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Telomere Length from Blood Cells and Breast Cancer Risk: Investigations in two case-control studies

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Abstract

Purpose—Telomere dysfunction, which leads to genomic instability, is hypothesized to play a causal role in the development of breast cancer. However, the few epidemiologic studies that assessed the relationship between telomere length in blood cells and breast cancer risk have been inconsistent. We conducted two case-control studies to further understand the role of telomere length and breast cancer risk.

Methods—Overall telomere lengths were measured by telomere quantitative fluorescent *in situ* hybridization (TQ-FISH) and telomere quantitative real-time PCR (TQ-PCR). The associations between telomere length from blood leucocytes and risk of breast cancer were examine in two breast cancer case-control studies that were conducted at Roswell Park Cancer Institute (RPCI) and Lombardi Comprehensive Cancer Center (LCCC).

Results—Using the 50th percentile value in controls as a cut point, women who had shorter telomere length were not at significantly increased risk of breast cancer compared with women who had longer telomere length in the RPCI study (odds ratio [OR] = 1.34, 95% confidence interval [CI] = 0.84 to 2.12), in the LCCC study (OR = 1.18, 95% CI = 0.73 to 1.91), or in the combined RPCI and LCCC studies (OR = 1.23, 95% CI = 0.89 to 1.71). There was no significant dose-response relationship across quartiles of telomere length and no significant difference when comparing women in the lowest to highest quartile of telomere length.

Conclusions—Overall telomere length from blood leucocytes was not significantly associated with the risk of breast cancer.

Keywords

Telomere length; blood leucocytes; breast cancer; biomarkers; genetic susceptibility

INTRODUCTION

Telomeres are the specialized DNA-protein structures that cap the ends of linear chromosomes. Functional telomeres require sufficient length of telomeric DNA repeats and are essential for maintaining chromosomal stability. Severely short and/or dysfunctional telomeres lead to chromosome end-to-end fusion, chromosomal rearrangements, and instability that is a common cause and hallmark of cancer [1]. In genetically engineered

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mouse models with a telomerase deficiency, the resulting telomere dysfunction has been shown to induce epithelial cancers, including mammary cancers [2]. Very short telomeres are frequently observed in pre-malignant breast lesions, i.e., atypical hyperplasia and ductal carcinoma in situ (DCIS), in humans [3–8], indicating telomere dysfunction is an early, and possible initiating event in breast cancer development. Epidemiologic studies found that having shorter overall telomere length from blood leucocytes was significantly associated with an increased risk of human bladder, head and neck, lung, and renal cell cancers [9;10]. Having a shorter overall telomere length in buccal cells was significantly associated with an increased risk of bladder cancer [11]. However, whether overall telomere length from blood cells is associated with breast cancer risk remains unclear. Four recent case-control studies examined the relationship of overall telomere length from blood leucocytes and breast cancer risk and have reported contradictory findings [12–15]. We conducted two independent case-control studies to investigate further whether the overall telomere length from blood leucocytes is associated with breast cancer risk.

MATERIALS AND METHODS

Study Populations

Analyses were conducted using data and samples from two independent breast cancer case-control studies: Roswell Park Cancer Institute Breast Cancer Study (RPCI Breast Cancer Study) and Lombardi Comprehensive Cancer Center Breast Cancer Study (LCCC Breast Cancer Study).

RPCI Breast Cancer Study—Data and samples from breast cancer cases and controls were provided by the Roswell Park Cancer Institute Data Bank and BioRepository (DBBR), a shared core resource [16]. In the DBBR, patients newly diagnosed with cancer consent to provide a blood sample and to complete a self-administered in-depth epidemiologic questionnaire. Visitors and family members of patients are consented as healthy controls, and familial relationships with patients and each other tracked so that they are not included in the same study population, avoiding over-matching. The refusal rate for participation in DBBR is approximately 10% and the questionnaire response rate is 75%. Only participants with complete questionnaire data are included in the present analyses. Blood samples are drawn by trained phlebotomists, processed and frozen within one hour of phlebotomy. For these analyses, we obtained DNA and data from the DBBR for 152 patients with histologically confirmed breast cancer who had no prior history of cancer and no systemic therapy prior to sample collection. Controls (n=176) were free of malignant diseases at the time of sample collection, with the exception of non-melanoma skin cancer, and were frequency-matched to cases on age (5 year intervals) and race.

The LCCC Breast Cancer Study—Breast cancer cases (N = 153) were recruited at the Georgetown University Hospital clinics (Lombardi Comprehensive Cancer Center's Division of Medical Oncology, Department of Surgery and the Betty Lou Ourisman Breast Cancer Clinic). Cases included women with a diagnosis of breast cancer within the prior 6 months, who had not been treated with chemotherapy and/or radiotherapy, and could provide informed consent in English. Women with a prior history of cancer, who had already received chemotherapy or radiotherapy, or had an active infection or immunological disorder requiring antibiotics or immunosuppressive medication within the prior month were excluded. From 2006 through 2008, a total of 228 newly diagnosed eligible breast cancer patients were identified and 153 (67%) participated in our study. The most common reasons for non-participation were: too busy or not interested (24%), overwhelmed by cancer diagnosis (5%), or not responsive to phone call or e-mail contact (4%).

Controls (N =159) were randomly selected from healthy women who visited the mammography screening clinic at Georgetown University Hospital, and were frequency matched to cases by age (5-year interval), race, and state of residence (D.C., Maryland or Virginia). Other inclusion and exclusion criteria for controls were the same as for cases. Additionally, women who had a recent breast biopsy (within 6 months) or were pregnant or breast feeding were not eligible. The overall participation rate among the eligible women was 57% for controls. The major reasons for non-participation were having no extra time for the study interview at the appointment for their routine mammography screening (19%) or not interested (23%).

After providing informed consent, women received a structured, in-person interview assessing prior medical history, tobacco smoke exposures, alcohol use, current medications, family medical history, reproductive history, and socioeconomic characteristics. Venous blood was obtained by trained phlebotomists using heparinized tubes.

The studies were approved by the IRB of Roswell Park Cancer Institute and IRB of MedStar Research Institute-Georgetown University.

Telomere Length Measurement

Two methods, real-time telomere quantitative PCR (TQ-PCR) [17] and telomere quantitative fluorescent in situ hybridization (TQ-FISH) [18], were used for the telomere length measurements. Both methods are well established and have been shown to reliably measure the telomere length in previous epidemiological studies [12;19;20] and in basic research [18;21;22]. Telomere length was measured by TQ-PCR using available DNA samples isolated from frozen buffy coats in the RPCI Breast Cancer Study. Telomere length was measured by TQ-FISH using cultured blood lymphocytes in the LCCC Breast Cancer Study.

Telomere Quantitative PCR—The real-time quantitative PCR method described by Cawthon [17] was used. For this study, the β-globin gene (hgb) was used as the single copy reference gene and the primer sequences for telomere amplification and β-globin gene were published before [17]. Real time kinetic quantitative PCR determines the fractional cycle (Ct) number at which the well's accumulating fluorescence crosses a set threshold of 40 that is several standard deviations above baseline fluorescence [23]. A plot of Ct versus log amount of input target DNA is linear, allowing relative quantization of unknown samples by comparison to a standard curve derived from amplification, in the same plate, of serial dilutions of a reference DNA sample. Since the amount of the PCR product approximately doubles in each cycle of the PCR, the T/S ratio is $[2^{Ct(telomere)}/2^{Ct(\beta\text{-globin})}]^{-1}$. Because the size of single copy gene products (S) is 0.076 kb, the overall telomere length (TL) is calculated based on the formula: $TL = 0.076\ kb\ (S) \times [2^{Ct(telomere)}/2^{Ct(\beta\text{-globin})}]^{-1}$. Each sample was assayed in triplicate and only the samples with a coefficient of variation (CV) among the triplicates less than 15% were accepted. Samples that failed to meet this criterion were repeated (13.5%); if the CV was again unacceptable, the sample was rejected from statistical analysis (1.3%). So for the samples used in this study the average CV was 8.1%.

Telomere Quantitative Fluorescent In Situ Hybridization (TQ-FISH)—TQ-FISH was performed on chromosome spreads of cultured blood lymphocytes. Lymphocyte cultures were prepared from fresh blood within 48 hours after the samples were obtained, as previously described [24]. The chromosome preparations were dropped onto clean microscopic slides. Slides were then dehydrated through an ethanol series, and air dried. Fifteen microliters of hybridization mixture consisting of 0.3 μg/ml Cy3-labeled telomere-specific peptide nucleic acid (PNA) probe, 50% formamide, 10 mM Tris-HCl, pH 7.5, 5%

blocking reagent, and 1× Denhart's solution was applied to each slide. Slides were cover-slipped, then placed in a Hybex microarray hybridization oven where the DNA was denatured by incubating at 75°C for five minutes, followed by hybridizing at 30°C for three hours. After hybridization, the slides were sequentially washed; once in 1 × SSC, once in 0.5 × SSC, and once in 0.1 × SSC; each wash was 10 min at 42°C. The slides were then mounted in anti-fade mounting medium containing 300 ng/ml 4'-6-diamidino-2-phenylindole (DAPI).

The slides were analyzed using a Leica DM 4000 epifluorescence microscope equipped with a charge-coupled device (CCD) camera. Images of chromosome spreads were captured and digitized fluorescent telomere signals were quantified using a semi-automated script, TeloMeter, which was written with image analysis software (ImageJ). This software permits measurement of telomere signals in defined regions, i.e., single cell [5]. Telomere length was expressed as fluorescent intensity units (FIU). For each patient, 15 metaphase spreads were analyzed to estimate the mean telomere length across all chromosomes for each sample. A control slide containing cells with known telomere length was included in each FISH assay batch. The control slide was used to generate data for the normalization of batch variations and for quality control purposes. In this study, the CV of telomere length from 20 repeated control slides was 12.4%.

Statistical Methods

Student's t-test was used to compare the means of telomere length between cases and controls because the normality test indicated that telomere length was symmetrically distributed. Chi-square tests were used to compare the distribution of categorical variables between cases and controls. Pearson correlations (r) were used to examine the associations between telomere length and age.

We examined the associations between the telomere length and the risk of breast cancer for each study separately and the two studies combined, using unconditional logistic regression. Telomere length was dichotomized as short/long based on the 50^{th} percentile values in the controls as a cut point for each study. Quartile categories of telomere length were derived according to the distribution in controls for each study. Odds ratios were adjusted for age, race, smoking status, education, and income. Given the different techniques to measure overall telomere length, the combined analysis used telomere data categorized according to each study-specific control telomere length distribution. The p-values were two-sided and considered statistically significant if $p < 0.05$. All analyses were performed using SAS software, version 9 (SAS Institute Inc., Cary, NC).

RESULTS

Characteristics of Study Population

Table 1 lists the characteristics of the study subjects from both LCCC study and the RPCI study. The mean age was approximately 3 years older in the RPCI study compared to that in the LCCC study. There were no significant case-control differences in the distributions of mean age, race, menopausal status, tobacco smoking, and alcohol use. There were significant differences in the distribution of levels of education between cases and controls in the RPCI study and borderline significant in the LCCC study, with controls having attained higher education levels. Family history of cancer was significantly higher among controls than cases (p=0.007) in RCPI study. In LCCC study, the distribution of family history of cancer was similar between case and controls. There were no significant differences in the distribution of the levels of household income between cases and controls among those who reported household income. Overall, the LCCC study population had

higher level of education and higher level of household income (median ~ $100,000) compared to the RPCI study population (median ~ $50,000, Table 1), representing the different demographics of patients seen at the RPCI and the LCCC.

Correlations of Telomere Length and host factors

In the RPCI study, we observed a moderate inverse correlation between telomere length and age in all subjects ($r = -0.26$, $p < 0.01$), in controls ($r = -0.21$, $p < 0.01$) and in cases ($r = -0.30$, $p < 0.01$). Among controls, there were no significant differences in mean telomere length between subgroups defined by race, smoking status, family history of cancer, hormone replacement therapy, and household income. Longer telomere lengths were observed among premenopausal compared with postmenopausal women ($p = 0.03$) and among women reporting ever drinking alcohol compared with those who never drank alcohol ($p = 0.02$). Among cases, longer telomeres were observed among African American women compared with white women ($p < 0.01$), among premenopausal compared to postmenopausal women ($p = 0.02$), and among women reporting high household income compared to those reporting low household income ($p < 0.01$).

In the LCCC study population, we observed a weak inverse correlation between telomere length and age in all subjects ($r = -0.16$, $p < 0.01$), in control subjects ($r = -0.15$, $p = 0.06$), and in case subjects ($r = -0.19$, $p = 0.03$). Among controls, there were no significant differences in the mean telomere length between subgroups defined by race, smoking status, alcohol drinking, family history of cancer, menopausal status, hormone replacement therapy, and household income. Among cases, longer telomeres were observed in African American women compared with white women ($p = 0.03$). Therefore, age, race, menopausal status, alcohol use and household income were included in the logistic models for adjustment.

Telomere Length of Blood Leucocytes and Breast Cancer Risk

Overall, there were no significant differences in mean telomere length between cases and controls in either study population (Table 1). No significant case-control differences were seen when the comparisons were stratified by age, race, menopausal status, cigarette smoking status, alcohol use status, family history of cancer, hormone replacement therapy status and household income.

Table 2 shows the logistic regression analysis examining the association between telomere length and breast cancer risk. Using the 50^{th} percentile value in controls as a cut point, women who had shorter telomere length were not at a significantly increased risk of breast cancer compared with women who had longer telomere length in the RPCI study (odds ratio [OR] = 1.34, 95% CI = 0.84 to 2.12), in the LCCC study (OR = 1.18, 95% CI = 0.73 to 1.91), or in the combined RPCI and LCCC studies (OR = 1.23, 95% CI = 0.89 to 1.71). Odds ratios were adjusted for age, race, education, smoking status, alcohol consumption, and household income. When the telomere length was categorized into quartiles, there was no significant dose-response relationship across quartiles of overall telomere length nor was there a statistically significant difference comparing women in the lowest to those in the highest quartile of overall telomere length (Table 3).

DISCUSSION

In this report, we did not find any significant associations between overall telomere length measured from peripheral blood leucocytes and breast cancer risk in two independent case-control studies with a total of 292 newly diagnosed, pre-treatment breast cancer cases and 335 unrelated healthy women controls. Telomere length in blood leucocytes has been proposed as a potentially useful biomarker to explore individual susceptibility to cancer in

epidemiologic studies, largely because telomere dysfunction is a characteristics of malignant epithelial tumors [25;26] and because previous studies indicated that short telomeres in blood cells were associated with increased risk for bladder [9;11], lung [27], head and neck, and renal cell cancers [10]. Four recent studies examined the association between telomere length in blood leucocytes and breast cancer risk and reported contradictory results. In a study of 287 breast cancer cases and 350 sister controls, Shen *et al* reported that having shorter overall telomere length in blood leucocytes was not significantly associated with breast cancer risk in the overall study population (OR = 1.26, 95% CI = 0.86, 1.83) [12]. The authors noted that shorter telomere length may be associated with breast cancer risk in premenopausal women (85 cases and 121 controls) with an odds ratio of 1.37 (95% CI = 0.70, 2.27) and a suggestive dose-response trend (p-for-trend = 0.14). In a study of 212 cases and 1804 controls, Barwell *et al* reported that there was no significant difference in the mean overall telomere length in blood leucocytes between breast cancer patients and normal controls [14]. When restricting the analysis to a subset of newly diagnosed, pretreatment cases and matched controls (140 cases and 108 controls), they confirmed that there was no significant difference in the mean overall telomere length in blood leucocytes between cases (mean = 6.65 kb) and controls (mean = 6.60 kb, P = 0.592). One prospective study (the Nurses' Health Study) of 1122 cases and 1147 controls just published recently and found that telomere in blood leucocytes was not significantly associated with postmenopausal breast cancer risk (OR = 1.23, 95% CI = 0.94, 1.60) [15]. However, Svenson *et al* reported that having longer overall telomere length in blood leucocytes was strongly associated with an increased breast cancer risk in a case-control study (265 cases and 446 controls) [13]. The authors did not offer a biologically plausible explanation for this observation and acknowledged that the result was unexpected and the differences in methodology related to telomere length assessment can not be ruled out. This study used the same method (TQ-PCR) to measure the telomere length as the two of other three breast cancer studies [12;15]. Our data are in agreement with the first three breast cancer studies [12;14;15] and suggest that telomere length in blood cells is not significantly associated with breast cancer risk.

It is not surprising to find that overall telomere length in blood cells is not associated with breast cancer risk. A study of genetically engineered mice provided evidence that the overall telomere length in a cell may be less important for cancer risk than short telomeres on specific chromosome arms, because chromosome fusions associated with telomere dysfunction occur preferentially on chromosome arms with the shortest telomeres [28]. In humans, there are total of 92 telomeres, and chromosome arm specific telomere lengths are highly polymorphic between chromosomal arms, and between allelic arms [29–31]. Several reports have demonstrated that chromosome arms with the shortest telomeres were more often found in the telomere fusions leading to chromosome instability [28;32]. It is well documented that frequent chromosomal abnormalities in breast cancers are not random, but concentrate on certain chromosome arms, including gains of 1q, 8q, 17q, and 20q, and losses of 8p, 9p, 16q and 17p [33–35]. Thus, overall telomere length is not likely to be very informative for cancer risk as we found in our two studies, due to the inclusion of large number of "irrelevant" telomeres in the measurement.

CONCLUSIONS

Our data provide further evidence that overall telomere length from blood leucocytes is not significantly associated with breast cancer risk. Future studies should aim to identify chromosome arm specific telomeres that potentially have better predictive power for breast cancer.

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REFERENCES

1. Gray JW, Collins C. Genome changes and gene expression in human solid tumors. Carcinogenesis. 2000; 21:443–452. [PubMed: 10688864]
2. Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, DePinho RA, Greider CW. Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell. 1997; 91:25–34. [PubMed: 9335332]
3. van Heek NT, Meeker AK, Kern SE, Yeo CJ, Lillemoe KD, Cameron JL, Offerhaus GJ, Hicks JL, Wilentz RE, Goggins MG, De Marzo AM, Hruban RH, Maitra A. Telomere shortening is nearly universal in pancreatic intraepithelial neoplasia. Am J Pathol. 2002; 161:1541–1547. [PubMed: 12414502]
4. Meeker AK, Hicks JL, Platz EA, March GE, Bennett CJ, Delannoy MJ, De Marzo AM. Telomere shortening is an early somatic DNA alteration in human prostate tumorigenesis. Cancer Res. 2002; 62:6405–6409. [PubMed: 12438224]
5. Meeker AK, Hicks JL, Gabrielson E, Strauss WM, De Marzo AM, Argani P. Telomere shortening occurs in subsets of normal breast epithelium as well as in situ and invasive carcinoma. Am J Pathol. 2004; 164:925–935. [PubMed: 14982846]
6. Engelhardt M, Drullinsky P, Guillem J, Moore MA. Telomerase and telomere length in the development and progression of premalignant lesions to colorectal cancer. Clin Cancer Res. 1997; 3:1931–1941. [PubMed: 9815582]
7. Kinouchi Y, Hiwatashi N, Chida M, Nagashima F, Takagi S, Maekawa H, Toyota T. Telomere shortening in the colonic mucosa of patients with ulcerative colitis. J Gastroenterol. 1998; 33:343–348. [PubMed: 9658312]
8. O'Sullivan JN, Bronner MP, Brentnall TA, Finley JC, Shen WT, Emerson S, Emond MJ, Gollahon KA, Moskovitz AH, Crispin DA, Potter JD, Rabinovitch PS. Chromosomal instability in ulcerative colitis is related to telomere shortening. Nat Genet. 2002; 32:280–284. [PubMed: 12355086]
9. McGrath M, Wong JY, Michaud D, Hunter DJ, De V I. Telomere length, cigarette smoking, and bladder cancer risk in men and women. Cancer Epidemiol Biomarkers Prev. 2007; 16:815–819. [PubMed: 17416776]
10. Wu X, Amos CI, Zhu Y, Zhao H, Grossman BH, Shay JW, Luo S, Hong WK, Spitz MR. Telomere dysfunction: a potential cancer predisposition factor. J Natl Cancer Inst. 2003; 95:1211–1218. [PubMed: 12928346]
11. Broberg K, Bjork J, Paulsson K, Hoglund M, Albin M. Constitutional short telomeres are strong genetic susceptibility markers for bladder cancer. Carcinogenesis. 2005; 26:1236–1271.
12. Shen J, Terry MB, Gurvich I, Liao Y, Senie RT, Santella RM. Short telomere length and breast cancer risk: a study in sister sets. Cancer Res. 2007; 67:5538–5544. [PubMed: 17545637]
13. Svenson U, Nordfjall K, Stegmayr B, Manjer J, Nilsson P, Tavelin B, Henriksson R, Lenner P, Roos G. Breast cancer survival is associated with telomere length in peripheral blood cells. Cancer Res. 2008; 68:3618–3623. [PubMed: 18483243]
14. Barwell J, Pangon L, Georgiou A, Docherty Z, Kesterton I, Ball J, Camplejohn R, Berg J, Aviv A, Gardner J, Kato BS, Carter N, Paximadas D, Spector TD, Hodgson S. Is telomere length in

peripheral blood lymphocytes correlated with cancer susceptibility or radiosensitivity? Br J Cancer. 2007; 97:1696–1700. [PubMed: 18000505]

15. De V I, Prescott J, Wong JY, Kraft P, Hankinson SE, Hunter DJ. A prospective study of relative telomere length and postmenopausal breast cancer risk. Cancer Epidemiol Biomarkers Prev. 2009; 18:1152–1156. [PubMed: 19293310]
16. Ambrosone CB, Nesline MK, Davis W. Establishing a cancer center data bank and biorepository for multidisciplinary research. Cancer Epidemiol Biomarkers Prev. 2006; 15:1575–1577. [PubMed: 16985014]
17. Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res. 2002; 30:e47. [PubMed: 12000852]
18. Slijepcevic P. Telomere length measurement by Q-FISH. Methods Cell Sci. 2001; 23:17–22. [PubMed: 11741140]
19. Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA. Association between telomere length in blood and mortality in people aged 60 years or older. Lancet. 2003; 361:393–395. [PubMed: 12573379]
20. Finley JC, Reid BJ, Odze RD, Sanchez CA, Galipeau P, Li X, Self SG, Gollahon KA, Blount PL, Rabinovitch PS. Chromosomal instability in Barrett's esophagus is related to telomere shortening. Cancer Epidemiol Biomarkers Prev. 2006; 15:1451–1457. [PubMed: 16896031]
21. Leach NT, Rehder C, Jensen K, Holt S, Jackson-Cook C. Human chromosomes with shorter telomeres and large heterochromatin regions have a higher frequency of acquired somatic cell aneuploidy. Mech Ageing Dev. 2004; 125:563–573. [PubMed: 15336914]
22. Vera E, Canela A, Fraga MF, Esteller M, Blasco MA. Epigenetic regulation of telomeres in human cancer. Oncogene. 2008; 27:6817–6833. [PubMed: 18762811]
23. Higuchi R, Fockler C, Dollinger G, Watson R. Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. Biotechnology. 1993; 11:1026–1030. [PubMed: 7764001]
24. Zheng YL, Loffredo CA, Alberg AJ, Yu Z, Jones RT, Perlmutter D, Enewold L, Krasna MJ, Yung R, Shields PG, Harris CC. Less efficient g2-m checkpoint is associated with an increased risk of lung cancer in African Americans. Cancer Res. 2005; 65:9566–9573. [PubMed: 16230422]
25. Bailey SM, Murnane JP. Telomeres, chromosome instability and cancer. Nucleic Acids Res. 2006; 34:2408–2417. [PubMed: 16682448]
26. Charames GS, Bapat B. Genomic instability and cancer. Curr Mol Med. 2003; 3:589–596. [PubMed: 14601634]
27. Jang JS, Choi YY, Lee WK, Choi JE, Cha SI, Kim YJ, Kim CH, Kam S, Jung TH, Park JY. Telomere length and the risk of lung cancer. Cancer Sci. 2008; 99:1385–1389. [PubMed: 18452563]
28. Hemann MT, Strong MA, Hao LY, Greider CW. The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. Cell. 2001; 107:67–77. [PubMed: 11595186]
29. Lansdorp PM, Verwoerd NP, van de Rijke FM, Dragowska V, Little MT, Dirks RW, Raap AK, Tanke HJ. Heterogeneity in telomere length of human chromosomes. Hum Mol Genet. 1996; 5:685–691. [PubMed: 8733138]
30. Graakjaer J, Bischoff C, Korsholm L, Holstebroe S, Vach W, Bohr VA, Christensen K, Kolvraa S. The pattern of chromosome-specific variations in telomere length in humans is determined by inherited, telomere-near factors and is maintained throughout life. Mech Ageing Dev. 2003; 124:629–640. [PubMed: 12735903]
31. Martens UM, Zijlmans JM, Poon SS, Dragowska W, Yui J, Chavez EA, Ward RK, Lansdorp PM. Short telomeres on human chromosome 17p. Nat Genet. 1998; 18:76–80. [PubMed: 9425906]
32. Der-Sarkissian H, Bacchetti S, Cazes L, Londono-Vallejo JA. The shortest telomeres drive karyotype evolution in transformed cells. Oncogene. 2004; 23:1221–1228. [PubMed: 14716292]
33. Baudis M. Genomic imbalances in 5918 malignant epithelial tumors: an explorative meta-analysis of chromosomal CGH data. BMC Cancer. 2007; 7:226. [PubMed: 18088415]
34. Gorgoulis VG, Koutroumbi EN, Kotsinas A, Zacharatos P, Markopoulos C, Giannikos L, Kyriakou V, Voulgaris Z, Gogas I, Kittas C. Alterations of p16-pRb pathway and chromosome locus 9p21-22 in sporadic invasive breast carcinomas. Mol Med. 1998; 4:807–822. [PubMed: 9990866]

35. An HX, Niederacher D, Picard F, van RC, Bender HG, Beckmann MW. Frequent allele loss on 9p21-22 defines a smallest common region in the vicinity of the CDKN2 gene in sporadic breast cancer. Genes Chromosomes Cancer. 1996; 17:14–20. [PubMed: 8889502]

Table 1

Descriptive characteristics of breast cancer cases and controls

Host factors	RPCI Breast Cancer Study			LCCC Breast Cancer Study		
	Cases (n=152)	Controls (n=176)	p-value	Cases (n=140)	Controls (n=159)	p-value
Age (years), mean (SD)	56.1 (12.3)	56.8 (12.8)	0.62	52.7 (10.6)	53.3 (9.9)	0.66
BMI (kg/m^2), mean (SD)	27.9 (8.2)	27.3 (5.9)	0.39	27.1 (6.6)	27.3 (6.3)	0.86
Race, N (%)						
Caucasian	144 (94.7)	162 (92.1)		104 (74.2)	118 (74.2)	
African American	5 (3.3)	12 (6.8)		28 (20.0)	34 (21.4)	
Other	3 (2.0)	2 (1.1)	0.30	8 (5.7)	7 (4.4)	0.85
Menopausal status, N (%)						
Pre/perimenopausal	63 (41.5)	57 (32.4)		56 (42.8)	67 (42.4)	
Postmenopausal	89 (58.6)	119 (67.6)	0.09	75 (57.3)	91 (57.6)	0.95
Cigarette smoking status, N (%)						
Never	82 (53.9)	89 (50.6)		81 (60.0)	93 (58.9)	
Ever	70 (46.0)	87 (49.5)	0.54	54 (40.0)	65 (41.1)	0.84
Alcohol consumption, N (%)						
Never	42 (27.6)	33 (18.8)		15 (11.5)	12 (7.6)	
Ever	110 (72.4)	143 (81.2)	0.06	116 (88.6)	147 (92.5)	0.23
HRT[§] use, N (%)						
Never	41 (26.9)	64 (36.4)		86 (65.7)	94 (59.1)	
Ever	108 (71.1)	112 (63.6)	0.09	45 (34.4)	65 (40.9)	0.25
Education, N (%)						
High school or less	53 (34.9)	31 (17.6)		18 (13.4)	9 (5.7)	
Post high school – 4 years college	99 (65.1)	145 (82.4)	**<0.01**	60 (44.8)	73 (45.9)	
> 4 years college				56 (41.8)	77 (48.4)	0.06

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Host factors	RPCI Breast Cancer Study			LCCC Breast Cancer Study		
	Cases (n=152)	Controls (n=176)	p-value	Cases (n=140)	Controls (n=159)	p-value
Family history of cancer[^]						
No	62 (40.8)	47 (26.7)		50 (39.7)	73 (46.5)	
Yes	90 (59.2)	129 (73.3)	**0.007**	76 (60.3)	84 (53.5)	0.25
Household income[†]*, N (%)*						
Low	72 (47.4)	66 (37.5)		46 (32.9)	56 (35.2)	
High	63 (41.5)	88 (50.0)		51 (36.4)	77 (48.4)	
Missing data	17 (11.2)	22 (12.5)	0.18	43 (30.7)	26 (16.4)	**0.01**

[§] hormone replacement therapy

[^] defined as any cancer in the first degree blood relatives

[†] RPCI study household income low < $50,000, high ≥ $50,000; LCCC study household income low ≤ $100,000 and high > $100,000.

Table 2

Case-Control Comparison of Mean Telomere Lengths, by Host Factors

Host factors	RPCI Breast Cancer Study[a]			LCCC Breast Cancer Study[b]		
	Cases (n=152) Mean (SD)	Controls (n=176) Mean (SD)	P_1	Cases (n=140) Mean (SD)	Controls (n=159) Mean (SD)	P_1
All subjects	8.64[a] (2.24)	8.25 (1.85)	0.89	4.36[b] (0.99)	4.58 (1.01)	0.07
Age						
≤ median	8.81 (2.07)	8.59 (2.00)	0.49	4.41 (1.02)	4.70 (1.03)	0.07
> median	7.70 (2.28)	7.94 (1.65)	0.46	4.26 (0.95)	4.46 (0.99)	0.20
P_2	**<0.01**	**0.02**		0.49	0.19	
Race, N (%)						
Caucasian	8.17 (2.06)	8.30 (1.87)	0.56	4.29 (1.01)	4.55 (1.03)	0.06
African American	11.01 (5.17)	7.92 (1.33)	0.08	4.75 (0.92)	4.57 (0.84)	0.43
P_2	**<0.01**	0.50		**0.03**	0.91	
Menopausal status						
Pre/perimenopausal	8.77 (1.96)	8.68 (2.03)	0.82	4.27 (0.92)	4.52 (1.06)	0.13
Postmenopausal	7.93 (2.37)	8.04 (1.73)	0.71	4.40 (1.04)	4.67 (0.95)	0.07
P_2	**0.02**	**0.03**		0.33	0.36	
Cigarette smoking status						
Never	8.48 (1.89)	8.22 (1.94)	0.39	4.36 (1.03)	4.55 (1.01)	0.22
Ever	8.05 (2.58)	8.28 (1.77)	0.51	4.43 (0.95)	4.57 (0.98)	0.43
P_2	0.24	0.84		0.67	0.89	
Alcohol consumption						
Never	7.89 (2.61)	7.59 (1.40)	0.53	4.14 (0.82)	4.18 (1.35)	0.93
Ever	8.43 (2.07)	8.41 (1.91)	0.91	4.44 (1.03)	4.61 (0.98)	0.17
P_2	0.18	**0.02**		0.22	0.30	
HRT use						

Host factors	RPCI Breast Cancer Study[a]			LCCC Breast Cancer Study[b]		
	Cases (n=152) Mean (SD)	Controls (n=176) Mean (SD)	P_1	Cases (n=140) Mean (SD)	Controls (n=159) Mean (SD)	P_1
Never	8.51 (2.37)	8.39 (2.04)	0.70	4.42 (0.98)	4.58 (0.98)	0.30
Ever	7.81 (1.79)	8.00 (1.45)	0.54	4.42 (1.02)	4.58 (1.07)	0.44
P_2	0.12	0.15		0.99	0.99	
Family history of cancer						
No	8.16 (2.48)	8.17 (1.77)	0.98	4.54 (0.94)	4.68 (1.01)	0.42
Yes	8.36 (2.06)	8.28 (1.89)	0.76	4.37 (1.02)	4.51 (1.01)	0.41
P_2	0.59	0.74		0.35	0.27	
Household income						
Low	7.53 (1.86)	8.07 (2.15)	0.11	4.45 (0.99)	4.53 (0.94)	0.69
High	9.07 (2.25)	8.45 (1.56)	0.06	4.40 (1.05)	4.51 (0.99)	0.54
Missing data	8.55 (2.67)	7.99 (1.96)	0.46	4.23 (0.92)	4.87 (1.21)	**0.03**
P_2†	**<0.01**	0.32		0.79	0.92	

[a] In the RPCI study, the unit of telomere length is kb

[b] In the LCCC study, the unit of telomere length is fluorescent intensity units in million (MFIU)

P_1 is the p-value comparing mean telomere length between cases and controls

P_2 is the p-value comparing mean telomere length between groups defined by host factors

P_2† is the p-value comparing mean telomere length between low income and high income women

Table 3

Logistic Regression Analysis Examining the Association between Telomere Length and Breast Cancer Risk

	RPCI Breast Cancer Study		LCCC Breast Cancer Study		RPCI and LCCC studies combined	
	Case /control	**OR (95% CI)**	**Case /control**	**OR (95% CI)**	**Case /control**	**OR (95% CI)**
All subjects						
≥ median	68/89	1.00	63/79	1.00	131/168	1.00
< median	84/87	1.34 (0.84–2.12)	77/80	1.18 (0.73–1.91)	161/167	1.23 (0.89–1.71)
Categorized by quartiles in controls						
Q4	35/43	1.00	25/39	1.00	60/82	1.00
Q3	33/46	0.92 (0.48–1.78)	38/40	1.56 (0.78–3.13)	71/86	1.13 (0.71–1.81)
Q2	41/41	1.33 (0.70–2.56)	30/40	1.29 (0.63–2.64)	71/81	1.22 (0.76–1.95)
Q1	43/46	1.23 (0.64–2.37)	47/40	1.76 (0.87–3.55)	90/86	1.42 (0.89–2.27)
P_{trend}		0.35		0.20		0.13
Pre/peri-menopausal women						
≥ median	36/38	1.00	30/36	1.00	66/74	1.00
< median	27/19	1.51 (0.69–3.29)	26/31	1.09 (0.52–2.29)	53/50	1.26 (0.74–2.12)
Categorized by quartiles in controls						
Q4	11/9	1.00	12/17	1.00	33/33	1.00
Q3	15/22	0.66 (0.25–1.76)	18/19	1.39 (0.51–3.84)	33/41	0.91 (0.46–1.81)
Q2	16/10	1.42 (0.48–4.20)	11/17	1.05 (0.35–3.15)	27/27	1.13 (0.54–2.37)
Q1	11/9	0.98 (0.30–3.19)	15/14	1.65 (0.55–4.93)	26/23	1.27 (0.59–2.75)
P_{trend}		0.65		0.50		0.40
Post-menopausal women						
≥ median	32/51	1.00	33/42	1.00	65/93	1.00
< median	57/68	1.27 (0.70–2.30)	42/49	1.17 (0.60–2.28)	99/117	1.16 (0.76–1.78)
Categorized by quartiles in controls						
Q4	14/27	1.00	13/21	1.00	27/58	1.00
Q3	18/24	1.34 (0.53–3.44)	20/21	1.49 (0.54–4.09)	38/45	1.37 (0.71–2.62)

	RPCI Breast Cancer Study		LCCC Breast Cancer Study		RPCI and LCCC studies combined	
	Case /control	OR (95% CI)	Case /control	OR (95% CI)	Case /control	OR (95% CI)
Q2	25/31	1.41 (0.58–3.38)	16/23	1.20 (0.44–3.27)	41/54	1.23 (0.65–2.32)
Q1	32/37	1.54 (0.66–3.61)	26/26	1.75 (0.66–4.60)	58/63	1.52 (0.82–2.79)
P_{trend}		0.34		0.35		0.25

Odds ratios were adjusted for age, race, education, alcohol consumption, household income and smoking status