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Mean Telomere Length and Risk of Incident Colorectal Carcinoma: A Prospective, Nested Case-Control Approach

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Abstract

Recent studies have shown telomere-length shortening in colorectal carcinoma. However, to date, no prospective, epidemiological data are available on examining mean leukocyte telomere length as a risk predictor. Using leukocyte DNA samples collected at baseline in a prospective cohort of 14,916 initially healthy American men, we examined the relationship of mean telomere repeat copy number to single gene copy number (T/S ratio), using a modified quantitative polymerase chain reaction protocol, amongst 191 incident colorectal carcinoma cases, matched to 306 controls by age, smoking status, and length of follow-up; all white males. An inverse correlation between T/S ratio and age was observed in our sample population ($p=0.038$). However, the T/S ratios were similar between cases and controls ($p=0.650$). Furthermore, in a multivariable adjusted analysis, we found no evidence for an association of the observed T/S ratios with colorectal carcinoma risk (adjusted odds ratio=1.249, 95%CI=0.863-1.808, $p=0.238$). In summary, the present investigation found no evidence for an association of leukocyte mean telomere length with risk of incident colorectal carcinoma, and further suggests that leukocyte mean telomere length may not be a useful indicator for risk assessment.

Keywords

mean leukocyte telomere length; colorectal carcinoma; risk predictor

Introduction

Telomeres are tandem repeats of DNA sequences—special chromatin structures—located at the ends of eukaryotic chromosomes. These structures are believed to protect the telomeric regions from recombination and degradation, thus avoiding a DNA damage cellular response (1,2). Genome instability is a hallmark of tumorigenesis, and is a widely accepted view as a major contribution to the development of cancer, including colorectal carcinoma (CRC) (1, 3). Furthermore, 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. However, to date, no studies have been conducted to examine the relationship of telomere length as a risk predictor with incident CRC.

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We thus prospectively examined the possible association of mean peripheral blood leukocyte (PBL) telomere length with risk of incident CRC using a nested, matched case-control sample drawn from the prospective Physicians' Health Study (PHS) cohort.

Materials and Methods

Study Design

We employed a nested case-control design within the PHS, a randomized, double-blinded, placebo-controlled trial of aspirin and beta-carotene initiated in 1982 among 22,071 male, predominantly white (>94%), U.S. physicians, 40 to 84 years of age at study entry (12). Before randomization, 14,916 participants provided an EDTA-anticoagulated blood sample that was stored for genetic analysis. All participants were free of prior myocardial infarction (MI), stroke, transient ischemic attacks, deep venous thrombosis/pulmonary embolism and cancer at study entry. Yearly follow-up self-report questionnaires provided reliable updated information on newly developed diseases. For all reported incident CRC events occurring after study enrollment, medical records were requested and reviewed by an end-points committee.

The nested case-control design has been previously described (13). For each case, one or two controls matched by age $\pm$ 2 years, smoking history (never, past, or current) and length of follow-up were chosen among those subjects who remained free of CRC. The present study consisted of white males only: 76 1-to-1 matched pairs, and 115 1-to-2 matched pairs. Median length of follow-up since randomization for the cases was 6.02 years (interquartile range: 3.22-8.95). The 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 and validated, unified quantitative polymerase chain reaction (qPCR) protocol (14). 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 (T/S) ratio 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. Each 5 μ L amplification reaction volume contained 1 $\times$ Qiagen Quantitect Sybr Green Master Mix (Qiagen, Chatsworth, CA) and 10ng of template DNA. The primer sequences used were described elsewhere (14,15). 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-genotyping was performed. The Ct values generated were used to calculate the T/S ratio 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

As previously noted, the observed telomere-repeat copy number to single gene copy number ratios (T/S ratios) had a skewed distribution, the data were log_e-transformation. The T/S ratios between cases and controls were compared using the non-paired t-test. Spearman's correlation analysis was used to assess the effects of age, current smoking, bodymass index (BMI), alcohol use, and exercise on relative T/S ratios amongst all subjects. Risk ratio of CRC associated with log_e-transformed T/S ratios were calculated by conditional logistic regression analysis, adjusting for age, smoking status, and length of follow-up since randomization, and further

controlling for randomized treatment assignment, BMI, alcohol use (daily/weekly/rarely), and exercise (daily/weekly/rarely). 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

Baseline characteristics of the study participants are shown in Table 1. In concordance with published data, an inverse relationship of the observed T/S ratios with age amongst all subjects was found (Spearman correlation = -0.093 , $p=0.038$; Supplemental Data Table 1), but not BMI, current smoking, daily alcohol use, nor daily exercise (Supplementary Data Table 1). However, the observed T/S ratios were similar between cases and controls ($p=0.650$; Table 1). Furthermore, no association of mean T/S ratios with risk of incident CRC was found in the regression analysis (adjusted odds ratio = 1.249 , 95% CI = 0.863 - 1.808 , $p=0.238$; Table 2). Stratified analysis by median follow-up time since randomization was also performed, and again similar null findings were observed. The coefficients of variation of the telomere, single-gene, and T/S ratio duplicate assays were all $<2\%$, respectively.

Discussion

To the best of our knowledge, the present nested, matched case-control investigation is the first to examine the relationship of mean leukocyte telomere length with risk of incident CRC, and we found no evidence for an association. In concordance with previous reports, the present study found an inverse correlation between mean leukocyte telomere length and age.

Recent studies have shown telomere length shortening as an independent marker for the progression and/or prognosis of CRC (4-11). However, these studies were based on telomere length measurements in colonocytes of paired cancerous-noncancerous tissue specimens from the same individuals, as opposed to PBL as used in the current investigation. As previously noted, Craig *et al.* found no correlation of telomere length with age in normal colonocytes from normal individuals; however, telomere shortening with age was observed in other tissues including blood (16). Furthermore, the study by O'Sullivan *et al.* also observed an association between telomere length in PBL and gastric tissue, but not between PBL and colon (10). Taken altogether, these data suggest that telomere dynamics in colonocytes differ from other tissues including PBL (10,16), and may be partly related to the dynamics of telomere-telomerase complex in cell proliferation (8), and exposure/responses to oxidative damage (17). As no prospective, epidemiological data on mean PBL telomere length and risk of incident CRC are available, a cross-reference comparison cannot be achieved on the present null findings in relation to CRC risk. Nevertheless, the present investigation suggests that PBL telomere-length may not play a role in the underlying pathogenesis of CRC.

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 males only so the data cannot be generalized to other ethnic groups, women, and populations with different socioeconomic status. Furthermore, no baseline information on colorectal screening is available in the present sample population (for example, removal of polyp during colonoscopy). Thus, the potential confounding effect of colorectal screening cannot be assessed in the present context. 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 true difference in the mean telomere length ratio of <-0.153 or >0.153 . Because of the stringent matching criteria used, 76 cases could only be matched to one control; thus power to detect differences may differ by matched case-control subgroups.

As telomere biology represents a rapidly expanding research field, further thoughts on future development of qPCR technique is worthwhile. Of relevant note, a multiplex monochrome qPCR method for telomere length measurement has recently been described (18). Thus, future methodological comparison to the gold-standard Southern Blot method is worthwhile.

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

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Table 1

Baseline characteristics of study participants.

	Controls (N=306)	Cases (N=191)	<i>p</i> ^{**}
Age (years)	58.05±7.95	60.52±8.66	0.0012
Smoking Status (%)			0.67
Never	39.22	35.60	
Past	54.25	56.54	
Current	6.53	7.86	
Body-mass Index (kg/m ²)	24.82±2.55	25.19±2.86	0.14
Alcohol use (%)			0.59
Daily	29.93	31.41	
Weekly	47.37	49.74	
Rarely	22.70	18.85	
Exercise (%)			0.96
Daily	6.58	6.88	
Weekly	67.44	68.26	
Rarely	25.99	24.86	
Aspirin use (%)	53.27	50.26	0.51
Beta-carotene use (%)	50.65	53.93	0.48
*Median length of follow-up since randomization (years)	6.60 [3.80-9.19]	6.02 [3.22-8.95]	0.30
Cancer site (%)			--
Colon	--	78.79	
Rectum	--	21.21	
Log _e -transformed T/S ratio	3.51±0.58	3.53±0.61	0.65

Mean±SD unless otherwise stated.

T/S=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 2Conditional logistic regression analysis of shortening of log_e-transformed mean telomere length.

T/S ratio	Crude	Adjusted
Colorectal carcinoma	OR, 95%CI, <i>p</i> 0.837, 0.584-1.198, 0.331	OR, 95%CI, <i>p</i> 0.801, 0.553-1.159, 0.238

Crude=adjusting for age, smoking status, length of follow-up.

Adjusted=further controlling for randomized treatment group, body-mass index, alcohol use, and exercise.