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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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**Subject Category:** Colon/Small Bowel

## INTRODUCTION

Telomeres cap linear chromosomes to maintain stability<sup>1</sup> and shorten with successive rounds of DNA replication during sequential cell division.<sup>2</sup> 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<sup>3</sup> associated with genetic instability<sup>4</sup> and oncogenesis.<sup>5,6</sup>

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,<sup>7–9</sup> renal,<sup>10</sup> lung,<sup>11</sup> esophageal,<sup>12</sup> and colorectal<sup>13–16</sup> cancers.<sup>5,6,17</sup> 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).<sup>7</sup> Studies of telomere dynamics in colorectal cancer (CRC), specifically, have reported inconsistent results,<sup>13–16</sup> with one recent study reporting that both long and short telomeres were associated with an increased CRC risk.<sup>14</sup> 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).<sup>18–21</sup>

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),<sup>22</sup> 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;<sup>23</sup> 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,<sup>24,25</sup> 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<sup>17</sup> 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,<sup>17</sup> 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.<sup>26</sup> 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



**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

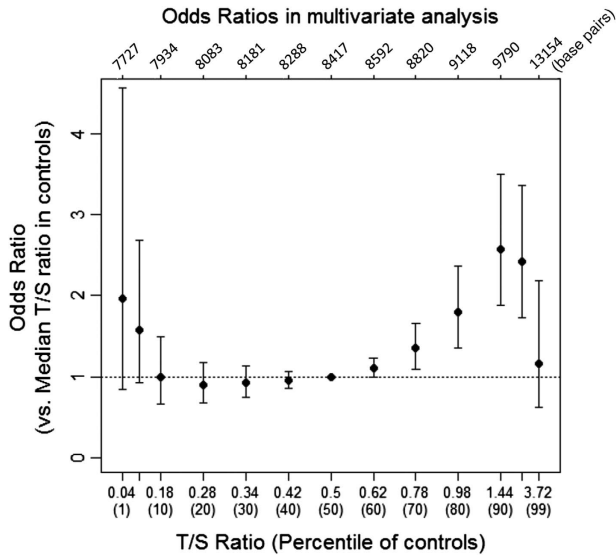
percentiles. For those with telomeres shorter than the 80th percentile, telomere length was not statistically significantly associated with CRC risk.

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



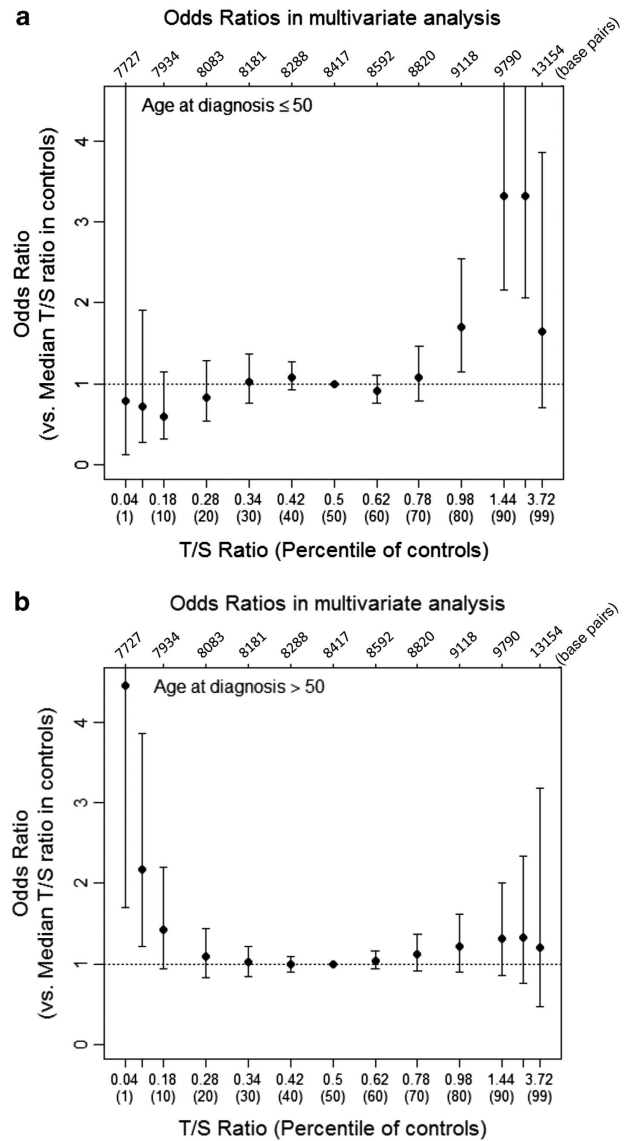
**Figure 1** Odds ratios (OR; solid line) and 95% confidence intervals (dotted lines) for the association between peripheral blood leukocyte (PBL) telomere length and colorectal cancer (CRC). OR for CRC for the 1%, 5%, and each decile from 10 to 90, 95 to 99% of T/S ratio, compared with the median of 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.

telomeres have been associated with some cancers, including renal cell, lung, pancreas, head and neck, and urinary bladder cancer,<sup>7–12</sup> whereas longer telomere length has been associated with an increased risk for breast cancer,<sup>29,30</sup> Non-



**Figure 2** (a) Association between telomere length and risk of colorectal cancer (CRC) among those with age of diagnosis  $\leq 50$  ( $n = 318$ ) 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 ( $n = 1,430$ ).

Hodgkin's lymphoma,<sup>31</sup> and melanoma.<sup>32</sup> Studies of telomere dynamics in CRC, specifically, have reported inconsistent results, with some studies reporting greater CRC risk associated with shorter<sup>13</sup> or longer<sup>14</sup> telomeres, or reporting null findings.<sup>15,16</sup> 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.<sup>14</sup> 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

Percentile (in all controls)	T/S ratio	Age at diagnosis					
		< 50			> 50		
		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	<b>12.29</b>	1.06	142.82
5	0.16	0.50	0.08	3.07	<b>3.53</b>	1.35	9.25
10	0.22	0.62	0.28	1.35	<b>1.91</b>	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	<b>1.56</b>	1.01	2.41	1.11	0.74	1.67
90	1.34	<b>2.59</b>	1.57	4.26	1.23	0.69	2.22
95	1.87	<b>3.50</b>	2.02	6.07	1.36	0.61	3.05
99	2.57	<b>5.67</b>	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.<sup>33,34</sup> 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.*<sup>35</sup> 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,<sup>13</sup> 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.<sup>36</sup> 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;<sup>17</sup> 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 biorepository (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.<sup>37</sup>

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.<sup>38</sup> Similarly, high correlations have been reported between PBL telomere length and other epithelial tissues including tongue ( $r=0.84$ ) and skin ( $r=0.79$ ).<sup>39,40</sup> 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.<sup>41</sup> 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

**Guarantor of the article:** Lisa A. Boardman, MD.

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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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- Blackburn EH. Structure and function of telomeres. *Nature* 1991; **350**: 569–573.
- Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. *Nature* 1990; **345**: 458–460.
- Cawthon R, Smith K, O'Brien E *et al.* Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* 2003; **361**: 393–395.
- Rampazzo E, Bertorelle R, Serra L *et al.* Relationship between telomere shortening, genetic instability, and site of tumour origin in colorectal cancers. *Br J Cancer* 2010; **102**: 1300–1305.
- Wentzensen IM, Mirabello L, Pfeiffer RM *et al.* The association of telomere length and cancer: a meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2011; **20**: 1238–1250.
- Ma H, Zhou Z, Wei S *et al.* Shortened telomere length is associated with increased risk of cancer: a meta-analysis. *PLoS One* 2011; **6**: e20466.
- Wu X, Amos CI, Zhu Y *et al.* Telomere dysfunction: a potential cancer predisposition factor. *J Natl Cancer Inst* 2003; **95**: 1211–1218.
- McGrath M, Wong JY, Michaud D *et al.* Telomere length, cigarette smoking, and bladder cancer risk in men and women. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 815–819.
- Broberg K, Bjork J, Paulsson K *et al.* Constitutional short telomeres are strong genetic susceptibility markers for bladder cancer. *Carcinogenesis* 2005; **26**: 1263–1271.
- Shao L, Wood CG, Zhang D *et al.* Telomere dysfunction in peripheral lymphocytes as a potential predisposition factor for renal cancer. *J Urol* 2007; **178**: 1492–1496.
- Jang JS, Choi YY, Lee WK *et al.* Telomere length and the risk of lung cancer. *Cancer Sci* 2008; **99**: 1385–1389.
- Risques RA, Vaughan TL, Li X *et al.* Leukocyte telomere length predicts cancer risk in Barrett's esophagus. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 2649–2655.
- Pooley KA, Sandhu MS, Tyrer J *et al.* Telomere length in prospective and retrospective cancer case-control studies. *Cancer Res* 2010; **70**: 3170–3176.
- Cui Y, Cai Q, Qu S *et al.* Association of leukocyte telomere length with colorectal cancer risk: nested case-control findings from the Shanghai Women's Health Study. *Cancer Epidemiol Biomarkers Prev* 2012; **21**: 1807–1813.
- Zee RYL, Castonguay AJ, Barton NS *et al.* Mean telomere length and risk of incident colorectal carcinoma: a prospective, nested case-control approach. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 2280–2282.
- Lee IM, Lin J, Castonguay AJ *et al.* Mean leukocyte telomere length and risk of incident colorectal carcinoma in women: a prospective, nested case-control study. *Clin Chem Lab Med* 2010; **48**: 259–262.
- Cunningham J, Johnson R, Litzelman K *et al.* Telomere length varies by DNA extraction method: implications for epidemiologic research. *Cancer Epidemiol Biomarkers Prev* 2013; **11**: 2047–2054.
- Adloff M, Arnaud J-P, Schloegel M *et al.* Colorectal cancer in patients under 40 years of age. *Dis Colon Rectum* 1986; **29**: 322–325.
- Gryfe R, Kim H, Hsieh ETK *et al.* Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. *N Engl J Med* 2000; **342**: 69–77.
- Moore P, Dilawari R, Fidler W. Adenocarcinoma of the colon and rectum in patients less than 40 years of age. *Am Surg* 1994; **50**: 10–15.
- Zorluoglu A, Yilmazlar T, Ozguc H *et al.* Colorectal cancers under 45 years of age. *Hepato-Gastroenterology* 2004; **51**: 118–120.
- Newcomb PA, Baron J, Cotterchio M *et al.* Colon Cancer Family Registry: an international resource for studies of the genetic epidemiology of colon cancer. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 2331–2343.
- Wang L, Baudhuin LM, Boardman LA *et al.* MYH mutations in patients with attenuated and classic polyposis and with young-onset colorectal cancer without polyps. *Gastroenterology* 2004; **127**: 9–16.
- Schroder CP, Wisman GBA, Sd Jong *et al.* Telomere length in breast cancer patients before and after chemotherapy with or without stem cell transplantation. *Br J Cancer* 2001; **84**: 1348–1353.
- Smogorzewska A, van Steensel B, Bianchi A *et al.* Control of human telomere length by TRF1 and TRF2. *Mol Cell Biol* 2000; **20**: 1659–1668.
- Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res* 2002; **30**: e47.
- Dozoi EJ, Boardman LA, Suwanthanma W *et al.* Young-onset colorectal cancer in patients with no known genetic predisposition: can we increase early recognition and improve outcome? *Medicine (Baltimore)* 2008; **87**: 259–263.
- Limburg PJ, Harnsen WS, Chen HH *et al.* Prevalence of alterations in DNA mismatch repair genes in patients with young-onset colorectal cancer. *Clin Gastroenterol Hepatol* 2011; **9**: 497–502.
- Gramatges MM, Telli ML, Balise R *et al.* Longer relative telomere length in blood from women with sporadic and familial breast cancer compared with healthy controls. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 605–613.
- Svenson U, Nordfjall K, Stegmayr B *et al.* Breast cancer survival is associated with telomere length in peripheral blood cells. *Cancer Res* 2008; **68**: 3618–3623.
- Lan Q, Cawthon R, Shen M *et al.* A prospective study of telomere length measured by monochrome multiplex quantitative PCR and risk of non-Hodgkin lymphoma. *Clin Cancer Res* 2009; **15**: 7429–7433.
- Nan H, Qureshi AA, Prescott J *et al.* Genetic variants in telomere-maintaining genes and skin cancer risk. *Hum Genet* 2011; **129**: 247–253.
- Artandi SE, DePinho RA. Telomeres and telomerase in cancer. *Carcinogenesis* 2010; **31**: 9.
- DePinho R, Polyak K. Cancer chromosomes in crisis. *Nat Genet* 2004; **36**: 932.
- Jones AM, Beggs AD, Carvajal-Carmona L *et al.* TERC polymorphisms are associated both with susceptibility to colorectal cancer and with longer telomeres. *Gut* 2012; **61**: 248–254.
- Hastie ND, Dempster M, Dunlop MG *et al.* Telomere reduction in human colorectal carcinoma and with ageing. *Nature* 1990; **346**: 866–868.
- Wacholder S, Chatterjee N, Hartge P. Joint effect of genes and environment distorted by selection biases: implications for hospital-based case-control studies. *Cancer Epidemiol Biomarkers Prev* 2002; **11**: 885–889.
- Risques RA, Lai LA, Brentnall TA *et al.* Ulcerative colitis is a disease of accelerated colon aging: evidence from telomere attrition and DNA damage. *Gastroenterology* 2008; **135**: 410–418.
- Friedrich U, Griese E, Schwab M *et al.* Telomere length in different tissues of elderly patients. *Mech Ageing Dev* 2000; **119**: 89–99.
- Nakamura K, Izumiyama-Shimomura N, Sawabe M *et al.* Comparative analysis of telomere lengths and erosion with age in human epidermis and lingual epithelium. *J Invest Dermatol* 2002; **119**: 1014–1019.
- Nordfjall K, Svenson U, Norrback K-F *et al.* The individual blood cell telomere attrition rate is telomere length dependent. *PLoS Genet* 2009; **5**: e1000375.



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