Telomere length and cancer mortality in American Indians: the Strong Heart Study

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Abstract The objective of this study was to investigate whether leukocyte telomere length (LTL) predicts the risk for cancer mortality among American Indians participating in the Strong Heart Study (1989–1991). Participants (aged 45–74 years) were followed annually until December 2015 to collect information on morbidity/mortality. LTL was measured by qPCR using genomic DNA isolated from peripheral blood. The association between LTL and risk for cancer mortality was examined using a multivariable Cox proportional hazard model, adjusting for age, gender, education, study site, smoking, alcohol use, physical activity, systolic blood pressure, fasting blood glucose, obesity, and low- and high-density lipoprotein. Of 1945 participants (mean age 56.10 ± 8.17 at baseline, 57% women) followed for an average 20.5 years, 220 died of cancer. Results showed that longer LTL at baseline significantly predicts an increased risk of cancer death among females (HR 1.57, 95% CI 1.08–2.30), but not males (HR 0.74,

95% CI 0.49–1.12) (p for interaction 0.009). Specifically, compared with the women with the longest LTL (fourth quartile), those in the third, second, and first quartiles showed 53%, 41%, and 44% reduced risk for cancer death, respectively. The findings highlight the importance of sex-specific analysis in future telomere research.

Keywords Telomere length · Biological aging · Cancer mortality · American Indians · Strong Heart Study

Introduction

Cancer is the second leading cause of death in the United States (US) (Heron 2016). While recent trend shows a decrease in the nationwide cancer mortality rates (Jemal et al. 2017), cancer mortality among American Indians has remained stagnant or even increased

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over the past decades (Jemal et al. 2017; CDC 2016). Indeed, cancer has overtaken cardiovascular disease as the leading cause of death for American Indian women (Espey et al. 2014). Identification of novel biomarkers could prove crucial for early detection of cancer and will likely provide insights into disease mechanisms underlying this disparity.

Identification of early biomarkers for cancer remains challenging despite substantial efforts and significant progresses. Recent evidence suggests that leukocyte telomere length (LTL) may serve as a new biomarker predictive of cancer onset and progression in many populations (De Vivo et al. 2009; Duggan et al. 2014; Willeit et al. 2011; Mons et al. 2017; Kotsopoulos et al. 2014). Telomeres are repetitive DNA sequences and their associated proteins localized at the distal ends of chromosomes (de Lange 2005). Telomeres are critical in maintaining chromosomal stability during mitotic cell proliferation. Telomere length shortens during each round of cell division, and when it reaches a critical length, also known as the “Hayflick limit” (Stewart and Weinberg 2006), cells stop dividing and become senescent (Siderakis and Tarsounas 2007). This phenomenon is closely related to cancer onset and development as tumor cells may bypass replicative senescence even after telomeres reach a critical length (Greider and Blackburn 1996). Indeed, telomere length has been associated with a variety of cancers such as lung cancer, breast cancer, ovarian cancer, and pancreatic cancer (Jeon et al. 2014; Frías et al. 2008; Sanchez-Espiridion et al. 2014; Russo et al. 2014; De Vivo et al. 2009; Duggan et al. 2014; Yang et al. 2017; Campa et al. 2014; Wentzensen et al. 2011; Wu et al. 2003). However, the association between telomere length and cancer is inconsistent, with some studies showing that longer telomere length increases the risk of some types of cancer while others showing the opposite (Campa et al. 2014; Yang et al. 2017; Russo et al. 2014; Kachuri et al. 2016; Willeit et al. 2010; Zhang et al. 2015a). For example, in a case-control study including 1385 cases of lung cancer, longer LTL was found to be protective against squamous cell carcinoma, but it increased the odds of having adenocarcinoma (Sanchez-Espiridion et al. 2014). Another case-control study reported that breast cancer patients were over five times as likely to have longer telomere length compared with controls (Svenson et al. 2008). A prospective study by De Vivo et al. (2009) did not find an association between telomere length and risk for post-menopausal

breast cancer. Apart from these mixed results, existing studies were largely derived from Caucasian populations. However, given the genetic control of telomere metabolism (Donate and Blasco 2011), the relationship between telomere length and cancer risk may vary across different racial/ethnic groups. To date, no study has examined the prospective association between LTL and cancer mortality in American Indians.

The goal of this study is to examine whether baseline LTL predicts the risk for cancer mortality among American Indians using mortality data collected by the Strong Heart Study (SHS).

Methods

Study population

The SHS is a multicenter, population-based longitudinal study of cardiovascular diseases (CVD), diabetes, and their risk factors in American Indians in 13 tribes residing in Arizona, North and South Dakota, and Oklahoma. The study design, survey methods, and laboratory measurements of the SHS have been described previously (Lee et al. 1990). Briefly, the first clinical examination of the SHS was conducted between 1989 and 1991 by enrolling 4549 tribal members aged 45–74 years. Living participants were reexamined twice in an interval of 4–5 years. At each visit, participants completed a personal interview and underwent physical examination. Information on demographic factors, socioeconomic status, medical history, medication use, and lifestyle factors was collected during personal interview using standard questionnaires. The physical examination included measurements of blood pressure, height, weight, and waist circumference among others. Fasting blood samples were collected for laboratory tests, including fasting glucose, glycosylated hemoglobin, insulin, lipids, lipoproteins, creatinine, and biomarkers. In addition, all study participants were followed annually to collect information on morbidity and mortality due to cardiovascular disease, cancer, and other health conditions. Informed consent was obtained from all participants. The SHS protocols were approved by the Institutional Review Boards from the Indian Health Service and the participating centers.

For the current analysis, we excluded participants whose tribe later withdrew their participation in the study ($n = 1033$), those diagnosed with cancer ($n = 122$), and those missing information on cancer mortality

($n = 30$), LTL ($n = 1416$), or age ($n = 1$) at baseline. To remove outliers, we further excluded participants ($n = 2$) whose log-transformed LTL was outside of $\pm 4 \times \text{IQR}$ (interquartile range). We used a wider range of log-LTL because telomere lengths have been shown to vary broadly among humans at any age (Calado and Dumitriu 2013). The final sample is composed of 1945 participants.

Measurement of LTL

Detailed methods for the measurement of LTL in the SHS have been described previously (Zhao et al. 2014). Briefly, genomic DNA from peripheral blood drawn from baseline exam was isolated according to standard methods. LTL was measured using quantitative PCR at Dr. Blackburn's lab at the University of California, San Francisco, using a high-throughput telomere length assay system. The telomere length assay determines the ratio of telomeric product/single copy gene (T/S) obtained using quantitative PCR according to protocols reported previously (Cawthon 2002). The T/S ratio was calculated by taking the ratio between the mean of two T values and two S values attained for each of the three replicates. These three T/S ratios were averaged, and standard deviation and coefficient of variation (%CV, standard deviation/mean) were calculated. The T/S ratios were normalized to the mean of all samples and reported. For quality control, seven control DNA samples from various cancer cell lines were included in each assay plate. These control samples allowed us to create 8 standard curves, which were then integrated into a composite standard curve, used for T and S concentration calculations. In addition, about 20% of the samples (randomly selected) were measured twice. Intra- and inter-assay %CV were 4.6% and 6.9%, respectively.

Follow-up and cancer mortality

Baseline information in the SHS was collected in 1989–1991. All living participants were followed through December 2015. Detailed methods for ascertainment of deaths and their causes in the SHS have been described previously (Zhang et al. 2015b; Lee et al. 1998; Best et al. 2015). Briefly, death of a participant was identified from the Indian Health Service hospital records and direct contact with the family members. The cause of death was determined by physicians on the

SHS Mortality Review Committee using medical records, autopsy reports, and informant interview. For this study, information on mortality was retrieved from the mortality decision form used for cardiovascular disease surveillance in the SHS. Cancer mortality was defined as any death with a primary cause of death listed as malignant neoplasm, as determined independently by two members of the SHS Mortality Committee after reviewing information including death certificates and medical records including pathology reports as well as informant interviews, when needed.

Assessments of baseline covariates

Sociodemographic information (e.g., age, sex, and education years) was collected by standard questionnaires. Participants were classified into “current smokers” and “non-smokers.” Current smokers were defined as those who had smoked at least 100 cigarettes in their lifetime and were currently smoking during the time of interview. Non-smokers included persons who had never smoked a cigarette as well as former smokers. Alcohol use was also classified into “current drinkers” and “non-drinkers.” Current drinkers were those who had consumed any alcohol during the past year. Participants reported the leisure activities (walking, gardening/yard work, hunting, dancing, running, swimming, bicycling, and skating) they had engaged in the past year, and using the estimates of frequency and duration of each activity, average hours per week spent for leisure activities was calculated. Additionally, information on work-related physical activity was collected by asking the number of hours spent in moderate to hard activity at work. Measure of total physical activity was calculated by adding past-year hours per week spent in leisure activities and at work doing moderate to hard activity (Strong Heart Study 2001). Body weight (kg) and height (cm) were measured by trained staff when participants wore light clothes and no shoes. Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters (kg/m^2). Systolic blood pressure (SBP) was the average of two measurements taken by trained staff using a standard mercury sphygmomanometer. The participants rested for at least 5 min before the measurements were taken. Fasting glucose, serum creatinine, and

blood lipids, including total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), were measured by standard laboratory methods (Lee et al. 1990). Information on use of medications against hypertension, diabetes, and hypercholesterolemia were obtained by medical records review and standard questionnaires.

Statistical analysis

We conducted an analysis to examine whether baseline LTL predicts risk of cancer deaths in SHS participants. Prior to statistical analysis, we first log-transformed LTL to improve normality. Then, we regressed log-LTL on chronological age and used the residuals (age-adjusted log-LTL) in subsequent data analyses. Baseline characteristics of study participants are presented according to quartiles of age-adjusted log-LTL.

We constructed hierarchical Cox proportional hazard regression models, in which time (in years) to death (due to any type of cancer) was the dependent variable and baseline LTL (age-adjusted log-LTL as continuous variable or categorized into quartiles) was the independent variable, adjusting for potential confounding variables. In model 1, we adjusted for sociodemographic factors (sex, educational level), study center, prevalent CVD, lifestyle factors (current smoking, current drinking, physical activity, and BMI), and metabolic factors (SBP, fasting glucose, LDL-C, and HDL-C) at baseline. In addition, we tested for interaction between sex and LTL. To examine whether prevalent diabetes and CVD status influenced our results, we excluded participants with overt diabetes ($n=675$) or CVD ($n=130$) at baseline (model 2). Model 3 additionally adjusted for use of medications (yes/no) for hypertension, diabetes, and hypercholesterolemia at the baseline.

In the above-described models, the follow-up period was calculated from the date of clinical exam at baseline to the date of death or truncated to the date of last follow-up. Assumptions for the proportional hazard model were tested using Schoenfeld residuals (Allison 2010). P values less than 0.05 were considered statistically significant. All statistical analyses were conducted using SAS statistical software (version 9.4, Cary, NC). R

Version 3.4.1 (R Foundation for Statistical Computing, Vienna, Austria) was used to plot survival curves.

Results

Participants were aged 45 to 75 years at baseline (mean 56.1 ± 8.2 years), and women accounted for 56.9% of the study population. The median follow-up was 20.5 years (follow-up period ranges from 0.02 to 26.6 years) during which 220 (11.3%) participants died of cancer. Table 1 shows the baseline characteristics of study participants according to quartiles of age-adjusted log-LTL. Except for sex, participants with longer LTL did not differ from those with shorter LTL at baseline.

Compared with participants who did not die of cancer, those who died of cancer were significantly older ($P<0.001$), were more likely to be current smokers ($P<0.001$), were less educated ($P=0.035$), and had lower fasting plasma glucose ($P=0.028$) at baseline. Compared with men, women were less likely to be current smokers ($P=0.002$) or current drinkers ($P<0.001$). Moreover, women were physically inactive ($P<0.001$), had lower blood pressure ($P=0.009$), and were less likely to be diagnosed with CVD ($P<0.001$). However, women had higher BMI ($P<0.001$), fasting plasma glucose ($P=0.001$), and HDL ($P<0.001$). The age-adjusted log-LTL was significantly longer in women compared with men (0.02 vs -0.03 , $P=0.034$) (Table 2).

Our multivariable Cox regression analyses using age-adjusted log-LTL as a continuous variable showed that longer LTL at baseline significantly predicts an increased risk of cancer mortality in females (HR 1.57, 95% CI 1.08–2.30), but not in males (HR 0.74, 95% CI 0.49–1.12) (p for interaction 0.009). In the model with LTL quartiles, women in the first quartile (Q1; age-adjusted log-LTL < -0.275) (HR 0.56, 95% CI 0.32–0.97), the second quartile (Q2; age-adjusted log-LTL -0.275 to -0.033) (HR 0.59, 95% CI 0.35–0.99), and the third quartile (Q3; age-adjusted log-LTL -0.032 to 0.281) (HR 0.47, 95% CI 0.27–0.82) had a reduced risk for cancer death compared with those with the longest LTL (Q4; age-adjusted log LTL > 0.281) (Table 3). This sex-specific association was independent of age, education, study center, current smoking, current alcohol use, physical activity, BMI, fasting blood glucose, SBP, HDL-C, and LDL-C. After excluding participants with

Table 1 Baseline characteristics of SHS participants according to age-adjusted log-LTL quartiles ($n = 1945$)

Characteristics ^a		Total	Q1	Q2	Q3	Q4	<i>P</i> trend
Age, years		56.1 (8.2)	56.0 (8.0)	56.2 (8.5)	56.3 (8.0)	56.0 (8.2)	0.999
Sex, <i>n</i> (%)	Females	1107 (56.9)	244 (50.1)	278 (57.2)	291 (60.0)	294 (60.4)	0.006
	Males	838 (43.1)	243 (49.9)	208 (42.8)	194 (40.0)	193 (39.6)	
Current smoking, <i>n</i> (%)		823 (42.4)	218 (44.7)	190 (39.2)	205 (42.3)	210 (43.1)	0.999
Current drinkers, <i>n</i> (%)		872 (45.0)	237 (48.9)	221 (45.7)	215 (44.4)	199 (41.0)	0.093
CVD at baseline, <i>n</i> (%)		130 (6.7)	33 (6.8)	39 (8.0)	32 (6.6)	26 (5.3)	0.874
Education, years		11.4 (3.1)	11.3 (3.0)	11.2 (3.1)	11.6 (3.1)	11.6 (3.0)	0.581
Physical activity, hours/week		79.4 (82.7)	82.4 (81.9)	76.0 (81.5)	75.9 (80.8)	83.2 (86.4)	0.999
Body mass index, kg/m ²		30.2 (5.9)	30.1 (5.6)	30.2 (6.1)	30.1 (6.0)	30.3 (6.0)	0.999
Systolic blood pressure, mmHg		125.4 (18.8)	125.3 (18.0)	126.0 (18.5)	124.7 (19.3)	125.6 (19.5)	0.999
Fasting plasma glucose, mg/dL		137.7 (68.1)	137.1 (61.4)	132.9 (62.1)	139.8 (71.4)	141.2 (76.5)	0.831
LDL cholesterol, mg/dL		121.5 (32.9)	120.1 (31.9)	121.1 (32.5)	121.9 (33.3)	122.9 (34.1)	0.849
HDL cholesterol, mg/dL		45.7 (14.1)	46.2 (15.6)	46.6 (14.2)	45.8 (13.6)	44.4 (12.9)	0.323
Antihypertensive medication, <i>n</i> (%)		381 (19.6)	85 (17.5)	103 (21.2)	95 (19.6)	98 (20.1)	0.979
Hypoglycemic medication, <i>n</i> (%)		433 (22.3)	111 (22.8)	99 (20.4)	109 (22.5)	114 (23.4)	0.999
Lipid-lowering medication, <i>n</i> (%)		9 (0.5)	2 (0.4)	3 (0.6)	3 (0.6)	1 (0.2)	0.999

^aResults are expressed as mean (SD) unless otherwise noted*Q* quartile, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein

prevalent diabetes and CVD (model 2, Table 4), the association between the first quartile of log-LTL and cancer mortality disappeared (HR 0.55, 95% CI 0.29–1.06), but the association became more prominent for

Table 2 Baseline characteristics of SHS participants by cancer mortality and sex ($n = 1945$)

Characteristics ^a		Cancer death			Sex		
		Yes ($n = 220$)	No ($n = 1725$)	<i>P</i> ^b	Females	Males	<i>P</i> ^c
Age, years		58.8 (8.2)	55.8 (8.1)	< 0.001	56.34 (8.14)	55.79 (8.19)	0.140
Sex, <i>n</i> (%)	Females	112 (50.9)	995 (57.7)	0.056	–	–	–
	Males	108 (49.1)	730 (42.3)		–	–	
Age-adjusted log-LTL		0.02 (0.6)	− 0.003 (0.5)	0.529	0.02 (0.5)	− 0.03 (0.5)	0.034
Current smoking, <i>n</i> (%)		119 (54.1)	704 (40.9)	< 0.001	435 (39.3)	388 (46.4)	0.002
Current drinkers, <i>n</i> (%)		87 (39.6)	785 (45.7)	0.084	403 (36.5)	469 (56.2)	< 0.001
CVD diagnosis at baseline, <i>n</i> (%)		12 (5.6)	118 (6.8)	0.438	46 (4.2)	84 (10.0)	< 0.001
Education, years		11.0 (3.3)	11.5 (3.0)	0.035	11.4 (3.1)	11.4 (3.1)	0.788
Physical activity, hours/week		76.6 (82.8)	79.7 (82.7)	0.606	65.5 (67.9)	98.0 (96.0)	< 0.001
Body mass index, kg/m ²		29.4 (6.1)	30.3 (5.9)	0.158	30.7 (6.1)	29.5 (5.7)	< 0.001
Systolic blood pressure, mmHg		127.1 (18.5)	125.3 (18.8)	0.185	124.4 (19.5)	126.7 (17.8)	0.006
Fasting plasma glucose, mg/dL		129.3 (57.3)	138.8 (69.3)	0.028	142.1 (73.4)	132.0 (60.2)	0.001
LDL cholesterol, mg/dL		122.7 (30.6)	121.3 (33.2)	0.568	120.2 (33.4)	123.1 (32.2)	0.062
HDL cholesterol, mg/dL		45.3 (16.6)	45.8 (13.8)	0.702	48.0 (13.8)	42.8 (14.0)	< 0.001

^aResults are expressed as mean (SD) unless otherwise noted^bFor difference in baseline characteristics by cancer death^cFor difference in baseline characteristics by gender*Q* quartile, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein

Table 3 Sex-specific association between baseline LTL and all-cancer mortality in American Indians ($n = 1721$)

Variables	Females		Males	
	Number of cancer deaths (incidence density ^a)	HR (95% CI)	Number of cancer deaths (incidence density ^a)	HR (95% CI)
Continuous age-adjusted log-LTL ^b	112 (5.49)	1.57 (1.08–2.30)	108 (7.87)	0.74 (0.49–1.12)
Categorical age-adjusted log-LTL ^b				
Q1	23 (5.18)	0.56 (0.32–0.97)	37 (9.24)	1.38 (0.78–2.45)
Q2	25 (4.85)	0.59 (0.35–0.99)	21 (5.95)	0.87 (0.46–1.63)
Q3	21 (3.85)	0.47 (0.27–0.82)	25 (7.99)	1.21 (0.66–2.25)
Q4	43 (8.04)	Reference	25 (8.18)	Reference

^a Incidence density: the number of cancer deaths per 1000 person-years

^b The models (with continuous log-LTL and categorical log-LTL) were adjusted for sex, study site, education, current smoking, current drinking, physical activity, baseline CVD, BMI, systolic blood pressure, fasting glucose, and low- and high-density lipoprotein cholesterol P for interaction between sex and log-LTL = 0.009

the second quartile in females (HR 0.43, 95% CI 0.22–0.81). The HRs remained unchanged after additional adjustment for use of medications (model 3, Table 5). Figure 1 shows the survival curves by age-adjusted LTL quartiles for females and males, respectively.

Discussion

In this large prospective cohort of American Indians, we found that longer LTL significantly predicts an increased risk of cancer mortality in females, but not males. Specifically, one unit increase in the baseline age-adjusted log-LTL was, on average, associated with a 57% increase in the risk for cancer death among female American Indians. Compared with women with the longest LTL (the fourth quartile), those in the third

quartile had the highest reduction in cancer mortality (53%) followed by those in the first (44%) and second quartiles (41%).

Although previous studies have consistently reported that longer LTL is positively associated with non-neoplastic chronic conditions, such as diabetes (Salpea et al. 2010; Zhao et al. 2013, 2014), coronary artery diseases (Ogami et al. 2004), CVD (Mwasongwe et al. 2017; Fitzpatrick et al. 2006; Said et al. 2017), liver cirrhosis (Carulli 2015; Wiemann et al. 2002), and chronic obstructive pulmonary diseases (Savale et al. 2009), the relationship between LTL and cancer has been controversial, with some studies reporting that longer telomere length increases the risk of cancer, whereas others reporting the opposite direction (Russo et al. 2014; Yang et al. 2017;

Table 4 Association between baseline LTL and all-cancer mortality after excluding participants with diabetes and CVD at baseline ($n = 1087$)

Baseline LTL	Females HR (95% CI)	Males HR (95% CI)
Continuous age-adjusted log-LTL ^a	1.85 (1.18–2.90)	0.88 (0.54–1.43)
Categorical age-adjusted log-LTL ^a		
Q1	0.55 (0.29–1.06)	1.20 (0.61–2.37)
Q2	0.43 (0.22–0.81)	0.81 (0.39–1.66)
Q3	0.44 (0.23–0.86)	1.27 (0.62–2.62)
Q4	Reference	Reference

^a The models were adjusted for sex, study site, education, current smoking, current drinking, physical activity, baseline CVD, BMI, systolic blood pressure, fasting glucose, and low- and high-density lipoprotein cholesterol

Table 5 Association between baseline LTL and all-cancer mortality in American Indians after additional adjustment for medication use ($n = 1721$)

Variables	Females HR (95% CI)	Males HR (95% CI)
Continuous age-adjusted log-LTL ^a	1.57 (1.07–2.30)	0.74 (0.49–1.12)
Categorical age-adjusted log-LTL ^a		
Q1	0.56 (0.32–0.97)	1.38 (0.78–2.45)
Q2	0.59 (0.35–0.99)	0.87 (0.46–1.63)
Q3	0.48 (0.27–0.82)	1.21 (0.65–2.25)
Q4	Reference	Reference

^a Both models were adjusted for sex, study site, education, current smoking, current drinking, physical activity, baseline CVD, BMI, systolic blood pressure, fasting glucose, low- and high-density lipoprotein cholesterol, hypertension medication, hypoglycemic medication, and lipid-lowering medication

Campa et al. 2014; Willeit et al. 2010; Svenson et al. 2008; Pezzolo et al. 2015). Our results revealed a sex-dependent association between LTL and risk of cancer deaths.

Our findings in American Indians appear to be in line with several previous studies demonstrating that longer LTL increases the risk of death due to cancer (Svenson et al. 2008, 2009; Zhang et al. 2014; Pezzolo et al. 2015; Liu et al. 2012). For instance, in a study by Svenson et al. (2008) including 227 women with newly diagnosed breast cancer, breast cancer mortality was nearly twofold higher for women with longer telomere length than those with shorter telomere length.

Another study including 70 patients with esophageal squamous cell carcinoma showed that patients with longer telomere length had poor survival outcomes compared with those with shorter telomere length (Zhang et al. 2014). In a recent large-scale Mendelian randomization analysis using genetic variants in *OBFC1*, *TERT*, and *TERC* genes as instrument variables, 200-base pair decrease in genetically determined telomere length was found to be associated with decreased cancer mortality (HR 0.86, 95% CI 0.76–0.96) (Rode et al. 2015).

However, our finding was inconsistent with several other population-based prospective cohort

Fig. 1 Kaplan-Meier Plots for survival function of cancer by gender. P value was obtained using log-rank test. Q1, first quartile; Q2, second quartile; Q3, third quartile; Q4, fourth quartile. **a** Females. **b** Males

studies. For example, a longitudinal study that followed 787 Italians (aged 40 to 79 years) for 10 years found that 1-SD decrease in log-transformed telomere length was associated with a 60% increase in the risk of cancer mortality (Willeit et al. 2010). However, this study included only 44 cancer deaths, of which only two cancer deaths were observed among the group with the longest LTL. Another study that followed 47,102 participants from the Danish population (1730 events, median follow-up 6 years) found that individuals with shorter LTL (fourth quartile) were 1.31 times more likely to die of cancer compared with those with longer LTL (first quartile) (Weischer et al. 2013), suggesting protective effect of longer LTL on cancer death. More research is needed to determine the relationship between telomere length and cancer risk across different ethnic groups.

The mechanisms behind the relationship between telomere length and cancer mortality could be highly complex and warrant further investigation. In experimental studies, telomerase activation (and thus longer telomere length) was observed in more than 90% of cancer cells (Greider and Blackburn 1996; Jafri et al. 2016). Thus, the detrimental effects of longer telomeres on cancer could be due to its role in promoting cell division and genomic instability (Greider and Blackburn 1996; Jafri et al. 2016; Stewart and Weinberg 2006).

In this study, we identified a sex-specific association between LTL and cancer mortality among American Indians. While the precise mechanisms underlying the gender difference in the relationship between LTL and cancer mortality remain to be determined, it is possible that the observed sex-specific association could be due to genes located on the X chromosome (Barrett and Richardson 2011; Nawrot et al. 2004). For instance, the *DKC1* gene, which locates on the X chromosome, encodes dyskerin, a protein that plays a role in telomerase stabilization and maintenance (Nawrot et al. 2004; Bessler et al. 2004). Overexpression of this gene may promote cell division and cause genomic instability, thereby contributing to sex-specific cancer such as prostate cancer (Sieron et al. 2009), breast cancer (Montanaro et al. 2006, 2008), and ovarian carcinoma (Schaner et al. 2003). Another possible explanation for the

observed gender-dependent effect of LTL on cancer mortality could attribute to the estrogenic effects on telomerase activation (Kyo et al. 1999; Bayne et al. 2007; Misiti et al. 2000). There is evidence demonstrating that estrogen receptor signaling promotes *hTERT* gene expression and enhances telomerase activity, which may stimulate cell proliferation (Misiti et al. 2000) and contribute to gender differences in cancer susceptibility.

To our knowledge, this is the first study to assess the prospective association between LTL and cancer mortality among American Indians. Our study has several strengths including a prospective design, large sample size, long follow-up period, and low loss to follow-up rate. In addition, our statistical analyses adjusted for many potential confounders. However, our study also has some limitations. The impact of LTL on the risk of different types of cancer may be different. However, due to the small number of specific cancer cases in our population, we were unable to examine the association of LTL with cancer type-specific mortality. In addition, as all observational studies, we cannot determine the causal role of LTL in risk of cancer deaths. Finally, as women account for majority of our study population, we cannot exclude the possibility that the observed gender-specific association is due to lack of power for our analysis in males.

In conclusion, in a large prospective study of American Indians, we found that longer LTL at baseline significantly increased the risk of cancer mortality in women but not men. Our results suggest that telomeres may play different roles in males and females and highlight the importance of sex-specific analysis in future telomere research.

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Compliance with ethical standards

Ethical approval All procedures performed in this study were in accordance with ethical standards of Indian Health Service

Institutional Review Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of interest The authors declare that they have no conflict of interest.

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