Short Telomere Length and Breast Cancer Risk: A Study in Sister Sets

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Abstract

Telomeres consist of a tandem repeats of the sequence TTAGGG at the ends of chromosomes and play a key role in the maintenance of chromosomal stability. Previous studies indicated that short telomeres are associated with increased risk for human bladder, head and neck, lung, and renal cell cancer. We investigated the association between white blood cell telomere length and breast cancer risk among 268 family sets (287 breast cancer cases and 350 sister controls). Telomere length was assessed by quantitative PCR. The mean telomere length was shorter in cases (mean, 0.70; range, 0.03–1.95) than in unaffected control sisters (mean, 0.74; range, 0.03–2.29), but no significant difference was observed (P = 0.11). When subjects were categorized according to the median telomere length in controls (0.70), affected sisters had shorter telomeres compared with unaffected sisters after adjusting for age at blood donation and smoking status [odds ratio (OR), 1.3; 95% confidence interval (95% CI), 0.9–1.8], but the association was not statistically significant. The association by quartile of telomere length (Q4 shortest versus Q1 longest) also supported an increase in risk from shorter telomere length, although the association was not statistically significant (OR, 1.6; 95% CI, 0.9–2.7). This association was more pronounced among premenopausal women (OR, 2.1; 95% CI, 0.8–5.5) than postmenopausal women (OR, 1.3; 95% CI, 0.5–3.6 for Q4 versus Q1). If these associations are replicated in larger studies, they provide modest epidemiologic evidence that shortened telomere length may be associated with breast cancer risk. [Cancer Res 2007;67(11):5538–44]

Introduction

Telomeres are special structures consisting of a variable number of repeated sequences (TTAGGG) at the ends of chromosomes (1). Telomeres play a key role in the maintenance of chromosomal stability (2), and short telomere length, as a measure of telomere dysfunction, was significantly related to baseline and mutagen-induced genetic instability (3). Telomeric DNA is dynamic, being progressively lost with each cell division due to incomplete replication of the termini of linear DNA molecules (the end-replication problem). Thus, age has been recognized as a factor inversely associated with telomere length, but there is high variability between individuals of the same age (4). In addition, cigarette smoking, oxidative stress, chronic inflammation, and epigenetic changes may also cause telomere shortening (1, 5–9). Previous data suggested that the distribution of telomere lengths among chromosomes is genetically determined and, therefore, might be considered a polymorphic quantitative trait (10). Correlations have been observed between short telomeres and human diseases associated with aging, such as vascular dementia (11), atherosclerosis (12), myocardial infarction (13), Alzheimer's dementia (14), ulcerative colitis (15), liver cirrhosis (16), and premature aging syndromes, as well as solid tumors (1). Short telomere length, measured in peripheral blood lymphocytes or buccal cells, seems to be associated with increased risk for human bladder, head and neck, lung, and renal cell cancers and may interact with cigarette smoking (3, 17). They seem to be one of the earliest and most prevalent genetic alterations acquired in the multistep process of malignant transformation (10, 18).

Several studies examining telomere length in humans using Southern blot telomere restriction fragment (TRF) analysis found that breast carcinomas had shorter telomeres than normal breast tissue; high grade (grade III of III) invasive carcinomas had shorter telomeres than low grade (grade I of III) invasive carcinomas (19, 20). Dysfunctional telomeres are considered an early initiating event in breast cancer development, inducing chromosomal instability (18, 21). However, results are mixed with respect to associations between tumor telomere length and clinicopathologic features, such as histologic grade, tumor size, lymph node status, or hormone receptor status (19, 22, 23). Only two studies suggested that short telomeres are associated with smoking-related cancer risk (3, 17). Thus far, the relationship between telomere length and breast cancer susceptibility has not been reported. We hypothesized that individuals with shorter telomeres have a higher susceptibility for developing breast cancer.

Materials and Methods

Study design and characteristics of study population. The study population was selected from families participating in the Metropolitan New York Registry (MNYR), one of six international collaborating sites[3] of the Breast Cancer Family Registry. The description of the sources of study participants and recruitment and data collection methods have been described in detail elsewhere (24). Briefly, the MNYR has been recruiting high-risk breast and/or ovarian cancer families from clinical and community settings within the metropolitan New York area since 1995 who met one of the following criteria: (*a*) one or more members with breast cancer or ovarian cancer diagnosed at <45 years of age; (*b*) one or more members with both breast and ovarian cancer; (*c*) two or more first-degree relatives with breast or ovarian cancer diagnosed at age ≥45 years; (*d*) any male with breast cancer; and (*e*) a known *BRCA* mutation. Epidemiology and family history questionnaires were administered to each consenting,

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participating family member on recruitment into the Registry and collected information on demographics, ethnicity, history of all cancers, smoking and alcohol consumption, reproductive history, hormone use, weight, height, and physical activity. A self-administered dietary questionnaire was also provided with return by mail. In addition, a sample of peripheral blood was collected from participants. The present study included 268 family sets (287 breast cancer cases and 350 sister controls) from families in the MNYR with at least two sisters discordant for breast cancer.

Laboratory methods. Genomic DNA was extracted from white blood cells by the salting out procedure. White blood cells were lysed with SDS (final concentration 0.66% SDS) in a nuclei lysis buffer and treated with RNase A (final 133 μg/mL) and RNase T1 (final 20 units/mL) to remove RNA. Proteins were coprecipitation with NaCl (330 μL of saturated NaCl added per 1 mL solution) by centrifugation. Genomic DNA was recovered from the supernatant by precipitation with 100% ethanol, washed in 70% ethanol, and dissolved in the Tris-EDTA buffer. Telomere length quantification was done with the quantitative PCR (Q-PCR) method described by Cawthon (25). Telomere length measurement by the Q-PCR assay involved determining the relative ratio of telomere (T) repeat copy number to a single copy gene (S) copy number (T/S ratio) in experimental samples using standard curves. This ratio is proportional to the average telomere length. Then, the ratio for each sample was normalized to a reference DNA to standardize between different runs. 36B4, encoding acidic ribosomal phosphoprotein P0 was used as the single copy gene. Telomere and 36B4 gene PCRs were always done in separate 96 wells with each sample run in duplicate. Further modifications to the protocol were as follows: two master mixes of PCR reagents were prepared, one with telomere primer pairs (Tel-1, 5′-GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT-3′; Tel-2, 5′-TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA-3′) and the other with 36B4 primer pairs (36B4u, 5′-CAGCAAGTGGGAAGGTGTAATCC-3′; 36B4d, 5′-CCCATTCTATCATCAACGGGTACAA-3′) with the same final concentration (2.4 nmol/L). An aliquot of 25 ng (5 μL) template DNA was added to each reaction containing 12.5 μL SYBR Green PCR Master Mix (Applied Biosystems) and 7.5 μL primers mixture. The DNA quantity standards were serial dilutions of a reference DNA sample (a mixture of several unknown DNAs) to produce five final concentrations (0.4, 0.8, 1.2, 1.6, and 2.0 ng/μL). In each run, a standard curve and a negative control (water) were included. The PCR was done on a real-time PCR instrument (ABI 7500, Applied Biosystems). The thermal cycling profile for the telomere amplification was 95°C for 10 min followed by 30 cycles of 95°C for 15 s and 54°C for 2 min and for the 36B4 amplification was 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Following amplification, a dissociation curve was done to confirm the specificity of the reaction. Standard and dissociation curves were generated with the ABI Prism 7500 SDS software. R^2 for each standard curve was >0.98. The assay was done with the laboratory personnel blinded to the subject's case-control status. Evaluation of the variation of the Q-PCR method among 40 duplicated samples indicated that most of the data varied within the 95% confidence intervals (95% CI) of the mean (data not shown). The Pearson and Spearman correlation coefficients were 0.50 ($P < 0.001$) and 0.47 ($P < 0.002$), respectively. The intrabatch and interbatch variability [coefficient of variation (CV)] in present study was 19% and 28%, respectively.

Statistical methods. Telomere length was analyzed as a continuous variable and a categorical variable. ANOVA was used to determine the differences in telomere length as a continuous variable by case-control status, age group at blood donation (<40, 40–49, 50–59, and ≥60 years old), body mass index (BMI; <25 and ≥25), smoking history (never and ever), cigarette smoking per day (<10 and ≥10), and smoking pack-years (<7 and ≥7). Medians in controls were used to categorize the intervals of BMI, cigarettes per day, and pack-years. As a categorical variable, short telomeres were defined as less than the median (0.70) telomere length in controls, and χ^2 tests were used to assess case-control differences in frequencies of short telomeres. Quartiles of telomere length, based on control values (Q1, ≥0.96; Q2, 0.70–0.95; Q3, 0.49–0.69; and Q4, <0.49), were used to evaluate the dose-response. Because age might modify the association between telomere length and breast cancer risk, we further analyzed telomere-breast cancer relationships separately in two subgroups stratified by menopausal status. All analyses were stratified by family sets through conditional logistic regression with SAS version 9.0 (SAS Institute) to estimate the strength of the associations as odds ratios (OR) and corresponding 95% CI. All models were adjusted by age of blood donation and smoking status.

Results

The distributions of selected characteristics by case-control status are shown in Table 1. The age of blood donation differed significantly between cases (50.1 years) and controls (48.3 years). Cases and controls had similar ethnic distribution, smoking status, smoking amount, and mean BMI. Mean telomere length was shorter for cases (0.70 ± 0.33; range, 0.03–1.95) than controls (0.74 ± 0.35; range, 0.03–2.29), although no significant difference was observed ($P = 0.11$). The medians and the 25th and the 75th percentiles of telomere length in cases and controls are shown in Fig. 1.

The effects of covariates on telomere length by case-control status are shown in Table 2. With increasing age of blood donation, a

Table 1. Characteristics of sisters by case-control status

Characteristics	Cases (n = 287)	Controls (n = 350)	P
Age at blood donation, mean (SD)	50.1 (11.3)	48.3 (11.4)	0.04
Ethnicity, n (%)			
White	194 (67.6)	225 (64.3)	0.46
Hispanic	57 (19.9)	84 (24.0)	
Other	36 (12.5)	41 (11.7)	
Smoking status, n (%)			
Nonsmoker	168 (58.5)	202 (57.7)	0.83
Ever smoker	119 (41.5)	148 (42.3)	
No. cigarettes/d*, mean (SD)	14.2 (10.3)	13.5 (11.2)	0.59
Smoking pack-years*, mean (SD)	13.6 (16.6)	11.4 (13.3)	0.23
BMI, mean (SD)	25.5 (5.7)	25.4 (4.9)	0.81
Telomere length, mean (SD)	0.70 (0.33)	0.74 (0.35)	0.11

*Among ever smokers.

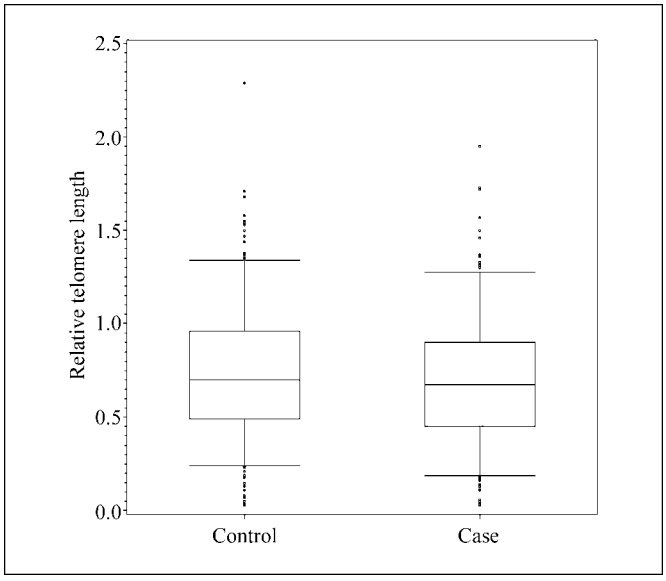

Figure 1. Box plot of the distributions of relative telomere length in the breast cancer cases and controls. *X axis,* case-control status; *Y axis,* relative telomere length. *Boxes,* interquartile range of the distribution (25th–75th percentile); *horizontal line within the box,* median; *vertical lines,* 5th and 95th percentiles; *closed circles,* outliers.

marginally significant shortening of telomere length was observed only in the control group ($P = 0.05$) but not among cases. Pearson correlation analysis between telomere length and age as a continuous variable also displayed similar patterns, with a negative correlation only observed among controls (Pearson correlation coefficient, −0.10; $P = 0.06$). No significant differences in means of telomere length were found for the covariables of BMI, smoking status, and smoking levels (cigarette per day and pack-years) by case-control status. A borderline significant difference in telomere length was observed between cases (0.69 ± 0.35) and controls (0.76 ± 0.37) only in never smokers ($P = 0.046$). No other significant case-control differences were observed for telomere length according to age groups of blood donation, BMI status, and smoking levels.

When subjects were categorized according to the median of telomere length in controls (0.70), we observed a statistically nonsignificant increased breast cancer risk for shorter telomere length (OR, 1.26; 95% CI, 0.86–1.83) compared with the reference group after adjusting by age of blood donation and smoking status (Table 3). Using the quartile with the highest telomere length (Q1, ≥0.96) as the referent group, the percentage of subjects in quartiles Q2, Q3, and Q4 were, respectively, 24.4%, 25.4%, and 28.6% in cases and 25.3%, 24.8%, and 24.8% in controls. The adjusted ORs for breast cancer increased from 1.35 (95% CI, 0.79–2.29) to 1.47 (95% CI, 0.86–2.52) to 1.55 (95% CI, 0.88–2.73) as telomere length shortened from 0.96 to <0.49, but no statistically significant dose-response was observed ($P_{trend} = 0.14$). The relationships between telomere length and breast cancer in subgroups categorized by menopausal status are shown in Table 4. Increased breast cancer risk for shorter telomeres was noted only in premenopausal women, although the association did not reach statistical significance. The adjusted OR in premenopausal women was 1.37 (95% CI, 0.70–2.71), similar to the nonsignificant OR observed among all women (1.26). The dose-response effect of shortened telomere length for increased breast cancer risk was more pronounced in premenopausal women. The adjusted ORs were, respectively, 1.65, 1.59, and 2.09 for Q2, Q3, and Q4 but still did not reach statistical significance ($P_{trend} = 0.17$).

To explore the potential effect of chemotherapy or radiation treatment on telomere length, we determined telomere length in 32 additional participants in the MNYR who donated blood before their breast cancer diagnosis. Telomere length was slightly longer in those cases who donated blood before diagnosis (0.76 ± 0.32) than in the cases who donated blood after diagnosis (0.70 ± 0.33; $P = 0.34$). However, the mean age of cancer diagnosis was significantly different (44.1 years in the former and 51.6 years in the latter; $P < 0.001$).

Discussion

Telomere length measured in peripheral blood DNA is a potentially useful biomarker, as a proxy of target tissue, to explore individual susceptibility to disease in epidemiologic studies because dynamic telomere length is determined by both genetic (26, 27) and environmental factors (1, 5–9). In the present study, short telomere length was associated with a nonsignificant increased breast cancer risk, and the dose-response trend was positive (with decreasing quartiles of telomere length, an increased breast cancer risk observed). The association was more pronounced among premenopausal women but was not statistically significant for either premenopausal or postmenopausal sister sets. This result is consistent with two previous studies that suggested that short telomeres are associated with increased risk for bladder, head and neck, lung, and renal cell cancers (3, 17). Wu et al. (3) observed that telomeres were statistically significantly shorter in patients with smoking-related cancers (head and neck, bladder, lung, and renal cell cancer) than in control subjects ($P < 0.001$). Individuals with constitutionally short telomeres were found to be at a considerably higher risk for developing bladder cancer (17). Until now, no studies have explored the potential role of menopausal status on modulating the telomere length and breast cancer relationship. Only one study indicated that menopausal status may affect telomere length and its relation with insulin resistance and inflammation (28). The validity of our finding that the association of shortened telomere length with increased breast cancer risk seemed to be more pronounced in premenopausal women is uncertain as is an appropriate explanation. One potential explanation is the dramatic difference in ovarian steroid hormones, particularly estrogen, during the premenopausal and postmenopausal periods. Estrogen may be linked to telomere dynamics through its anti-inflammatory and antioxidant attributes and its ability to stimulate telomerase, a reverse transcriptase that elongates telomere ends (29). Although only limited epidemiologic data are available thus far, these results indicate that telomere length may be a useful susceptibility biomarker to evaluate cancer risk. However, we must recognize that telomere length is not the only biomarker to indicate telomere dysfunction. Other genetic and nongenetic factors related to the stability of the telomere structure or that affect telomere length may also play important roles in this pathway and should be considered to confirm the real role of short telomere length in tumorigenesis.

Our results are biologically plausible and consistent with most previous studies exploring the relationship between telomere length and genomic instability or tumorigenesis using *in vivo* or *in vitro* experiments with highly sensitive cell lines or animals. The most important function of telomeres is maintaining genomic integrity and stability (2, 21). When telomeres are shortened to a

critical length, the result is telomere dysfunction with no further cell division (30). Recently, Lin et al. (31) reviewed the dynamics of telomere length in different cancers. In tumor cells, telomere length has a wide range of variability, and its equilibrium depends on the balance between telomere shortening from cell division and telomere elongation that results from telomerase activity (32). Many previous experiments showed that tumor cells have extremely short and stable telomeres, and their stability is achieved by the activation of telomerase (33, 34). Other studies on telomere length and carcinogenesis indicated that telomere dysfunction (caused by telomere shortening) is a very early and prevalent genetic alteration acquired in the multistep process of malignant transformation (10, 18), and telomere shortening leads to increased frequencies of chromosome instability (35, 36). Animal studies have shown that mice with shorter telomeres have an increased incidence of tumors and enhanced risk of epithelial cancers due to the formation of complex nonreciprocal translocations (35, 37). Clinical observations of tumor tissues showed that telomere length in colorectal carcinoma patients is shorter than in normal controls and subjects with colonic polyps (38). Maruyama et al. (39) observed that telomere length in intestinal metaplasia cases is statistically significantly shorter than in normal controls, but the telomere length in gastric carcinoma cases has no significant difference compared with controls. Sommerfeld et al. (40) measured telomere length in matched samples of normal benign prostatic hyperplasia (BPH) and prostate cancer tissues taken from radical prostatectomies. The telomeres from prostate cancer tissues were significantly and consistently shorter than the telomeres from cells in either the adjacent normal or BPH tissues. These results can be explained by the two outcomes of telomere dysfunction in somatic cells, depending on the integrity of checkpoint mechanisms (21, 41–43). On the one hand, when telomeres shorten to below a critical length due to cell division, cell division ceases and the cell undergoes either replicative senescence or apoptosis (44). On the other hand, if these two processes are bypassed, the cell continues to proliferate through activation of telomerase and genomic instability is initiated. The accumulated mutations, genetic lesions, and inactivated tumor suppressor checkpoints will ultimately result in cancer (1, 45). These data suggest that telomere shortening may be either a biomarker of susceptibility or resistance to cancer, depending on the balance of cellular checkpoint functions.

Overall, we did not observe significant relationships between telomere length and age at blood donation, BMI, smoking status, and smoking levels (cigarette per day and pack-years) either in cases or controls (Table 2), except for a marginally significant shortening of telomere length in controls with increasing age at blood donation. This result indicates a partial contribution of age at blood donation to telomere length, consistent with previous observations (46, 47). Valdes et al. reported a significant reverse dose-dependent relationship of telomere length with smoking pack-years among 1,122 White women ages 18 to 76 years. Another study found that cigarette smoking was associated with lower telomerase reverse transcriptase (hTERT) mRNA expression but had no detectable effect on telomere length (46). We did not observe inverse relationships between telomere length and

Table 2. Effects of covariates on mean telomere length by case-control status

Characteristic	Cases (n = 287)		Controls (n = 350)		P*
	n	mean (SD)	n	mean (SD)	
Age at blood donation, y					
<40	48	0.72 (0.31)	78	0.82 (0.37)	0.11
40–49	109	0.71 (0.34)	125	0.76 (0.38)	0.28
50–59	83	0.66 (0.30)	92	0.69 (0.28)	0.53
≥60	40	0.72 (0.41)	46	0.69 (0.31)	0.71
P*		0.67		0.05	
BMI, kg/m^2					
<25 †	148	0.69 (0.32)	190	0.74 (0.36)	0.16
≥25	134	0.70 (0.35)	154	0.73 (0.33)	0.47
P*		0.77		0.75	
Smoking status					
Never	164	0.69 (0.32)	200	0.76 (0.37)	0.046
Ever	119	0.71 (0.35)	147	0.72 (0.32)	0.92
P*		0.48		0.28	
Cigarettes per day					
<10 †	37	0.78 (0.38)	56	0.71 (0.34)	0.35
≥10	82	0.68 (0.33)	91	0.72 (0.31)	0.42
P*		0.15		0.86	
Smoking pack-years					
<7 †	49	0.76 (0.39)	73	0.72 (0.31)	0.51
≥7	67	0.67 (0.33)	72	0.73 (0.33)	0.34
P*		0.21		0.84	

*P value using two-sided one-way ANOVA.

†Medians in controls were used to categorize the intervals for BMI, cigarettes per day, and smoking pack-years.

Table 3. Associations between telomere length and breast cancer risk

Telomere status	Case-control status (268 sets, *n* = 637)		OR (95% CI)*	*P*
	Cases, *n* (%)	Controls, *n* (%)		
Categorized by median in controls				
≥0.70	130 (45.9)	175 (50.4)	1.00 (reference)	
<0.70	153 (54.1)	172 (49.6)	1.26 (0.86–1.83)	0.24
Categorized by quartile in controls				
≥0.96	61 (21.6)	87 (25.1)	1.00 (reference)	
0.70–0.95	69 (24.4)	88 (25.3)	1.35 (0.79–2.29)	0.27
0.49–0.69	72 (25.4)	86 (24.8)	1.47 (0.86–2.52)	0.16
<0.49	81 (28.6)	86 (24.8)	1.55 (0.88–2.73)	0.13
P_{trend}				0.14

*Adjusted by age of blood donation and smoking status.

cigarettes per day or pack-years, perhaps due to the small sample size with few heavy smokers 25% of the women smoked 20 or more pack-years. However, the different patterns for the telomere length-breast cancer relationship observed in never and ever smokers suggest that smoking may play a role in the process of telomere shortening.

The most widely used method to measure telomere length is TRF analysis. However, it suffers from several major drawbacks and is not high throughput, reducing its utility in epidemiologic studies. The Q-PCR assay, developed by Cawthon and used in present study, has the advantages of high throughput (96-well plates used) and high sensitivity (nanogram quantities of DNA can be analyzed). It also has relatively little variation because the measurement of telomeres by Q-PCR does not include the subtelomeric region, which is highly variable between individuals, from 2.5 to 6 kb (31). In addition, it is less affected by short telomeres and DNA quality (can be applied to degraded or fixed material; ref. 48). The means of telomere length and SEs in the present study are consistent with a prior study, but the CVs are higher than previously reported (47). This suggests that the Q-PCR method is reasonably reliable and useful in large-scale epidemiologic studies, although considerable variability exists, especially among different batches. To obtain more accurate and stable results in the future, triplicate or even quadruplicate assays might be useful for improving Q-PCR measurement of telomere length.

Measurement error may have occurred in categorizing subjects' telomere length by the Q-PCR assay because of the methodologic variability, but it should have randomly and equally affected cases and controls. Because sisters in the same family were assayed randomly, and the laboratory personnel were blinded to case-control status, any measurement error in the laboratory assay would be nondifferential and therefore likely bias the associations toward the null. The nondifferential measurement error cannot explain the positive trends we have observed here. Even if this bias was present, it should be of limited importance due to the increased ORs observed in those with much shorter telomere

Table 4. Associations between telomere length and breast cancer risk by menopausal status

Telomeres status	Premenopausal sisters (84 sets, *n* = 207)*				Postmenopausal sisters (95 sets, *n* = 204)*			
	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)†	*P*	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)†	*P*
By median								
≥0.70	37 (43.5)	65 (53.7)	1.00 (reference)		46 (46.0)	43 (42.2)	1.00 (reference)	
<0.70	48 (56.5)	56 (46.3)	1.37 (0.70–2.71)	0.35	54 (54.0)	59 (57.8)	1.01 (0.53–1.92)	0.98
By quartile								
≥0.96	16 (18.8)	34 (28.1)	1.00 (reference)		24 (24.0)	22 (21.6)	1.00 (reference)	
0.70–0.95	21 (24.7)	31 (25.6)	1.65 (0.63–4.34)	0.31	22 (22.0)	21 (20.6)	1.23 (0.47–3.25)	0.67
0.49–0.69	22 (25.9)	31 (25.6)	1.59 (0.58–4.36)	0.36	23 (23.0)	28 (27.5)	1.02 (0.39–2.66)	0.97
<0.49	26 (30.6)	25 (20.7)	2.09 (0.79–5.52)	0.14	31 (31.0)	31 (30.4)	1.34 (0.50–3.60)	0.57
P_{trend}				0.17				0.67

*Numbers of cases and controls by telomere length and menopausal status may be not equal to total number due to deletion of sister set discordant in menopausal status from the analysis.
†Conditional logistics regression analysis adjusted by age of blood donation and smoking status.

length (Q3 and Q4). Undiagnosed breast cancer cases might be included in controls, especially among the younger control sisters, and would produce misclassification of disease. This misclassification bias might underestimate the potential association between shorter telomere length and breast cancer risk. Although we have adjusted for age at blood donation and smoking status, potential confounders, in the analytic models, we cannot exclude residual confounding caused by unconsidered factors related to both the dynamics of telomere length and breast cancer risk in the present study. These factors, including oxidative stress, chronic inflammation, epigenetic modifications, and genetic polymorphisms in telomere-related genes, should be investigated in future studies to better understand the relationship between telomere length and breast cancer risk.

Compared with previous studies, our study has several advantages. One strength was the family-based design using sisters from the same families as cases and controls, which may be an efficient design in an association study compared with unrelated population controls (49). Any potential confounding related to population admixture was reduced, and some of the confounding due to differences in genetic susceptibility as well as behavioral and lifestyle factors that cluster within families was reduced. A major limitation is the case-control study design with telomere length measured in bloods collected after diagnosis of cancer eliminating our ability to determine the etiologic temporal sequence between telomere shortening and breast cancer risk. Previous reports, with small numbers of samples, are inconsistent on the role of chemotherapy or radiation treatment on telomere length (50–52). Our results indicated that telomere length in cases who donated blood before diagnosis was not significantly different from that of cases who donated blood after diagnosis ($P = 0.34$), but their age of cancer diagnosis was not comparable (44.1 versus 51.6 years, respectively). Therefore, we cannot exclude the possible effect of treatment (chemotherapy or radiation) on telomere length based on the present data. Further investigation focused on this issue in a well-designed follow-up study is needed.

In conclusion, our data provided modest evidence that short telomere length is associated with increased breast cancer risk and that this relationship may be more pronounce in premenopausal women. However, because of the large uncertainty surrounding our point estimates, further studies using large cohorts are needed.

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