Research Article

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Longer Relative Telomere Length in Blood from Women with Sporadic and Familial Breast Cancer Compared with Healthy Controls

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Abstract

Telomeres cap the ends of chromosomes and are composed of a series of noncoding hexamer repeats. Telomeres protect the integrity of DNA coding sequences and are integral to the maintenance of genomic stability. Previous studies have shown an association between shortened lymphocyte telomeres and increased risk for specific cancers. However, the association between telomere length and breast cancer risk is less clear. We examined the relative telomere length (RTL) in blood from women with no personal or family history of cancer (controls) compared with different populations of women with breast cancer and women at high genetic risk for developing breast cancer. RTL was determined as the telomere to single gene copy number ratio assessed by quantitative PCR. Breast cancer cases (low risk, $n = 40$; high risk, $n = 62$) had significantly longer RTL compared with unaffected controls ($n = 50$; mean RTL = 1.11 versus 0.84; $P < 0.0001$). The assessment of risk by RTL quartile showed an increased risk for breast cancer with each longer quartile, with the most significant risk observed in the longest quartile (odds ratio, 23.3; confidence interval, 4.4-122.3; $P < 0.0003$). Women without breast cancer but at high risk due to family history ($n = 30$) also showed longer telomeres than controls (mean RTL = 1.09 versus 0.84; $P < 0.0001$). Our analysis supports previous findings of longer RTL in breast cancer cases compared with controls, and is the first to observe longer RTL in women without breast cancer identified as high risk based on family history. *Cancer Epidemiol Biomarkers Prev; 19(2); 605–13. ©2010 AACR.*

Introduction

Telomeres are composed of a series of tandem repeats of TTAGGG nucleotides localized to eukaryotic chromosome ends. Telomeres maintain genomic stability by preventing chromosome end-to-end fusion, and prevent the loss of coding terminal bp during cell division. Telomere length is genetically determined (1) and varies widely both between individuals and between cell populations within a single individual. Length is inversely correlated with age, smoking, and body mass index, and shortens following significant chemotherapy exposure (2-9). During eukaryotic DNA replication, a portion of telomere DNA at the 5′ end of the lagging strand is lost with each mitotic division, a phenomenon described as the "end replication problem." This translates to a loss of approximately 40 to 80 bp per year depending on age (3). Critically short telomeres are recognized as DNA damage, triggering Rb and p53 signaling pathways to initiate either cell senescence or apoptosis (10). Telomere shortening, in some cases, may result in the upregulation of telomerase expression precluding senescence, a phenomenon observed in germ cells, hematopoietic cells, skin cells, and gastrointestinal tissue (11, 12). The activation of telomerase is also seen in >90% of malignant cell populations (13).

Telomere length in the hematopoietic compartment serves as a convenient proxy for average individual telomere length in nonmalignant tissues (14). Telomere shortening has been associated with various cancers, including bladder, small cell lung, basal cell, esophageal, and papillary thyroid cancers (15-20). Longer telomeres have been associated with clear cell renal carcinoma (21). Several studies have examined lymphocyte telomere length in relation to breast cancer risk with conflicting results. A nonsignificant trend for shorter telomeres has been observed in high-risk premenopausal women with breast cancer compared with unaffected sisters (22). Among postmenopausal women, no association with telomere length and risk has been observed (23). In contrast, significantly longer telomeres were observed in premenopausal breast cancer patients compared with unrelated female controls, with worse outcomes in the group with the longest telomeres (24). Most recently,

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results from two case-control studies revealed no significant association of risk for cancer with telomere length in blood leukocytes (25). Currently, there is no unifying explanation for this conflicting evidence. Our study was conceived to further assess the relationship between telomere length and the different populations of breast cancer patients, compared with unrelated controls without personal or family history of breast cancer.

Materials and Methods

Study Population

Nonsmoking women who were patients at the Stanford Breast Cancer Oncology Clinic or Cancer Genetics Clinic from 2003 to 2009 were eligible for participation in the comparison groups. To obtain a control population, subjects were recruited from nonsmoking female Stanford employees on a volunteer basis. Women in both the comparison and control groups were primarily residents of the San Francisco Bay Area in California, with similar socioeconomic status. All subjects were required to have no smoking history to remove the potential confounder of smoking on telomere length. After providing written informed consent, eligible subjects were grouped into one of four predefined categories: (*a*) affected by breast cancer with family history of breast cancer (High-Risk Case), (*b*) unaffected by breast cancer with family history of breast cancer (High-Risk Unaffected), (*c*) affected by breast cancer with no family history of cancer (Low-Risk Case), and (*d*) a control group unaffected by breast cancer with no personal or family history of cancer (Control; Table 1). The Control group was composed of graduate students, laboratory personnel, nurses, physicians, research scientists, medical assistants, residents, and fellows, with eligibility contingent upon having no personal history or first-degree relative history of any type of cancer. In the Control group, only data about age, race, family history, and menopausal status were available. Because eligibility criteria were listed in recruitment literature, participation rates were 100%. For the comparison groups, information on disease diagnosis, status, and treatment, as well as parity, age at menarche, menopausal status, and family history, was collected at time of enrollment and entered into a secure research database. Breast cancer histologic subtypes included both ductal and lobular carcinoma *in situ* as well as invasive ductal or lobular carcinoma. Positive family history in the High-Risk Unaffected and High-Risk Case groups was defined as one or more first-degree relatives with breast cancer diagnosed at age <60 y. In the Low-Risk Case group, subjects were not permitted to have a first-degree relative with any cancer history with the exception of skin cancer or breast cancer diagnosed at age >65 y. Participation rate for the Low-Risk Case group was 73%; participation rate for the High-Risk Case group was 49%; and participation rate for the High-Risk Unaffected groups was 28%. As consent for the High-Risk population was sought along with offer for BRCA testing, participation rates in the High-Risk groups may have been lower given the psychological implications of BRCA testing, as well as challenges in obtaining insurance authorization for this testing. All subjects had a single blood sample collected in an identical manner and frozen immediately at the time of consent. Time of consent for the High-Risk and Low-Risk Case groups was within 3 mo of diagnosis. The study protocol was approved by the Stanford University Institutional Review Board. Informed consent was obtained from all subjects, who were also consented to terms of use and disclosure of personal health information as outlined in the Health Insurance Portability and Accountability Act.

Laboratory Methods

DNA extraction from all blood samples was done in an identical manner. Genomic DNA was extracted from whole blood using the Gentra Puregene Blood kit, according to the manufacturer's instructions. DNA was quantified by NanoDrop after vortexing to ensure accurate and uniform concentration. Using quantitative real-time PCR, relative telomere length (RTL) was calculated from the telomere repeat to single gene copy number ratio, using a modified version of the method described by Cawthon et al. (26). The single gene used was *36B4*, encoding acidic ribosomal phosphoprotein P0. The assay was done on a high-throughput 384-well plate and was analyzed on an Applied Biosystems 7900HT sequence detection system. Telomere and single gene copy amplifications were run separately, with a negative control (water) and a standard curve run on each plate. The standard curve was composed of serial dilutions of pooled human genomic DNA (Roche) from 80 to 100 donors. For each plate preparation, a master mix of Platinum SYBR Green qPCR SuperMix-UDG with ROX (Invitrogen) was combined with either telomere or 36B4 primers. For each 10-mL reaction, 4 mL of DNA (0.4 ng/mL) was added to 5 mL of the master mix + 1 mL of primers. Telomere primer pairs were Tel 1 (5′-GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT-3′) at 270 nm, Tel 2 (5′-TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA-3′) at 900 nm, 36B4d (5′-CCCATTCTATCATCAACGGGTACAA-3′) at 500 nm, and 36B4u (5′-CAGCAAGTGGGAAGGTGTAATCC-3′) at 500 nm. 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. For the 36B4 amplification, the profile was 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 58°C for 1 min. Following amplification, a dissociation curve confirmed the specificity of the reaction. Both standard and dissociation curves were generated with the ABI 7900HT SDS software, with a R^2 for each standard curve of ≥0.98. Of note, samples from all four study groups were intermixed on each well plate, and each sample was assayed in triplicate. Outlying samples, defined as a sample with Ct deviation of >0.2 from the remaining two samples of the triplicate,

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were eliminated from statistical analysis. The remaining average coefficient of variation was 5.0%.

BRCA1 and BRCA2 mutation detection was done by Myriad on genomic DNA extracted from High-Risk peripheral blood samples by direct sequencing of all coding exons of BRCA1 and BRCA2 and partial flanking intronic sequences. Sequencing results were compared with the reference DNA sequences of the BRCA1 and BRCA2 gene using the SeqScape software (Applied Biosystems) and then reviewed manually. Tumor estrogen receptor expression status was determined by immunohistochemistry.

Statistical Methods

Power was calculated to detect a mean difference of 0.3 in RTL between the Control group and the Case groups, with $\alpha = 0.05$, $\beta = 0.2$, and an expected SD of 0.5 based on the information from recent literature (22-25). We determined a sample size of 45 controls and 45 cases would be required to detect this difference.

To compare differences between the groups, we conducted ANOVA on the continuous factors including age, parity, and menarche. For the categorical factors including menopause, race, and ER positivity, we conducted a χ^2 analysis or, where appropriate, Fisher's Exact Test.

Exploratory graphical data analysis suggested that RTL values could be modeled by a normal distribution. Because the distribution seemed slightly skewed, we repeated our analyses with log transformation of the data, based on λ approximating 0 given by a Box-Cox transformation (MASS package, R 2.9.1). We also conducted Wilcoxon analyses on each comparison. As the results from both the log scale representation and the nonparametric analysis afforded the same inferences, for ease of interpretation, we report the untransformed RTL values.

Mean RTLs between the groups were compared using an unpaired *t* test with a two-tailed significance level of 0.05. RTL values for the Control as well as High-Risk and Low-Risk Case groups were then divided into quartiles. Multivariate odds ratios (OR) for breast cancer were calculated through logistic regression with corresponding 95% confidence limits and Wald χ^2 tests for significance. These logistic models assessed each quartile relative to the shortest quartile. Hierarchical stepwise models were built to adjust for age, race, menopausal status, and RTL quartile. We were unable to adjust for parity, menarche, and estrogen receptor status, as this information was not known for or was not applicable to the Control group.

Despite no difference observed after the adjustment for menopausal status, given the potential effect of premenopausal versus postmenopausal status, we further analyzed the relationship between telomere length and breast cancer risk by removing the postmenopausal group. Linear modeling was used to assess the difference in telomere length as a continuous variable by presence or absence of breast cancer, age at time of blood donation, and race. A linear model and exploratory loess regression was also used to determine if a relationship existed between telomere length and parity/age at menarche. An additional model was built to rule out the possibility of an age by quartile interaction. All statistical analyses were done using SAS version 9.2.

Results

Participant Characteristics

A total of 182 women met the eligibility criteria for one of the four groups described in Table 1, and all were evaluable. The distribution of selected participant characteristics is also shown in Table 1. All subjects were nonsmokers. The difference in median age between the groups was statistically significant ($P < 0.0001$). However, the testing for interaction between age and quartiles in the logistic models described below revealed no evidence of a statistically significant interaction ($P < 0.25$ for all subjects and $P < 0.41$ for the premenopausal subjects). Race distribution was similar in each group and primarily Caucasian, with Asian as the second most commonly noted; however, the differences between the groups was significant ($P = 0.0302$). Parity and menarche data were not available on the Controls, but for the remaining three groups, differences were not significant for parity, but significant for age at menarche ($P < 0.006$). Each group had similar numbers of premenopausal and post menopausal women, except the High-Risk Case group having a relatively larger number of postmenopausal women, likely reflecting the combined effect of chemotherapy-induced menopause and incidence of elective oophorectomy, given concern for ovarian cancer risk based on family history ($P < 0.0001$). Breast cancer tumor estrogen receptor status was also different between the Case groups ($P = 0.0441$).

RTL by Subset

Correlation and regression analyses of RTL and age, stratified by race and disease status, did not suggest a statistically significant association between RTL and age for any subgroup in this narrow age range. Pearson's correlation coefficients for the four groups were as follows: non-Caucasian without cancer = −0.11 ($P < 0.8$), Caucasian without cancer = 0.11 ($P < 0.38$), non-Caucasian with cancer = −0.005 ($P < 0.98$), and Caucasian with cancer = −0.02 ($P < 0.88$).

The overall range of RTL including all groups was 0.47 to 1.95. No statistically significant difference was observed in telomere length between the following groups: Low-Risk Case (mean RTL = 1.10; $n = 40$) versus High-Risk Case (mean RTL = 1.12; $n = 62$), women with breast cancer who had received chemotherapy before blood sample draw (mean RTL = 1.15; $n = 48$) versus those who had not yet received chemotherapy (mean RTL = 1.08; $n = 84$), High-Risk Case with a BRCA mutation (mean RTL = 1.07; $n = 18$) versus High-Risk Case wild-type for BRCA (mean RTL = 1.13; $n = 44$), and High- and Low-Risk Case women who were estrogen receptor

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Table 1. Distribution of selected characteristics by group

Variable	Controls	Low-risk cases	High-risk cases	High-risk unaffected	*P*
Age range	30-60	39-63	30-65	33-60	
Age, mean ± SD	40.2 ± 8.6	49.2 ± 5.8	49.0 ± 9.4	46.1 ± 7.9	$P < 0.0001$
Number enrolled	50	40	62	30	
BRCA 1 or 2 mutation	NA	NA	18/62	8/30	
Criteria*					
History of breast cancer	No	Yes	Yes	No	
History of other cancer	No	No	Yes and No	No	
Family history of cancer†	No	No	Yes	Yes	
Race, *n* (%)					$P = 0.0302$
Caucasian	43 (86)	29 (72.5)	48 (77.4)	29 (96.7)	
Asian	7 (14)	11 (27.5)	13 (21.0)	1 (3.3)	
Black	0 (0)	0 (0)	1 (1.6)	0 (0)	
Menopausal status					$P < 0.0001$
Pre, *n* (%)	43 (86)	26 (76.5)	23 (37.1)	21 (70)	
Post, *n* (%)	7 (14)	8 (23.5)	39 (62.9)	9 (30)	
Not available, *n*	0	6	0	0	
Parity, mean ± SD	ND	1.3 ± 1.0	1.5 ± 1.0	1.5 ± 1.1	Ns
Age of menarche, mean ± SD	ND	12.6 ± 1.4	12.2 ± 1.4	13.2 ± 1.4	$P < 0.006$
Estrogen receptor					$P = 0.044$
Positive, *n* (%)	ND	34 (89.5)	41 (71.9)	NA	
Negative, *n* (%)	ND	4 (10.5)	16 (28.1)	NA	
Not available, *n*		2	5		
RTL, mean ± SD	0.8 ± 0.2	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	
RTL, median	0.8	1.1	1.1	1.1	

Abbreviations: ND, not determined; NA, not applicable; Ns, not significant.
*All subjects are female, nonsmokers.
†Positive family history is defined as ≥1 first-degree relative (parent, sibling, or offspring) with breast cancer diagnosed at age <60 y. Negative family history is defined as no first degree relatives with cancer, excluding breast cancer diagnosed at age >65 y.

positive (mean RTL = 1.14; n = 76) versus negative (mean RTL = 1.07; n = 20). In addition, within the High-Risk Case group wild-type for BRCA, the comparison between women with one first-degree relative with breast cancer (mean RTL = 1.21; n = 21) versus women who had two or more first-degree relatives with breast cancer (mean RTL = 1.06; n = 23) did not show a significant difference in RTL. No association was noted between RTL in relation to either parity and/or age at menarche, using both a linear model as well as exploratory loess regression (data not shown).

In comparing our Control group to each of the three comparison groups, a significant difference was noted when comparing RTL in the Controls (mean RTL = 0.84; n = 50) to the combined High- and Low-Risk Case groups (mean RTL = 1.11; n = 102), with longer telomeres in the combined High- and Low-Risk Case groups ($P <$ 0.0001; Fig. 1). The same magnitude of difference ($P <$ 0.0001) was noted in comparing the Control group (mean RTL = 0.84; n = 50) to the High-Risk Unaffected group (mean RTL = 1.09; n = 30; Fig. 2).

Significant findings from the t test comparison as illustrated in Fig. 1 were then analyzed by logistic regression. RTL values from the Control subjects, the High-Risk Case subjects, and the Low-Risk Case subjects were combined, ranked, and then divided into four quartiles. We confirmed our results using the Wald χ^2 test (degree of freedom = 3, 14.39, $P < 0.0024$) allowing for the calculation of ORs as delineated in Table 2, all relative to the shortest quartile with an OR set at 1.

In a multivariate logistic regression model adjusting for race, menopausal status, and age, neither race nor menopausal status were significantly related to RTL, and were therefore removed from the model. However, age at the time of sample draw was statistically significant; therefore, the unadjusted quartile model and the reduced adjusted model including both quartile and age is shown in Table 2. After all postmenopausal samples were removed from the analysis, again race was not significantly associated with RTL and was therefore excluded from the model. Once again, age at time of sample draw showed statistical significance. The unadjusted model and the reduced adjusted model for premenopausal women including both quartile and age are also shown in Table 2.

A significant association was observed between longer telomere length and risk for breast cancer (Table 2). This

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association was shown in both the unadjusted and adjusted ORs, and remained significant after the elimination of the postmenopausal subjects. The effect was most pronounced in the quartile with the longest telomeres, with an age-adjusted OR of 23.3 [95% confidence interval (CI), 4.4-122.3; $P < 0.0003$ (all subjects)] and an age-adjusted OR of 18.7 [95% CI, 2.8-124.7; $P < 0.004$ (premenopausal subjects only)]. The differences between the Control and Case groups shown in Fig. 1, also shown in Table 2, are quantifying the same effect. This difference is also observed as a linear trend across the four quartiles, which is highly significant, with an OR of 2.2 (95% CI, 1.5-3.1; $P < 0.0001$).

Discussion

Our study compares RTL in blood from women with no personal or family history of cancer (Control group) compared with women with breast cancer with or without family history of cancer (High- and Low-Risk Case groups). We showed longer blood RTL in the High- and Low-Risk Case groups, regardless of family history. The risk for breast cancer increased in a stepwise fashion with each increasing quartile of RTL, the longest RTL quartile showing a significantly increased risk, with an OR of 23.3 (95% CI, 4.4-122.3). Interestingly, women with no personal history of cancer but with a strong family history of breast cancer (High-Risk Unaffected) also had longer RTL, perhaps consistent with an increased risk for breast cancer in that group.

Our study shows longer blood RTL in breast cancer patients, consistent with findings observed by Svenson et al. (24), who showed the association of longer telomeres with breast cancer with an OR of 5.17 in the longest quartile. Our findings are unique in that we show longer blood RTL in women with breast cancer, regardless of the presence or absence of a family history of breast cancer or a BRCA mutation. We found our results to be particularly significant given the younger median age of the Control group, as telomeres generally shorten with age. Most importantly, our results are the first to show longer RTL in unaffected women with a strong breast cancer family history (High-Risk Unaffected), suggesting a marker for risk.

Cells with both very short and very long telomeres exist in environments that may promote carcinogenesis. The role of telomere shortening in inducing replicative senescence, thus limiting the proliferative potential of a cell, is thought to function as a tumor suppressor mechanism (13, 27). Excessively shortened telomere length may result in chromosome end-to-end joining, thus ending the DNA damage response and allowing the cell to enter mitosis. Chromosome breaks that occur from the resulting anaphase bridges create genome instability and potential for malignant transformation (28). This process is illustrated by a telomerase knockout mouse model that shows resistance to cancer (29, 30) except in the setting of p53 mutations (31), emphasizing the role of the p53 pathway in recognizing critically short telomeres as DNA damage, resulting in cell senescence or apoptosis. In contrast, significantly longer telomeres confer a survival advantage, allowing continued cellular proliferation and subsequently providing an environment perhaps more susceptible to malignant transformation. Telomerase

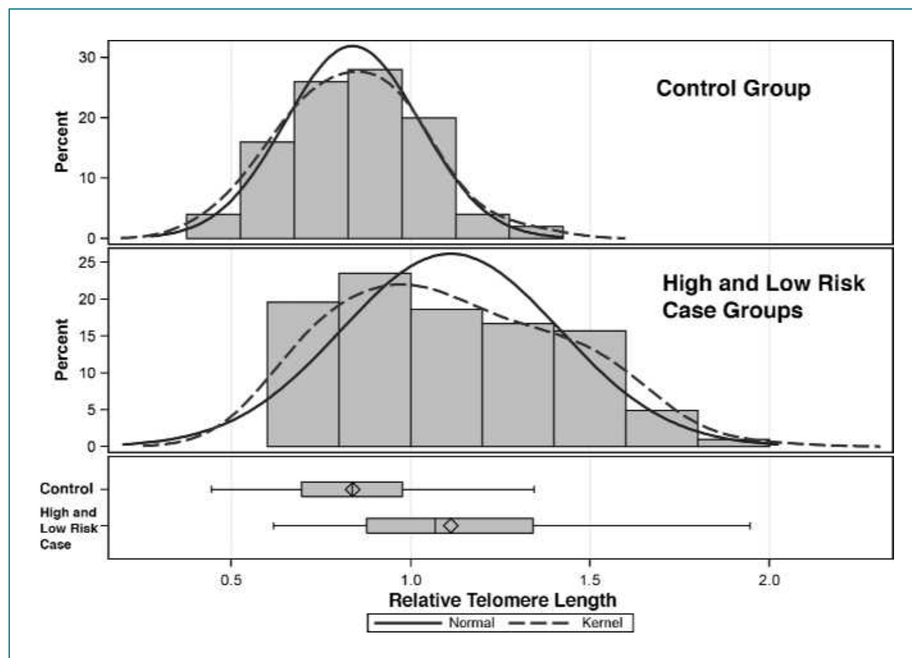

Figure 1. Control group compared with High and Low-Risk Case groups.

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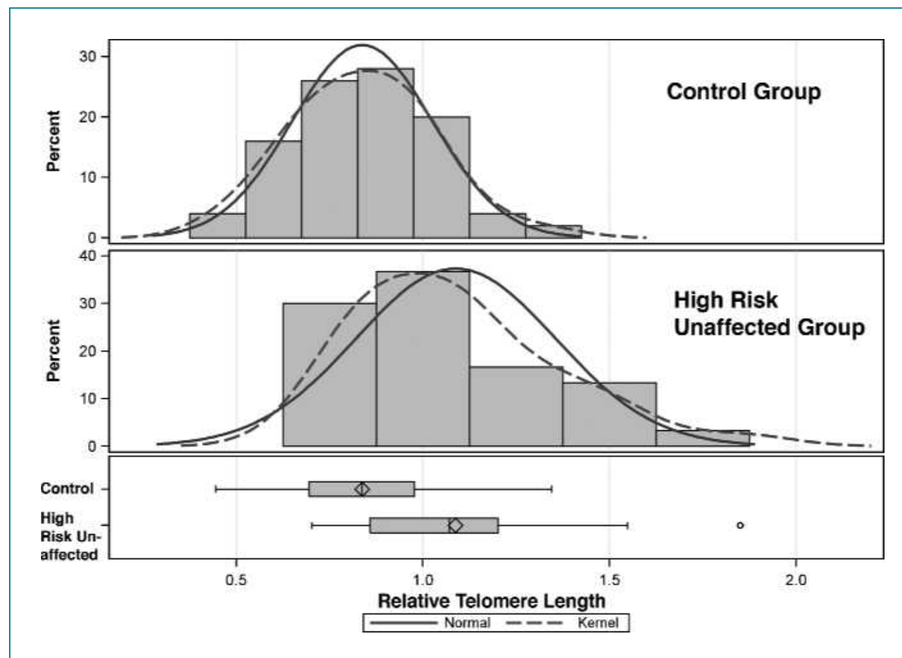

Figure 2. Control group compared with High-Risk Unaffected Group.

activation in human cell cultures has been shown to significantly increase cell life span (32). Telomerase overexpression in mice has been shown to promote mammary carcinogenesis (33), with multiple accelerated neoplasias occurring if combined with an impairment in p53 activity (34). Also in the mouse model, telomerase expression promotes cell growth independently of telomere length, with early telomerase expression in tumor cells even if telomere length is quite long (2). A potential mechanism for telomere lengthening in breast cancer patients may stem from variation in genes affecting telomerase expression, as recently described by Varadi et al. (35), who found an increased risk for breast cancer in a single nucleotide polymorphism variant within the tankyrase gene, a positive regulator of telomerase.

Longer telomeres associated with increased breast cancer risk may also be attributed to estrogen effect, as increased estrogen exposure is a well-known risk factor for breast cancer development. Estrogen affects telomere length directly through the activation of the human telomerase reverse transcriptase promoter (36) as well as by posttranscriptional human telomerase reverse transcriptase regulation (37). Telomere length is on average longer in females than males (38), although both sexes have equivalent lengths at birth (39), suggesting influence of gender on attrition rate. Whether this difference is genetic and/or related to hormonal exposure is not known. Previous investigations have examined pre and postmenopausal populations separately, due to the abrogation of telomere shortening in women treated with hormonal replacement therapy (40). Because data on hormonal replacement was not available in our postmenopausal population, we repeated our analyses on the premenopausal women only and found consistently significant results. As estrogen effect is one factor distinguishing breast cancer from other cancer types in which telomere shortening has been consistently observed, we explored tumor ER status for correlation to telomere length but found no association, nor did we find an association with either parity or age at menarche, as surrogates for lifetime estrogen exposure. Previous investigations have also failed to show a correlation between ER status and telomere length (23, 24), but this has yet to be evaluated prospectively on a large cohort including direct measurement of estradiol. Of note, tamoxifen, which blocks estrogen receptors, has been shown to inhibit the effect of estrogen on telomerase (41). Given the large proportion of women with breast cancer who are treated with tamoxifen, another potential explanation for longer telomere length in our breast cancer cases would be the early capture of our patient population before development of tamoxifen effect on telomerase and subsequent telomere shortening.

Limitations to our study include the use of whole blood in our analysis of RTL rather than using specific leukocyte populations. The use of hematopoietic cells as a proxy for average individual telomere length is complicated by the variation in telomere length observed within leukocyte subsets (42), as well as quantifiable changes in subset populations observed to occur with aging (43). Selection of a specific leukocyte subset, for example lymphocytes, would eliminate cell-to-cell variation and theoretically provide a more constant measure, although there is evidence that whole-blood telomere length

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correlates well with the mononuclear population (44). Our decision to use whole blood was primarily based on the storage of our familial samples as frozen blood without separation of the mononuclear layer, so that to process all samples in a consistent manner, the prospectively collected samples collected were also frozen immediately after collection. A third explanation for our findings may be the capture of a nonlymphocyte WBC subset that is more predictive of risk than targeted lymphocyte telomere length. Of note, telomere length is known to differ between chromosomes in a single cell (45, 46). Individual chromosome telomere length may more accurately predict cellular survival than average telomere length, and some studies have linked cancer risk to specific telomere length, albeit only with regard to shortening(47-49). Single telomere length analysis with quantitative PCR is limited by the generation of complex subtelomeric chromosome specific primers and, to date, is only available for chromosomes 2p, 11q, 12q, 17p, Xp, and Yp (50, 51). Given that our findings suggest *longer* RTL in High- and Low-Risk Case populations, and current evidence does not support biological effect of a lengthened single chromosome telomere, this analysis was not explored.

Additional limitations include our relatively small sample size resulting in our inability to age match, lack of information on body mass index (known to affect telomere length), lack of information on socioeconomic status, and lack of information on degree of exposure to secondhand tobacco smoke, which has an unknown but theoretical risk for shortened telomeres. As our groups did show differences in several demographic factors, we cannot exclude that the difference observed between telomere length in the Control and Low- and High-Risk groups may be due in part to these factors. That no significant association was observed between telomere length and age may be explained by two factors. First, the age range studied was narrow, and did not include subjects of age <30 or >65 years, where one would expect to observe higher attrition rates and less variability

Table 2. Association between RTL and breast cancer risk: controls versus cases, low and high risk

Unadjusted ORs

	All subjects (*n* = 152)				Premenopausal subjects (*n* = 92)			
RTL	**Controls, *n* (%)**	**Cases, low and high risk, *n* (%)**	**OR (95% CI)***	***P***	**Controls *n* (%)**	**Cases, low and high risk, *n* (%)**	**OR (95% CI)***	***P***
Quartile 1 (<0.8)	22 (44)	20 (20)	1.0		17 (40)	8 (16)	1.0	
Quartile 2 (0.8-1.0)	16 (32)	21 (21)	1.4 (0.6-3.5)	0.42	16 (37)	11 (22)	1.5 (0.47-4.6)	0.51
Quartile 3 (1.1-1.2)	10 (20)	25 (25)	2.7 (1.1-7.1)	0.04	8 (19)	13 (27)	3.4 (1.02-12)	0.046
Quartile 4 (>1.2)	2 (4)	36 (35)	20 (4.2-93)	0.0002	2 (5)	17 (35)	18.1 (3.3-98)	0.0008
Total	50	102			43	49		

Adjusted ORs*

	All subjects (*n* = 152)				Premenopausal subjects (*n* = 92)			
RTL	**Controls, *n* (%)**	**Cases, Low and High Risk, *n* (%)**	**OR (95% CI)***	***P***	**Controls *n* (%)**	**Cases, Low and High Risk, *n* (%)**	**OR (95% CI)***	***P***
Quartile 1 (<0.8)	22 (44)	20 (20)	1.0		17 (40)	8 (16)	1.0	
Quartile 2 (0.8-1.0)	16 (32)	21 (21)	2.0 (0.7-5.6)	0.2	16 (37)	11 (22)	1.4 (0.4-5.2)	0.6
Quartile 3 (1.1-1.2)	10 (20)	25 (25)	2.7 (0.9-7.8)	0.07	8 (19)	13 (27)	3.2 (0.8-12.6)	0.10
Quartile 4 (>1.2)	2 (4)	36 (35)	23.3 (4.4-122.3)	0.0002	2 (5)	17 (35)	18.7 (2.8-124.7)	0.003
Total	50	102			43	49		

*Conditional logistic regression analysis adjusted by age at time of blood donation.

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(52, 53). Second, whole-blood telomere length is inclusive of granulocyte DNA, recognized to show a less dramatic telomere attrition rate than lymphocytes (53). Given the level of interindividual variation in telomere length, age bias is a potential confounder in all telomere length studies, particularly in smaller sample sets such as the study described here. There is potential for selection bias in the use of employees in the Control group and patients in the comparison groups. Factors such as stress were not measured and could serve as potential confounders to our analysis, although stress alone, thought to contribute to more rapid telomere attrition, is unlikely to be a more significant factor in our Control group than our comparison groups (54, 55). Lastly, the timing of blood collection in this study (at first clinic visit or within the first several months of treatment) correlates well with previous studies, and no difference was observed in telomere length with regards to chemotherapy; however, a confounder may exist in the timing of the blood collection resulting in telomere lengthening in both our High- and Low-Risk Case groups and our High-Risk Unaffected group.

Our findings provide evidence for longer blood RTL in both women affected by breast cancer (High- and Low-Risk Case groups) and in women at high risk for breast cancer based on family history (High-Risk Unaffected). These results suggest that in some cancers, very long telomeres may provide an environment promoting cell survival, therefore increasing the risk for cancer development in a cell that has undergone the necessary steps toward malignant transformation. Demonstration of longer RTL in 30 samples from women at high genetic risk (High-Risk Unaffected) suggests that telomere lengthening may precede cancer diagnosis, and serve as a marker for risk. Our results caution against using relatives as a control group and may clarify why no significant difference was observed in one study of women at high genetic risk for breast cancer, which compared RTL between women affected with breast cancer and their nonaffected sisters (22), and another which used family members as controls in one of the groups (25). Based on the discrepancy in the literature about telomere length and breast cancer risk, the well-demonstrated effects of estrogen on telomerase, and the use of estrogen receptor inhibitors in the treatment of breast cancer, future study designs exploring this association should take into consideration direct measurement of serum estradiol in addition to surrogates for lifetime estrogen exposure and, if possible, should capture patient samples before tamoxifen exposure. In addition, further exploration of polymorphisms in telomere maintenance genes in a case-control format may contribute to our understanding of why longer telomeres have been observed in this group. Our observations suggest significantly increased risk in the group with the longest RTL (>1.2 in all women, >1.4 in premenopausal subjects), which could potentially serve as a threshold for screening purposes. A large prospective longitudinal study including unaffected women with affected first-degree relatives, along with unrelated age-matched controls, may improve our understanding of longer RTL as a potential predictive biomarker for breast cancer development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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