Median and interquartile range.

**Continuous and categorical variables were tested by non-paired t-test and Chi-square analysis, respectively.

Table 2

Spearman correlation analysis of TSR with several baseline variables amongst all participants.

TSR	Correlation coefficient; *p*
Age*	−0.008; 0.850
Bodymass index**	0.030; 0.506
Current smoking**	0.017; 0.713
Alcohol use**	−0.056; 0.220
Exercise**	−0.029; 0.520
Current HRT use**	−0.014; 0.753

*Spearman partial correlation coefficients adjusted for case-control status.

**Spearman partial correlation coefficients adjusted for age and case-control status.

HT, hormone therapy; TSR, telomere repeat copy number to single gene copy number.

Table 3

Conditional logistic regression analysis of shortening of $\log_e$-transformed TSR.

Colorectal carcinoma	**Crude**	**Adjusted**
	OR, 95%CI, *p*	**OR, 95%CI, *p***
All participants	0.982, 0.692–1.393, 0.921	0.943, 0.647–1.376, 0.762
Postmenopausal women only	1.005, 0.688–1.468, 0.979	1.013, 0.668–1.534, 0.952

Crude=adjusting for age, and smoking status.

Adjusted=further controlling for bodymass index, randomized treatment group, presence of colorectal polyps, alcohol use, exercise, postmenopausal status (if applicable), and hormone therapy use.

TSR, telomere repeat copy number to single gene copy number.