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The Association of Telomere Length with Colorectal Cancer Differs by the Age of Cancer Onset

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OBJECTIVES: Telomeres are nucleoprotein structures that cap the end of chromosomes and shorten with sequential cell divisions in normal aging. Short telomeres are also implicated in the incidence of many cancers, but the evidence is not conclusive for colorectal cancer (CRC). Therefore, the aim of this study was to assess the association of CRC and telomere length.
METHODS: In this case–control study, we measured relative telomere length from peripheral blood leukocytes (PBLs) DNA with quantitative PCR in 598 CRC patients and 2,212 healthy controls.
RESULTS: Multivariate analysis indicated that telomere length was associated with risk for CRC, and this association varied in an age-related manner; younger individuals ($\leq$50 years of age) with longer telomeres (80–99 percentiles) had a 2–6 times higher risk of CRC, while older individuals ($>$50 years of age) with shortened telomeres (1–10 percentiles) had 2–12 times the risk for CRC. The risk for CRC varies with extremes in telomere length in an age-associated manner.
CONCLUSIONS: Younger individuals with longer telomeres or older individuals with shorter telomeres are at higher risk for CRC. These findings indicate that the association of PBL telomere length varies according to the age of cancer onset and that CRC is likely associated with at minimum two different mechanisms of telomere dynamics.
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INTRODUCTION

Telomeres cap linear chromosomes to maintain stability[1] and shorten with successive rounds of DNA replication during sequential cell division.[2] In healthy cells, erosion of telomere length eventually leads to regulated cell senescence and apoptosis. However, in abnormal cells, continued cell division after telomere depletion can lead to end-to-end fusion of chromosomes and chromosomal instability. Telomere shortening, therefore, is a process of aging[3] associated with genetic instability[4] and oncogenesis.[5,6]

Previous research has shown that the depletion of constitutional telomere structure end sequences is associated with an increased risk for some cancers, including head and neck, urinary bladder,[7–9] renal,[10] lung,[11] esophageal,[12] and colorectal[13–16] cancers.[5,6,17] This work has also suggested that the association between telomere length and cancer risk may differ for those with a younger vs. older age of onset of cancer. In head and neck, urinary bladder, renal cell, and lung cancer patients, the associations between cancer risk and shorter telomere length for patients $<$55 years of age was three times higher than those who developed cancer after age 65 (odds ratio (OR) = 24 vs. OR = 8).[7] Studies of telomere dynamics in colorectal cancer (CRC), specifically, have reported inconsistent results,[13–16] with one recent study reporting that both long and short telomeres were associated with an increased CRC risk.[14] The nature of associations between telomere length and CRC risk remain unclear, and the way in which age-of-onset may operate in this association has not been explored. Although the majority of CRC occurs after age 65, up to 20% of CRC occurs in individuals 50 years of age or younger, and who do not have either of the known hereditary CRC conditions (Lynch syndrome or familial adenomatous polyposis).[18–21]

The relationship between telomere length in peripheral blood cells and CRC risk has not been systematically evaluated in relation to the age of cancer onset. In this multicenter, hospital-based case–control study, we measured telomere length in patients with microsatellite stable CRC and healthy controls across a broad range of ages to evaluate the association between PBL relative telomere length and CRC risk, and determine if this association is age dependent.

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METHODS

Study design. To assess the relationship between peripheral blood leukocytes (PBLs) telomere length and the risk for CRC, this case–control study compared the telomere with single-copy gene reference standard ratio (T/S ratio) measured by quantitative PCR from CRC patients to cancer-free controls, after controlling for demographic, environmental, and lifestyle variables including: sex, race, alcohol use, tobacco use, hormone replacement therapy in women, family history of CRC, nonsteroidal anti-inflammatory drugs and/or aspirin use, folate intake, calcium supplementation, amount of fruit or vegetable intake, red meat consumption level, age at blood sample draw, and age of mother and age of father at time of birth for the cases and controls.

Study population. The study population ($n=2,810$) consisted of 598 cases and 2,212 controls. All study participants were consented under institutional review board-approved protocols.

Data for this study were selected from four existing sources: (1) the Colon Cancer Family Registry (Colon CFR),[22] a multinational consortium with data on epidemiological risk factors, clinical data, and biospecimens on families at risk for CRC population-based or relative controls; (2) the Mayo Clinic Biobank for Gastrointestinal Health Research (BGHR), an ongoing institutional review board-approved collection of biospecimens from participants with normal colonoscopic examinations, colon polyps or CRC seen at Mayo Clinic Rochester from the year 2000 to the present; (3) the Mayo Biobank, a collection of health information and biospecimens from Mayo Clinic patients ages 18 and older; and (4) Pancreatic Cancer Registry controls from the Mayo Clinic SPORE in Pancreatic Cancer (MCSPC). CRC cases were eligible for this study if they had a diagnosis of CRC; intact expression of MMR proteins (hMLH1, hMSH2, or hMSH6); microsatellite markers (BAT26, D17S250, D5S346, ACTC, BAT40, BAT 25, BAT 34C4, D10S197, MYCL, and D18S55); no known history of inflammatory bowel disease, familial adenomatous polyposis, Lynch syndrome, or other hereditary CRC conditions; did not have biallelic germline MYH mutations;[23] and had a DNA sample available for analysis that had been extracted by Phenol/Chloroform or PureGene (Gentra AutoPure, Qiagen, Valencia, CA, USA). Cases were selected from the Colon CFR ($n=355$; 47% males; mean age at blood draw = 49.48) and the Mayo BGHR ($n=243$; 58% males; mean age at blood draw = 63.2). Cases ranged in age from 19 to 69 years, with an average age of 48. As some studies have reported that telomere length may be modified post-administration of chemo- and radiotherapy,[24,25] chemoradiotherapy naive specimens only were analyzed.

A control group of unrelated individuals (i.e., non-blood relatives and/or spousal participants) was selected from the Colon CFR ($n=427$), the Mayo Biobank ($n=534$), the Mayo BGHR ($n=288$), and the MCSPC registry ($n=963$) if they were healthy at the time of enrollment, did not have a history of cancer (except non-melanoma skin cancer), or a family history of Lynch syndrome or familial adenomatous polyposis, did not have inflammatory bowel disease, and had a DNA sample available for telomere analysis that had been extracted by Phenol/Chloroform or PureGene. Controls were frequency matched to the cases on age, gender, and geographic location. Colon CFR controls included non-blood relatives and/or spousal participants. Mayo Biobank controls were subjects from Olmsted County, MN, found to be healthy during a medical examination in the Department of Medicine divisions of Community Internal Medicine, Family Medicine or General Internal Medicine. Controls from the BGHR were consented participants with normal colonoscopies and no prior polyp or cancer history. Controls originating from the MCSPC registry included Caucasian individuals deemed healthy at primary-care routine check-up visits. Controls ranged in age from 21 to 91 years, and averaged 57.18 years of age.

Clinical and epidemiological data on cases and controls. Demographic, environmental, lifestyle, and clinical variables were collected via a self-administered questionnaire or abstracted from the medical record. These variables included sex, race (white vs. other), age at the time of the blood draw, body mass index, alcohol use (ever vs. never), tobacco use (ever vs. never), hormone replacement therapy use (among females only), diagnosis of diabetes, family history of CRC, use of nonsteroidal anti-inflammatory drugs and aspirin, current folate supplementation, current calcium supplementation, diet (fruit, vegetable, and red meat consumption; servings per day), and age of parents at birth.

Laboratory methods. Case T/S ratios were determined from PBL DNA samples. For cases, blood samples were drawn at CRC diagnosis or within a 2-year range of CRC diagnosis. Control blood samples were drawn at the time participant well-visit or enrollment into one of the control registries.

DNA extraction from PBLs for cases and control. DNA extraction was performed on all cases and controls using PureGene or Phenol/Chloroform chemistries[17] and quantified by ultraviolet absorbance. Although the Colon CFR registry contains some subjects with DNA extracted by QIAamp, QIAamp-extracted DNA may have truncated telomeres as an artifact of the extraction procedure itself rather than as a representation of actual biological telomere length,[17] and these subjects were, therefore, excluded from this study. DNA quality was assessed by 260/280 optical density ratio.

Assessment of telomere length by quantitative PCR. DNA was quantitated with PICO green and the same amount of DNA was used for each PCR reaction. Telomere length in PBL was measured using the PCR method described by Cawthon.[26] This PCR-based assay uses a set of primers to the telomeric hexamer repeats to amplify telomeric DNA. The average telomere length for each sample was measured by comparing the intensity of the sample's telomere signal (T) to the signal from a single-copy gene (S) to compute the T/S ratio. The T and S values were taken from the median of three repeats for each sample.

Two master mixes of PCR reagents were prepared, one with the T primer pair, the other with the S primer pair. Fifteen microliters of the T master mix were added to each sample well, control well, and standard curve well of the first plate and

15 μl of the S master mix were added to each sample well, control well, and standard curve well of the second plate. For each sample assayed, three identical 5 μl aliquots of the DNA sample (15 ng per aliquot) were added to plate 1 and another three aliquots were added to the same well position in plate 2. For each standard curve, one reference DNA sample was serially diluted in TE by 1:2 fold per dilution to produce six concentrations of DNA ranging from 0.78 to 25 ng/μl. Five microliters of each concentration was distributed to the standard curve wells on each plate. The plates were then sealed with a transparent adhesive cover, centrifuged briefly at 800 *g* and transported on ice to the ABI 7900HT instrument (Applied Biosystems, Life Technologies, Grand Island, NY, USA) for analysis.

The T and S PCRs were prepared identically with the exception of the oligonucleotide primers. The final concentrations of the reagents in the PCR were 20 mM Tris-HCl, 0.2 mM each dNTP's, 2.0 mM $MgCl^2$, 1% dimethylsulphoxide, 150 nM ROX dye, 0.2 X Sybr Green I (Molecular Probes, Life Technologies, Grand Island, NY, USA), 5 mM dithiothreitol, 1.25 U AmpliTaq Gold DNA polymerase (Applied Biosystems). The final telomere primer concentrations was tel 1b, 600 nM; tel 2b 900 nm.

The single-copy (S) control gene (B2-globin on chromosome 11) was B2-globin forward primer (HBG1) 300 nm; B2-globin reverse primer (HBG2) 700 nm. The primer sequences (written 5′–3′) were as follows:

tel 1b: CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT;
tel 2b: GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT;
HBG1: GCTTCTGACACAACTGTGTTCACTAGC;
HBG2: CACCAACTTCATCCACGTTCACC.

All PCRs were performed on the ABI Fast Real-Time 7900HT (Applied Biosystems). The thermal cycle conditions for both primers pairs began with a 95 °C incubation for 10 min to activate the AmpliTaq Gold DNA polymerase. For telomere PCR, this was followed by 40 cycles of 95 °C for 15 s, 54 °C for 2 min. For the HBG PCR, this was followed by 40 cycles of 95 °C for 15 s and 58 °C for 60 s. The data were then analyzed with the ABI SDS software (Applied Biosystems) to generate the standard curve for each plate. Intra-assay variability was 3.5%, similar to other studies. Intra-assay variability was determined by using stock DNA from one individual. For intra-assay variability, 25 telomere length measurements were compared from the one pair of T and S PCR plates. To determine inter-assay variability, 25 telomere length measurements were determined from one pair of T and S PCR plates and compared with a separate set of 25 measurements from another pair of T and S PCR plates. The T and S values were taken from the median of three repeats for each sample. For those 25 repeated samples, the coefficient of variation was 16%. However, for all the samples tested, those with a coefficient of variation of $>10\%$ were re-assayed in triplicate. Outliers on T/S ratio (mean ± 2*s.d. of log T/S ratio) were dropped from the analysis, resulting in a final sample of 580 cases and 2,081 controls.

Statistical methods. The purpose of the analysis was to (1) examine the relationship between telomere length and CRC and (2) assess whether young-onset CRC patients exhibit telomere lengths consistent with the shorter telomeres of a more aged population group. Multiple imputations (10 times with a predictive mean matching method using the aregImpute function in R R Foundation for Statistical Computing, Vienna, Austria) were performed for missing data of covariates, and their descriptive statistics were computed based on the average of 10 imputations. For univariate association or comparisons of the variables' distributions between cases and controls, Fisher's exact test or χ^2 test was used for categorical variables, and a two-sample *t*-test or Wilcoxon rank sum test was used for continuous variables. The association between T/S ratio and CRC was examined with and without multiple covariates (fruit, vegetable, and red meat consumption, alcohol, tobacco, and hormone replacement therapy (among females only) use, diabetes status, family history of CRC, nonsteroidal anti-inflammatory drug use, aspirin use, folate and calcium supplementation, age at blood draw, body mass index, age of father and mother at birth, and DNA extraction method) including the data source as a random effect in a generalized additive model (mgcv R package, R Foundation for Statistical Computing, Vienna, Austria) using a binomial distribution. Results were quantified in terms of imputation-adjusted ORs based on Rubin's rule along with 95% confidence intervals (CIs). These analyses were also conducted among those ≤ 50 years of age (consistent with the definition of young-onset CRC used in other studies[27,28]) and >50 years of age in order to examine whether the association differed for young-onset CRC (cases whose age at blood draw was ± 2 years from the cancer diagnosis were excluded). The distribution of T/S ratio in our study was skewed with a wide range of 0.01–12.33. For the analysis with a better model fit along with the reduction of possible measurement errors of T/S ratio, the ratio T/S was transformed to the natural log to generate an approximately normal distribution and outliers were excluded from the analysis. After excluding outliers, the T/S ratio ranged from 0.09 to 3.11, and all analyses were conducted using log T/S ratio.

RESULTS

Table 1 depicts demographic and lifestyle characteristics of cases and controls. Cases were younger than controls at the time of blood sample collection (48.26 vs. 56.80 years; $P<0.001$). Cases were also slightly less likely to have ever consumed alcohol (73% vs. 79%, $P=0.005$), more likely to have family history of non-hereditary syndrome-related CRC (29% vs. 12%; $P<0.001$), and less likely to report aspirin use (19% vs. 36%, $P<0.001$), folate supplementation (3% vs. 5%, $P=0.007$), or calcium supplementation (9% vs. 28%, $P<0.001$). Female cases reported less hormone replacement therapy use (32% vs. 44%; $P<0.001$). Cases also consumed less fruit per day than controls (56% 0–1 serving per day vs. 37%; $P<0.001$) as well as fewer daily portions of vegetables (44% 0–1 servings per day vs. 25%; $P<0.001$). PBL telomere lengths in CRC cases were longer than in controls (T/S ratio 0.85 vs. 0.68; $P<0.001$). Telomere shortened with age in both cases and controls; however, the association was not strong (Spearman correlation coefficient (95%

Table 1 Comparison of demographic characteristics and colorectal risk factors between CRC cases and controls

	Controls ($n=2,081$)	Cases ($n=580$)	*P* value for difference
Sex			0.378
Male	52%	50%	
Female	48%	50%	
Race			0.045
White	99%	98%	
Others	1%	2%	
Age at blood draw (mean ± s.d.)	56.80 ± 12.06	48.26 ± 8.32	<0.001
BMI (mean ± s.d.)	27.99 ± 5.70	27.60 ± 6.06	0.178
Ever consumed alcohol	79%	73%	0.005
Ever used tobacco	49%	52%	0.193
Hormone replacement therapy (females only)	44%	32%	<0.001
Diagnosed with diabetes	7%	7%	0.767
Family history of CRC	12%	29%	<0.001
Any NSAID use	32%	33%	0.884
Aspirin use	36%	19%	<0.001
Current folate Supplementation	5%	3%	0.007
Current calcium supplementation	28%	9%	<0.001
Fruit consumption (servings per day)			<0.001
0–1	37%	56%	
2	28%	25%	
3	19%	12%	
4	9%	5%	
5 or more	7%	2%	
Vegetable consumption (servings per day)			<0.001
0–1	25%	44%	
2	30%	31%	
3	23%	12%	
4	12%	7%	
5 or more	10%	6%	
Red meat consumptions (servings per day) (mean ± s.d.)	0.83 ± 0.70	0.80 ± 0.76	0.523
Age of mother at birth (mean ± s.d.)	27.88 ± 5.95	27.45 ± 5.80	0.196
Age of father at birth (mean ± s.d.)	31.29 ± 6.87	30.83 ± 6.75	0.273
T/S ratio (mean ± s.d.)	0.68 ± 0.52	0.85 ± 0.59	<0.001

BMI, body mass index; CRC, colorectal cancer; NSAID, nonsteroidal anti-inflammatory drug.

CI) = −0.09 (−0.17, −0.01), $P=0.031$ for cases and −0.2 (−0.24, −0.15), $P<0.001$ for controls).

In the multivariate analysis, telomere length was significantly associated with CRC risk in a non-linear manner (Figure 1; $P<0.001$). Both shorter (1st–5th percentile) and longer (70th–99th percentile) telomeres were associated with greater CRC risk, but this association was stronger and statistically significant for those with telomeres in the 80th percentile of length and longer. Those in the 80th percentile of telomere length were at 71% greater risk of CRC compared with those with the median telomere length among controls (Table 2; OR: 1.71, 95% CI: 1.23–2.37). The OR for CRC risk was greatest among those at the 90th percentile of telomere length compared with the 50th percentile (OR: 2.52, 95% CI: 1.78–3.56).

Telomere length was associated with varied risk for CRC in an age-dependent manner (Figure 2; Table 3). Individuals 50 years of age or younger who had longer telomere lengths were more likely to have CRC (Figure 2a). Those with telomere lengths in the 80th percentile were at >50% greater risk for CRC than those with telomeres in the 50th percentile (Table 3; OR: 1.56, 95% CI: 1.01–2.41). Risk increased to more than three times greater for individuals with extremely long telomeres in the 95th and 99th percentiles. For those with telomeres shorter than the 80th percentile, telomere length was not statistically significantly associated with CRC risk.

In contrast, individuals older than 50 had an increased risk for CRC when their PBL telomeres were the shortest length (Figure 2b). For those older patients telomeres in the 5th percentile, the risk for CRC was >3.5 times higher than for those with median telomere length (Table 3 OR: 3.53, 95% CI: 1.35–9.25), whereas those in the 10th percentile had almost double the risk (OR: 1.91, 95% CI: 1.07–3.41). The risk of CRC was not statistically significantly different for those with telomeres in the 20th percentile or higher, compared with the median telomere length.

DISCUSSION

We found that both longer and shorter telomere length measured in PBL DNA were associated with an increased risk for CRC. However, the association of longer telomeres with CRC risk was limited to those under the age of 50 years old, while extremely short telomeres were associated with greater CRC risk in those older than 50 years of age.

Previous research has established inconsistent associations between telomere length and cancer risk. Shorter PBL

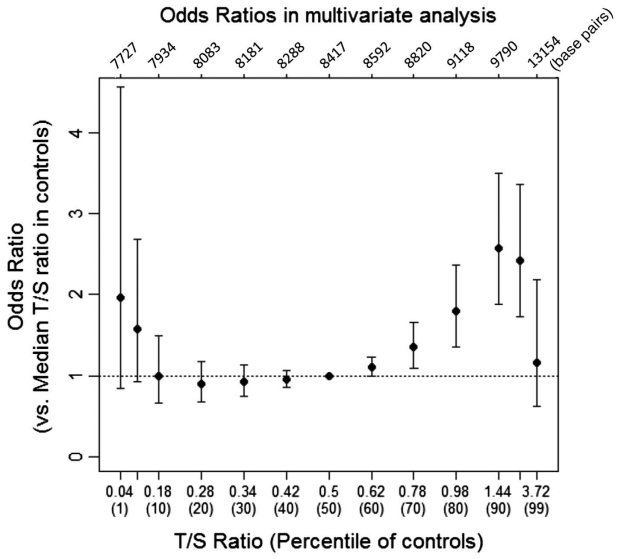

Figure 1 Odds ratios (OR; solid line) and 95% confidence intervals (dotted lines) for the association between peripheral blood leukocyte (PBL) telomere length and colorectal cancer (CRC). OR for CRC for the 1%, 5%, and each decile from 10 to 90, 95 to 99% of T/S ratio, compared with the median of T/S ratio in all controls. Both shorter (1st–5th percentile) and longer (70th–99th percentile) telomeres were associated with greater CRC risk, but this association was stronger and statistically significant for those with telomeres in the 80th–99th percentiles of length. Multivariate OR analysis adjusted for: fruit, vegetable, and red meat consumption, alcohol, tobacco, and hormone replacement therapy (among females only) use, diabetes status, family history of CRC, nonsteroidal anti-inflammatory drug (NSAID) use, aspirin use, folate and calcium supplementation, age at blood draw, body mass index (BMI), age of father and mother at birth, and DNA extraction method.

Table 2 Association between telomere length and CRC (vs. median T/S ratio in controls)

Percentile (in all controls)	T/S ratio	Odds ratio	95% Lower CI	95% Upper CI
1	0.10	1.84	0.81	4.19
5	0.16	1.03	0.63	1.70
10	0.22	0.79	0.52	1.19
20	0.3	0.83	0.59	1.16
30	0.36	0.92	0.70	1.21
40	0.43	1.00	0.86	1.16
50	0.52	1.00	1.00	1.00
60	0.63	0.99	0.83	1.18
70	0.78	1.16	0.87	1.54
80	0.97	1.71	1.23	2.37
90	1.34	2.52	1.78	3.56
95	1.87	2.18	1.47	3.22
99	2.57	2.15	1.26	3.69

BMI, body mass index; CI, confidence interval; CRC, colorectal cancer.
Multivariable odds ratios adjusted for: fruit, vegetable, and red meat consumption, alcohol, tobacco, and hormone replacement therapy use, diabetes status, family history of CRC, nonsteroidal anti-inflammatory drug use, aspirin use, folate and calcium supplementation, age at blood draw, BMI, age of father and mother at birth, and DNA extraction method.

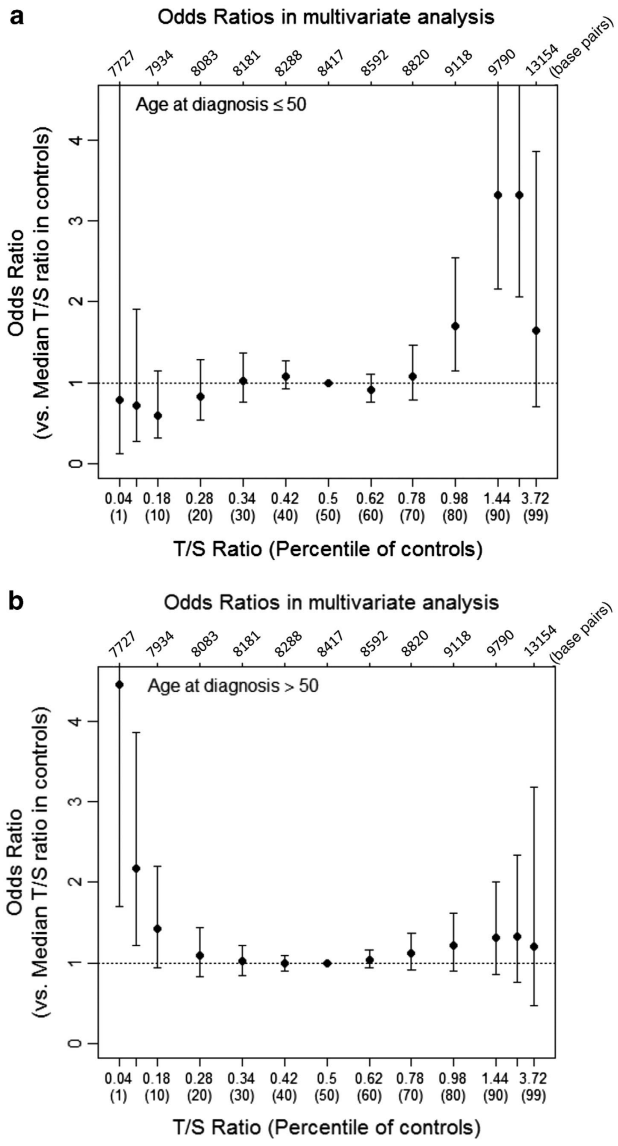

Figure 2 (**a**) Association between telomere length and risk of colorectal cancer (CRC) among those with age of diagnosis ≤ 50 ($n = 318$) and age-matched controls ($n = 651$). (**b**) Association between telomere length and risk of CRC among those with age of diagnosis > 50 ($n = 94$) and age-matched controls ($n = 1{,}430$).

telomeres have been associated with some cancers, including renal cell, lung, pancreas, head and neck, and urinary bladder cancer,[7–12] whereas *longer* telomere length has been associated with an increased risk for breast cancer,[29,30] Non-Hodgkin's lymphoma,[31] and melanoma.[32] Studies of telomere dynamics in CRC, specifically, have reported inconsistent results, with some studies reporting greater CRC risk associated with shorter[13] or longer[14] telomeres, or reporting null findings.[15,16] Our results are consistent with findings from the Shanghai Women's Health Study in which both long and short telomeres were associated with an increased CRC risk.[14] This study extends these findings to show that extremes of telomere length variation are associated with the patient's age at the time of cancer onset and the association between telomere length and CRC may therefore differ for young vs. older onset CRC.

Our findings suggest that older individuals (>50 years of age) with accelerated biological aging characterized by

Table 3 Association between telomere length and CRC (vs. median T/S ratio in controls), stratified by age at diagnosis

		Age at diagnosis					
		<50			>50		
Percentile (in all controls)	T/S ratio	Odds ratio	95% Lower CI	95% Upper CI	Odds ratio	95% Lower CI	95% Upper CI
1	0.1	0.34	0.00	54.10	**12.29**	1.06	142.82
5	0.16	0.50	0.08	3.07	**3.53**	1.35	9.25
10	0.22	0.62	0.28	1.35	**1.91**	1.07	3.41
20	0.30	0.77	0.49	1.22	1.25	0.86	1.83
30	0.36	0.87	0.62	1.22	1.10	0.85	1.43
40	0.43	0.95	0.79	1.15	1.03	0.89	1.18
50	0.52	1.00	1.00	1.00	1.00	1.00	1.00
60	0.63	1.02	0.83	1.26	1.01	0.86	1.18
70	0.78	1.16	0.81	1.65	1.05	0.78	1.40
80	0.97	**1.56**	1.01	2.41	1.11	0.74	1.67
90	1.34	**2.59**	1.57	4.26	1.23	0.69	2.22
95	1.87	**3.50**	2.02	6.07	1.36	0.61	3.05
99	2.57	**5.67**	1.35	23.81	1.70	0.36	8.07

BMI, body mass index; CI, confidence interval; CRC, colorectal cancer.
Multivariable odds ratios adjusted for: fruit, vegetable, and red meat consumption, alcohol, tobacco, and hormone replacement therapy use, diabetes status, family history of CRC, nonsteroidal anti-inflammatory drug use, aspirin use, folate and calcium supplementation, age at blood draw, BMI, age of father and mother at birth, and DNA extraction method. Significant odds ratios are highlighted with bold face font.

shorter PBL telomeres may be at higher risk for CRC, while among people $\leq$50 years old it appears that CRC may be more likely to develop if PBL telomere length is longer. Thus, the hypothesis that shorter telomeres exclusively lead to CRC may be true only in an older-aged population. Older patients with shorter telomeres may be subject to increased risk for various types of cancer due to telomere crisis and subsequent chromosomal instability, enabling cells to advance toward malignancy.[33,34] In younger individuals, on the other hand, extremely long telomeres may be indicative of dysregulation in telomere maintenance processes, leading to systemic increases in telomerase activation, or alternative telomere lengthening that may increase cancer risk. This potential pathway is supported by a recent study by Jones *et al.*[35] showing that individuals who carry a *TERC* SNP associated with longer constitutional telomeres had a higher risk for CRC.

Our results suggest that longer PBL telomeres may predispose younger individuals to CRC. Alternatively, telomere lengthening in younger individuals may be a marker for physiological changes associated with early CRC (reverse causality) in which cancer-related genetic events controlling regulation of telomere extension mechanisms may be operative in some CRC cases that occur in individuals younger than 50 years old. Nevertheless, this finding invites further investigation to determine genetic and mechanistic features that explain the increased hazard for those with longer PBL telomeres among younger individuals. Evaluation of a large, age-defined population of CRC patients for shorter and extremely longer telomeres may determine the relationship of short or long PBL telomeres and CRC and prove that telomere length assessment serves as an adequate biomarker flagging subgroups of patients with higher risk for CRC.

Telomere dynamics are driven by genetic and environmental factors,[13] both of which are key to the development of cancer. That short and extremely long PBL telomeres in both our study group and the Shanghai Women's Study were associated with an increased risk for CRC indicates that there may be a range of healthy telomere length and that the risk for diseases such as cancer may increase once telomere length falls outside the upper or lower limit of this range. Defining the relationship between PBL telomere lengths with tumor-specific telomere length may provide additional evidence necessary to determine if PBL telomere DNA length acts as a marker for the telomere dynamics ongoing at the level of the tumor. Indeed, studies of the disease- and organ-specific association of PBL telomere length compared with healthy somatic tissue and cancer tissue have been limited. In one study of a small sample of CRC cases, telomere length from CRC tumor DNA was found to be shorter than that of corresponding normal colon epithelial DNA, which was shorter than PBL DNA.[36] However, these relationships have not been studied in those CRC patients with extremely long PBL telomeres, and it remains unknown if changes in PBL telomere length correspond with changes in tissue-specific telomere length. ''Telomere typing'' solely on the basis of PBL telomere length may prove to be an adequate biomarker of risk for CRC prognostication of overall outcomes and/or responsiveness to chemotherapeutic regimens. Further investigation into the factors that regulate or abrogate normal telomere maintenance, such as aging and germline or epigenetic modification of telomere maintenance genes, may strengthen the role of telomere typing by including telomere length in combination with the factors influencing telomere maintenance pathways.

Our study benefits from several strengths, including a large multi-national sample and a rich data set of epidemiological and clinical covariates for analysis. However, our results should be interpreted in the context of some potential limitations. For example, the case–control design is susceptible to some forms of information bias and selection bias. Although our biological marker, PBL telomere length, is not susceptible to biased recall or reporting, a systematic and differential error in the measurement of telomere length could introduce a bias. While conducting this study, we identified

differences in the distribution of telomere lengths for different DNA extraction methods;[17] specifically, telomere lengths measured in DNA extracted using Qiagen kits were shorter on average and the distribution was truncated to lower values. A difference in the frequency of using Qiagen kits for cases and controls could introduce a bias. To minimize error, we excluded samples that had been extracted using the Qiagen kits. Another concern for bias may arise if differences exist in the distributions of telomere lengths arising from the process of selecting cases and controls (i.e., selection bias). Our study design included cases and controls sampled from multiple locations, and included controls who were either non-blood relatives (Colon CFR) or healthy participants in a bio-repository (Mayo BGHR and Mayo Biobank). The heterogeneity in the control samples would tend to minimize the chances for systematic differences in telomere length through selection and minimize concern for biased selection.[37]

A limitation of our study is that it assumes that telomere length in PBLs is a proxy for telomere length in colonic epithelium. Although we did not measure the correlation between leukocyte telomere length and telomere length in colon tissue directly, a separate report from 53 healthy individuals found that telomere length in both leukocytes and colonic epithelium declined with increasing age.[38] Similarly, high correlations have been reported between PBL telomere length and other epithelial tissues including tongue ($r = 0.84$) and skin ($r = 0.79$).[39,40] Moreover, although biological studies clearly point to telomere erosion leading to malignant transformation, we cannot exclude the possibility of reverse causation; namely, that some aspect of CRC caused changes in PBL telomere length. However, this is unlikely given the observation that rates of change in PBL telomere length at 10-year intervals were not different in individuals who developed cancer during a 10-year interval compared with those who did not.[41] Finally, in our analyses stratified by age, only 138 cases in our sample were diagnosed over the age of 50 and the CIs for this finding are imprecise because of the small sample size. Therefore, future work in a larger sample should confirm the age-related trends reported in this study. Our inter-assay variability when measured in a small subset of our cases and controls was 16%, which also impacts the precision of these results. However, in the overall sample studied, we aimed to minimize coefficient of variation by repeating all samples in which the coefficient of variation of the T/S ratio in the triplicated telomere length measurement was $>10\%$.

In conclusion, we observed an association between both longer and shorter telomeres in PBL DNA and increased risk for CRC. The nature of the associations was different depending on the age at onset of CRC. Future studies of CRC in relation to telomere length in PBL should investigate interaction by age of onset and should consider non-linear associations between telomere length and risk.

CONFLICT OF INTEREST

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Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- Telomeres are nucleoprotein structures that cap the end of chromosomes and shorten with sequential cell divisions in normal aging leading to regulated cell senescence and apoptosis.
- In abnormal cells, continued cell division after telomere depletion can lead to end-to-end fusion of chromosomes, chromosomal instability and oncogenesis.
- Telomere dynamics in colorectal cancer (CRC) indicate inconsistent results, with both long and short telomeres associated with an increased CRC risk.
- The relationship between telomere length in peripheral blood leukocytes (PBL) and CRC risk has not been systematically evaluated in relation to the age of cancer onset.

WHAT IS NEW HERE

- Telomere length in PBL is associated with risk for colorectal cancer; this association varied in an age-related manner.
- Younger individuals ($\leq$ 50 years of age) with longer telomeres had a two to six times higher risk of CRC, while older individuals ($>$50 years of age) with shortened telomeres had two to twelve times the risk for CRC.
- PBL telomere length varies according to the age of cancer onset and CRC is likely associated with at least two different mechanisms of telomere dynamics.

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