Published in final edited form as:
Breast Cancer Res Treat. 2012 July ; 134(1): 393–400. doi:10.1007/s10549-012-2058-9.

Genetic polymorphisms in telomere pathway genes, telomere length and breast cancer survival

Jing Shen[1,*], **Marilie D. Gammon**[2], **Mary Beth Terry**[3], **Patrick T. Bradshaw**[2], **Qiao Wang**[1], **Susan L. Teitelbaum**[4], **Alfred I. Neugut**[3], and **Regina M. Santella**[1]

[1]Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY 10032, USA

[2]Department of Epidemiology, University of North Carolina, Chapel Hill, NC 27599, USA

[3]Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY 10032, USA

[4]Department of Community and Preventive Medicine, Mt. Sinai School of Medicine, New York, NY 10029, USA

Abstract

The impact of genetic variants in telomere pathway genes on telomere length and breast cancer survival remains unclear. We hypothesized that telomere length and genetic variants of telomere pathway genes are associated with survival among breast cancer patients. A population-based cohort study of 1,026 women diagnosed with a first primary breast cancer was conducted to examine telomere length and 52 genetic variants of 9 telomere pathway genes. Adjusted Cox regression analysis was employed to examine associations between telomere length, genetic variants and all-cause and breast cancer-specific mortality. Longer telomere length was significantly correlated with all-cause mortality in the subgroup with HER-2/neu negative tumors (HR=1.90, 95%CI: 1.12–3.22). Carrying the *PINX1-33* (rs2277130) *G*-allele was significantly associated with increased all-cause mortality (HR=1.45, 95% CI: 1.06–1.98). Three SNPs (*TERF2-03* rs35439397, *TERT-14* rs2853677 and *TERT-67* rs2853669) were significantly associated with reduced all-cause mortality. A similar reduced trend for breast cancer-specific mortality was observed for carrying the *TERT-14* (rs2853677) *T*-allele (HR=0.57, 95% CI: 0.39–0.84), while carrying the *POT1-18* (rs1034794) *T*-allele significantly increased breast cancer specific-mortality (HR=1.48, 95% CI: 1.00–2.19). However, none of the associations remained significant after correction for multiple tests. A significant dose-response effect was observed with increased number of unfavorable alleles/genotypes (*PINX1-33 G*-allele, *POT1-18 T*-allele, *TERF2-03 GG*, *TERT-14 CC*, and *TERT-67 TT* genotypes) and decreased survival. These data suggest that unfavorable genetic variants in telomere pathway genes may help to predict breast cancer survival.

Keywords

Genetic variants; telomere length; breast cancer; survival

[*]Corresponding author: Jing Shen, Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, 650 West 168th Street, Black Building 1608, New York, NY 10032, USA, Phone: 212-305-8158, Fax: 212-305-5328, js2182@columbia.edu.

Competing interests. The authors declare that they have no competing interests.

INTRODUCTION

Breast cancer mortality has modestly decreased among women in the United States during recent years, but remains the second most common cancer death [1]. Clinical histopathological characteristics of breast cancer, including tumor size, lymph node status, metastases, histological grade and type, are important determinants for prognosis [2]. In addition, molecular markers in several biological pathways, such as estrogen/progesterone (ER/PR) receptors, HER-2/neu, epidermal growth factor receptor, insulin-like growth factor/receptor and PI3K/AKT/mTOR also predict breast cancer prognosis and survival (as reviewed by Morrow [3]). Germline inherited polymorphisms may also play a role in determining prognosis by influencing host susceptibility to tumor progression and metastasis [4;5]. Several genetic variations including those in tissue inhibitors of metalloproteinases (*TIMPs*) [6], *GPX4* in the antioxidant pathway [7], *COMT* in the steroid hormone metabolism pathway [8], interleukin-10 (*IL-10*) [9], and *IL-1RN* [10] have been reported to be associated with overall survival among women with breast cancer. However, due to the biological heterogeneity of breast cancer, further efforts are needed for exploration of novel biomarkers to identify molecular subgroups of prognostic significance [11;12]. Until now, little is known about the role of genetic polymorphisms in telomere pathway genes and telomere length on breast cancer survival.

Telomeres are essential chromosome end structures consisting of tandem repeats of the TTAGGG sequence, telomerase and a number of associated proteins. The functions of telomeres are to control cellular proliferation/replication and maintain genomic integrity and stability [13;14]. Genetic variations that effect telomere elongation, activation of telomerase and configuration of telomeric proteins could disrupt or reduce these functions, and affect clinical prognosis or outcome [15–17]. One study has reported that single nucleotide polymorphisms (SNPs) in telomere pathway genes (*TERF2*, *TNKS* and *TNKS2*) were associated with high histological grade, negative estrogen receptor status and lymph node metastases, but not with breast cancer survival during the follow-up period (median of 4.7 years) [17]. Several studies have reported that telomere length in solid tumor tissues may be a potential prognostic marker (as reviewed by Bisoffi and Svenson [18;19]), and may differ by tumor type. A recent study revealed that telomere length variation in normal epithelial cells adjacent to tumor is a strong predictor of breast cancer local recurrence after breast conserving surgery [20]. Another previous study conducted among 227 breast cancer patients reported that peripheral blood telomere length may carry significant prognostic information, and longer telomere length was associated with a worse outcome compared to shorter telomere length, especially for patients age 50 or younger [16]. Thus, the impact of common inherited genetic variants in telomere pathway genes on telomere length and breast cancer survival remains unclear.

In this report, 52 genetic variants in nine key telomere maintenance genes (*PINX1*, *POT1*, *RAD18*, *TERC*, *TERF1*, *TERF2*, *TERF2IP*, *TERT* and *TNKS*) were tested to evaluate the hypothesis that genetic variations in telomere pathway genes contain valuable prognostic information to predict breast cancer survival. The long-term follow-up of the population-based Long Island Breast Cancer Study Project (LIBCSP) includes outcome data with which to evaluate this hypothesis.

MATERIALS AND METHODS

Study design and patient population

The study protocol was approved by all institutional review boards of the collaborating institutions. Detailed study methods of the parent study have been described previously [21–23].

The parent LIBCSP included a population-based cohort of 1,508 women diagnosed with breast cancer. Subjects were identified among adult female residents of Nassau and Suffolk counties on Long Island, NY, who were any race, age 20 years or older at the time of diagnosis, spoke English, and were newly diagnosed with a first primary *in situ* or invasive breast cancer between August 1, 1996, and July 31, 1997.

Baseline data collection

In-person baseline interviews were conducted by trained personnel within a few months of each case's diagnosis. The structured, baseline questionnaire collected information on known and suspected factors associated with breast cancer. Medical records were abstracted to obtain hormone receptor status and other clinical characteristics of the first primary tumor.

Study outcomes

The LIBCSP follow-up study was conducted to obtain vital status among the cohort of LIBCSP breast cancer participants. The National Death Index was used to ascertain all-cause and breast cancer-specific mortality. By the end of 2005, a total of 308 (29.1%) deaths occurred, and 164 (53.2%) deaths were due to breast cancer based on International Classification of Diseases (ICD) codes 174.9 and C–50.9. The mean follow up time was 8.0 years (range: 0.2~9.4 years).

Biospecimens

Blood samples were donated by 1,102 breast cancer patients at the time of the baseline interview. Blood was processed using a standard protocol [22], and genomic DNA was isolated by standard phenol and chloroform/isoamyl alcohol extraction and RNase treatment as previously described [24]. The current project were restricted to utilizing samples donated by breast cancer patients with sufficient DNA for measuring telomere pathway markers (n = 1,026). There were 889 cases who donated blood prior to any chemotherapy, while 137 cases provided blood post chemotherapy. The comparisons between two groups of participants (with and without sufficient DNA samples) found no significant differences in age at diagnosis, menopausal status, race, body mass index (BMI = weight in kilograms divided by height in meters squared) and cigarette smoking status. No significant differences were observed for all-cause mortality (19.1% vs. 23.6%) and breast cancer-specific mortality (10.7% vs. 11.3%) between groups with and without DNA samples.

Genes and genetic variants

A total of 24 genes were identified within the human telomere pathway by a literature review [25–28]. The nine genes (*PINX1*, *POT1*, *RAD18*, *TERC*, *TERF1*, *TERF2*, *TERF2IP*, *TERT* and *TNKS*) considered as candidate targets in the current study are important genes coding for proteins involved in telomere maintenance pathway and there is previous evidence suggesting they are related to cancer development [29]. Totally, 9,768 SNPs are known for these genes. Potential functional variants were chosen from these genes according to the criteria: (a) a minor allele frequency ≥5% and heterozygosity ≥0.1 in Caucasians; (b) >80% homology between human and rat/mouse genomes; (c) located in exons (including untranslated regions), exon-intron junctions, or promoter regions; (d) previous evidence indicating a significant functional effect; and (e) previous findings indicating associations with telomere function or cancer prognosis. Fifty-five genetic variants were selected, and three SNPs were excluded because they were not in Hardy Weinberg equilibrium or minor allele frequency was <5%. Finally, 52 variants were evaluated in the final analyses.

Laboratory methods

Telomere length was measure by a quantitative PCR (Q-PCR) method described by Cawthon [30] to determine the relative ratio of telomere (T) repeat copy number to a single-copy gene (S) copy number (T/S ratio) according to a 5-point standard curve (final concentrations from 0.125 to 2 ng/ul using mixed human genomic DNA). Variations ranged from 16 to 21% within the triplicate samples. Genotyping was analyzed by the BioTrove OpenArray™ system. SNPs that could not be well genotyped on the BioTrove OpenArray™ system were genotyped by TaqMan assays on 384 well plates. Genotype reproducibility was verified by randomly duplicated DNAs. The overall consistent rates were ranged from 96.4–97.7% [29]. All assays were performed with the laboratory personnel blinded to the cases prognostic outcome status. Detailed procedures have been reported previously [29;31].

Statistical analysis

Telomere length was evaluated as a continuous variable or categorized by the median (≥0.73 vs. <0.73) of the study subjects [31]. We also examined the associations between longer telomere length and survival outcomes by known clinical prognostic factors, including tumor type (invasive vs. *in situ*), tumor size (≥2cm vs. <2cm), donated blood status (prior to vs. post chemotherapy), estrogen receptor (ER), progesterone receptor (PR) and HER-2/neu status (negative vs. positive). However, for several subgroups, the sample sizes were too small (<5), yielding unstable results; thus these results are not shown for select subgroups (including *in situ* stage, < 2 cm tumor size, donated blood sample after initiation of chemotherapy). Covariates considered as potential confounders included age at diagnosis (continuous), race (white vs. other) and family history of breast cancer (no vs. yes). None of the covariates confounded the association between telomere length and survival outcome; the final survival model was only adjusted by age at diagnosis as a continuous variable.

Allele frequencies of genetic variants were calculated, and Hardy-Weinberg equilibrium was determined for each SNP [32]. Genotyping data were examined by a dominant model to increase statistical power, and improve stability of the results. The Kaplan-Meier and log-rank test were used to examine the associations between genetic variants, telomere length and survival outcomes [33]. Cox proportional hazard regression was used to estimate the hazard ratio (HR) and 95% confidence interval (95%CI) for all-cause and breast cancer-specific mortality separately.

A backwards elimination strategy was used to evaluate confounding, and build separate models for each SNP. If eliminating a covariate from the full Cox regression model changed the main effect of genotyping on survival by 10% or more, the covariate was considered as a potential confounder, and adjusted for in the survival model. Covariates considered as potential confounders include age at diagnosis (continuous), race (white vs. other) and family history of breast cancer (no vs. yes). None of the covariates confounded the association between the genotyping and survival outcome; the final survival model was only adjusted by age at diagnosis as a continuous variable. To account for the multiple comparison problems of 52 SNPs analyzed in the current study, the Bonferroni approach was used to adjust *p*-values. All statistical analyses were completed using Statistical Analysis System 9.0 (SAS Institute, Cary, NC).

RESULTS

When categorized by the median of telomere length in subjects, no substantial relationship was found between longer telomere length (≥0.73) and overall and breast cancer-specific mortality (Table I). In a subgroup analysis by menopausal status, a similar non-significant correlation was found between telomere length and overall and breast cancer-specific

mortality. No significant associations between longer telomere length and survival were found in subgroup analyses by tumor type (invasive vs. *in situ*), estrogen receptor (ER), progesterone receptor (PR) status (negative vs. positive) and chemotherapy status (prior to vs. post) (data not shown for select subgroups due to small sample sizes – see Methods). Significant increased all-cause mortality was observed for longer telomere length among HER-2/neu negative breast cancer case (HR=1.90, 95%CI: 1.12–3.22). However, no significant influence of longer telomere length on all-cause or breast cancer specific mortality was found among HER-2/neu positive cases (Table I). In addition, several subgroups had small numbers of subjects limiting reliability.

In the age-adjusted Cox models analyzing for 52 genetic variants, there were a total of 5 SNPs showing a significant association with either overall and/or breast cancer survival at the $p < 0.05$ significance level (Table II). Carrying the *PINX1-33* (rs2277130) *G*-allele was significantly associated with increased all-cause mortality (HR=1.45, 95% CI: 1.06–1.98). Three SNPs (*TERF2-03* rs35439397, *TERT-14* rs2853677 and *TERT-67* rs2853669) were significantly associated with reduced all-cause mortality. The HRs were, respectively, 0.72 (95% CI: 0.52–0.99), 0.64 (95% CI: 0.48–0.86) and 0.71 (95% CI: 0.54–0.94). A similar reduced trend for breast cancer-specific mortality was observed for carrying the *TERT-14* (rs2853677) *T*-allele (HR=0.57, 95% CI: 0.39–0.84), while carrying the *POT1-18* (rs1034794) *T*-allele was associated with increased breast cancer specific-mortality (HR=1.48, 95% CI: 1.00–2.19). However, after Bonferroni adjustment, none of these five SNPs remain significant. Also, no statistically significant difference was observed for telomere lengths by the five SNPs. For the other 47 SNPs, no significant correlations with overall and breast cancer survival were obtained (supplemental Table).

To investigate the cumulative effects of carrying multiple putative risk alleles on overall and breast cancer survival, we evaluated the combined role of the five SNPs with $p<0.05$. A significant dose-response effect was observed with increased number of unfavorable alleles (*PINX1-33 G*-allele, *POT1-18 T*-allele, *TERF2-03 GG*, *TERT-14 CC*, and *TERT-67 TT* genotypes) and decreased survival after adjusting for age at diagnosis (Table III). Carrying 4–5 unfavorable alleles was associated with a significant increase in all-cause mortality (HR=2.24, 95% CI: 1.35–3.72) compared to those carrying 0–1 unfavorable alleles. The HRs for carrying 2–3 and 4–5 unfavorable alleles and breast cancer-specific mortality were, respectively, 2.15 (95% CI: 1.02–4.51) and 3.53 (95% CI: 1.64–7.62) compared to those carrying 0–1 unfavorable allele. No significant trend was observed with increased unfavorable alleles and telomere lengths (Table III).

DISCUSSION

This is a large prospective breast cancer study (1,026 patients) with a follow-up time up to 9.4 years. We did not observe a significant correlation between telomere length and overall breast cancer survival. However, longer telomere length was significantly associated with worse prognosis for the subgroup of HER-2/neu negative cases. Our finding is consistent with one prior study [16], which used the same Q-PCR assay to measure peripheral blood telomere length as we did in our study; these investigators found long telomeres associated with reduced survival compared with short telomeres in the subgroup patients with positive nodes or tumor size >16mm. Most previous studies measuring telomere content or telomere length in breast tumor tissues by Southern blot or slot blot found the opposite association, i.e. shorter telomere content was associated with reduced overall survival (reviewed in [19;34]). Variable methods (terminal restriction fragment (TRF) and Q-PCR) used in measurement of telomere length can be one reason for the discrepancy, although a good correlation between TRF and Q-PCR results was reported [35]. The down-regulated immunological activation observed in cancer patients with long telomere length was another

explanation [36]. Higher percentage of regulatory T cells indicating decreased immune response was found in a subset of HER-2/neu negative breast cancer patients [37], which may lead to less telomere attrition (long telomere length) due to fewer cell divisions, and reduce antitumor activity. The mechanisms of telomere length maintenance and compensation in the two types of tissue cells (breast epithelial and hematopoietic) may be different [16]. Breast epithelial cells are telomerase competent upon mitogenic stimulation, while hematopoietic cells usually have low but detectable telomerase [38]. Therefore, a compensation in telomerase expression or a triggered alternate telomeres lengthening mechanism in patients with poor prognosis may maintain the integrity of telomere length in blood cell [39].

Five SNPs in four telomere maintenance genes (*PINX1*, *POT1*, *TERF2* and *TERT*) were initially found significantly associated with all-cause or breast cancer-specific mortalities. Carrying the deleterious *PINX1-33* (rs2277130) *G*-allele or *POT1* (rs1034794) *T*-allele was correlated with increased all-cause or breast cancer specific-mortality, respectively. Three favorable alleles in *TERF2-03* (*A*-allele), *TERT-14* (*T*-allele) and *TERT-67* (*C*-allele) were significantly associated with reduced all-cause mortality. However, these SNPs were no longer significant after Bonferroni correction for multiple comparisons. Significant dose-response effects were observed for carrying more unfavorable alleles/genotypes (*PINX1-33 G*-allele, *POT1-18 T*-allele, *TERF2-03 GG*, *TERT-14 CC*, and *TERT-67 TT* genotypes) and increased overall and breast cancer-specific mortality. These data suggest that multiple genetic variants in telomere maintenance genes may provide useful information to improve prediction of breast cancer survival.

Previous studies suggest biological mechanisms for the effects of genetic changes in telomere maintenance genes on telomere function and cancer prognosis, including breast cancer. The potential impact of genetic variants was demonstrated in a study that found telomerase activity significantly increased in carriers of the *TERT-67 TT* genotype compared to those with the *CC* genotype (p=0.03) [40]. While there are no studies that directly link mRNA levels to SNPs of telomere maintenance genes, changes in expression have been associated with telomere length, tumor stage (*POT1*) [41], severity of breast cancer progression (*TERF2*) [15], recurrent disease (*TERT*) [42] and prediction of overall and disease-free survival (*TERT*) [15]. In other studies, two mutations in *PINX1* were found to force TERT accumulation within the nucleolus [43] and one SNP in *TERF2* (rs3785074) displayed significant associations with histologic grade and negative ER status [17]. Taken together, these results suggest that it is biologically plausible for SNPs in telomere pathway genes to impact on gene expression and function.

The strengths of the current study includes the population-based study design with a relatively large sample size, the longer follow-up time with detailed survival outcomes, a moderate number of genes/SNPs within the telomere pathway and an intermediate phenotype marker (telomere length) being examined simultaneously. Several genes in the telomere pathway were found to be separately associated with overall (*PINX1*, *TERF2* and *TERT*) or breast cancer-specific survival (*POT1* and *TERT*) indicating that the genetic variants may have potential value as prognostic markers. These data are important evidence for understanding the biological mechanisms of breast cancer prognosis, although their effects need confirmation in independent studies. Genetic variant within genomic DNA are stable and easily analyzed from the perspective of clinical practice compared to RNA or protein markers. This advantage makes the establishment of robust genetic predictive models of breast cancer survival feasible in the future. One potential drawback of the current study was that only the most common SNPs in telomere relevant genes were examined, and the effects of other variations with rare frequencies were not considered. We were unable to adequately evaluate the impact of SNPs and telomere length on breast cancer mortality

categorized by several clinical prognostic factors due to small sample size and/or missing data during the medical record abstraction. Another limitation was lack of biological functional studies on candidate geneticvariants and no telomerase activities were directly evaluated.

In summary, we found five genetic variants in telomere pathway genes (*PINX1*, *POT1*, *TERF2* and *TERT*) were significantly associated with breast cancer survival in a well-designed population-based study. A significant dose-response effect of carrying more unfavorable variant alleles and increased mortality provides critical data on the role of genetic polymorphisms in breast cancer prognosis. Longer telomere length was only correlated with all-cause mortality among the subgroup of HER-2/neu negative breast cancer patients. Independent studies to evaluate these findings are necessary to exclude potential false positive associations. Relevant functional studies and direct measurement of telomerase activity will clarify the link between telomere pathway genes and breast cancer survival, and contribute to clinical prediction of prognosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Grant support: National Cancer Institute and National Institute of Environmental Health Sciences grants R03CA125768, U01CA/ES66572, P30ES009089 and P30ES10126; and awards from the Breast Cancer Research Foundation, and Women At Risk (WAR).

Abbreviations

95% CI	95% confidence interval
BMI	body mass index
ER	estrogen receptor
HR	hazard ratio
ICD	International Classification of Diseases
LIBCSP	Long Island Breast Cancer Study Project
PINX1	PIN2-interacting protein 1
POT1	Protection of telomeres
PR	progesterone receptor
Q-PCR	quantitative PCR
SNP	single nucleotide polymorphism
TERF2	Telomeric repeat binding factor 2
TERT	Telomerase reverse transcriptase

References

1. Jemal A, Siegel R, Xu J, Ward E. Cancer Statistics, 2010. CA Cancer J Clin. 2010; 60:277–300. [PubMed: 20610543]
2. Fitzgibbons PL, Page DL, Weaver D, Thor AD, Allred DC, Clark GM, Ruby SG, O'Malley F, Simpson JF, Connolly JL, Hayes DF, Edge SB, Lichter A, Schnitt SJ. Prognostic factors in breast

cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med. 2000; 124:966–978. [PubMed: 10888772]

3. Morrow PK, Hortobagyi GN. Management of breast cancer in the genome era. Annu Rev Med. 2009; 60:153–165. [PubMed: 19630569]
4. Hsieh SM, Lintell NA, Hunter KW. Germline polymorphisms are potential metastasis risk and prognosis markers in breast cancer. Breast Dis. 2006; 26:157–162. [PubMed: 17473374]
5. Hunter KW. Host genetics and tumour metastasis. Br J Cancer. 2004; 90:752–755. [PubMed: 14970848]
6. Peterson NB, Beeghly-Fadiel A, Gao YT, Long J, Cai Q, Shu XO, Zheng W. Polymorphisms in tissue inhibitors of metalloproteinases-2 and -3 and breast cancer susceptibility and survival. Int J Cancer. 2009; 125:844–850. [PubMed: 19431211]
7. Udler M, Maia AT, Cebrian A, Brown C, Greenberg D, Shah M, Caldas C, Dunning A, Easton D, Ponder B, Pharoah P. Common germline genetic variation in antioxidant defense genes and survival after diagnosis of breast cancer. J Clin Oncol. 2007; 25:3015–3023. [PubMed: 17634480]
8. Udler MS, Azzato EM, Healey CS, Ahmed S, Pooley KA, Greenberg D, Shah M, Teschendorff AE, Caldas C, Dunning AM, Ostrander EA, Caporaso NE, Easton D, Pharoah PD. Common germline polymorphisms in COMT, CYP19A1, ESR1, PGR, SULT1E1 and STS and survival after a diagnosis of breast cancer. Int J Cancer. 2009; 125:2687–2696. [PubMed: 19551860]
9. Gerger A, Renner W, Langsenlehner T, Hofmann G, Knechtel G, Szkandera J, Samonigg H, Krippl P, Langsenlehner U. Association of interleukin-10 gene variation with breast cancer prognosis. Breast Cancer Res Treat. 2010; 119:701–705. [PubMed: 19437115]
10. Grimm C, Kantelhardt E, Heinze G, Polterauer S, Zeillinger R, Kolbl H, Reinthaller A, Hefler L. The prognostic value of four interleukin-1 gene polymorphisms in Caucasian women with breast cancer: a multicenter study. BMC Cancer. 2009; 9:78. [PubMed: 19267917]
11. Reis-Filho JS, Weigelt B, Fumagalli D, Sotiriou C. Molecular profiling: moving away from tumor philately. Sci Transl Med. 2010; 2:47ps43.
12. Weigel MT, Dowsett M. Current and emerging biomarkers in breast cancer: prognosis and prediction. Endocr Relat Cancer. 2010; 17:R245–R262. [PubMed: 20647302]
13. Blackburn EH. Structure and function of telomeres. Nature. 1991; 350:569–573. [PubMed: 1708110]
14. De Boeck G, Forsyth RG, Praet M, Hogendoorn PC. Telomere-associated proteins: crosstalk between telomere maintenance and telomere-lengthening mechanisms. J Pathol. 2009; 217:327–344. [PubMed: 19142887]
15. Salhab M, Jiang WG, Newbold RF, Mokbel K. The expression of gene transcripts of telomere-associated genes in human breast cancer: correlation with clinico-pathological parameters and clinical outcome. Breast Cancer Res Treat. 2007
16. 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]
17. Varadi V, Brendle A, Brandt A, Johansson R, Enquist K, Henriksson R, Svenson U, Tavelin B, Roos G, Hemminki K, Lenner P, Forsti A. Polymorphisms in telomere-associated genes, breast cancer susceptibility and prognosis. Eur J Cancer. 2009; 45:3008–3016. [PubMed: 19766477]
18. Bisoffi M, Heaphy CM, Griffith JK. Telomeres: prognostic markers for solid tumors. Int J Cancer. 2006; 119:2255–2260. [PubMed: 16858685]
19. Svenson U, Roos G. Telomere length as a biological marker in malignancy. Biochim Biophys Acta. 2009; 1792:317–323. [PubMed: 19419696]
20. Zhou X, Meeker AK, Makambi KH, Kosti O, Kallakury BV, Sidawy MK, Loffredo CA, Zheng YL. Telomere length variation in normal epithelial cells adjacent to tumor: potential biomarker for breast cancer local recurrence. Carcinogenesis. 2012; 33:113–118. [PubMed: 22072619]
21. Cleveland RJ, Eng SM, Abrahamson PE, Britton JA, Teitelbaum SL, Neugut AI, Gammon MD. Weight gain prior to diagnosis and survival from breast cancer. Cancer Epidemiol Biomarkers Prev. 2007; 16:1803–1811. [PubMed: 17855698]
22. Gammon MD, Neugut AI, Santella RM, Teitelbaum SL, Britton JA, Terry MB, Eng SM, Wolff MS, Stellman SD, Kabat GC, Levin B, Bradlow HL, Hatch M, Beyea J, Camann D, Trent M,

Senie RT, Garbowski G, Maffeo C, Montalvan P, Berkowitz GS, Kemeny M, Citron C, Schnabel F, Schuss A, Hajdu S, Vinceguerra V, Collman GW, Obrams G. The Long Island Breast Cancer Study Project: description of a multi-institutional collaboration to indentify environmental risk factors for breast cancer. Breast Cancer Res Treat. 2002; 74:235–254. [PubMed: 12206514]

23. Xu X, Gammon MD, Zhang Y, Bestor TH, Zeisel SH, Wetmur JG, Wallenstein S, Bradshaw PT, Garbowski G, Teitelbaum SL, Neugut AI, Santella RM, Chen J. BRCA1 promoter methylation is associated with increased mortality among women with breast cancer. Breast Cancer Res Treat. 2010; 121:685–692. [PubMed: 19921426]
24. Gammon MD, Santella RM, Neugut AI, Eng SM, Teitelbaum SL, Paykin A, Levin B, Terry MB, Young TL, Wang LW, Wang Q, Britton JA, Wolff MS, Stellman SD, Hatch M, Kabat GC, Senie R, Garbowski G, Maffeo C, Montalvan P, Berkowitz GS, Kemeny M, Citron M, Schnabel F, Schuss A, Hajdu S, Vinceguerra V. Environemntal toxins and breast cancer on Long Island. I Polycyclic aromatic hydrocarbon DNA adducts. Cancer Epidemiol Biomarkers & Prev. 2002; 11:677–685.
25. Andrew T, Aviv A, Falchi M, Surdulescu GL, Gardner JP, Lu X, Kimura M, Kato BS, Valdes AM, Spector TD. Mapping genetic loci that determine leukocyte telomere length in a large sample of unselected female sibling pairs. Am J Hum Genet. 2006; 78:480–486. [PubMed: 16400618]
26. Blasco MA. Telomeres and human disease: ageing, cancer and beyond. Nat Rev Genet. 2005; 6:611–622. [PubMed: 16136653]
27. Rodier F, Kim SH, Nijjar T, Yaswen P, Campisi J. Cancer and aging: the importance of telomeres in genome maintenance. Int J Biochem Cell Biol. 2005; 37:977–990. [PubMed: 15743672]
28. Savage SA, Stewart BJ, Liao JS, Helman LJ, Chanock SJ. Telomere stability genes are not mutated in osteosarcoma cell lines. Cancer Genet Cytogenet. 2005; 160:79–81. [PubMed: 15949576]
29. Shen J, Gammon MD, Wu HC, Terry MB, Wang Q, Bradshaw P, Teitelbaum SL, Neugut AI, Santella RM. Multiple genetic variants in telomere pathway genes and breast cancer risk. Cancer Epidemiology, Biomarkers & Prevention. 2010; 19:219–228.
30. Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res. 2002; 30:e47. [PubMed: 12000852]
31. Shen J, Gammon MD, Terry MB, Wang Q, Bradshaw P, Teitelbaum SL, Neugut AI, Santella RM. Telomere length, oxidative damage, antioxidants and breast cancer risk. Int J Cancer. 2009; 124:1637–1643. [PubMed: 19089916]
32. Cox DG, Canzian F. Genotype transposer: automated genotype manipulation for linkage disequilibrium analysis. Bioinformatics. 2001; 17:738–739. [PubMed: 11524375]
33. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc. 1958; 53:457–481.
34. Fordyce CA, Heaphy CM, Bisoffi M, Wyaco JL, Joste NE, Mangalik A, Baumgartner KB, Baumgartner RN, Hunt WC, Griffith JK. Telomere content correlates with stage and prognosis in breast cancer. Breast Cancer Res Treat. 2006; 99:193–202. [PubMed: 16752076]
35. Cawthon RM. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. Nucleic Acids Res. 2009; 37:e21. [PubMed: 19129229]
36. Liu HQ, An JZ, Liu J, Yang YF, Zhang HX, Zhao BY, Li JB, Yang HS, Chen ZN, Xing JL. Leukocyte telomere length predicts overall survival in hepatocellular carcinoma treated with transarterial chemoembolization. Carcinogenesis. 2012
37. Muraro E, Martorelli D, Turchet E, Miolo G, Scalone S, Comaro E, Talamini R, Mastorci K, Lombardi D, Perin T, Carbone A, Veronesi A, Crivellari D, Dolcetti R. A different immunologic profile characterizes patients with HER-2-overexpressing and HER-2-negative locally advanced breast cancer: implications for immune-based therapies. Breast Cancer Res. 2011; 13:R117. [PubMed: 22112244]
38. Weng NP. Telomere and adaptive immunity. Mech Ageing Dev. 2008; 129:60–66. [PubMed: 18199471]
39. Hodes RJ, Hathcock KS, Weng NP. Telomeres in T and B cells. Nat Rev Immunol. 2002; 2:699–706. [PubMed: 12209138]

40. Hsu CP, Hsu NY, Lee LW, Ko JL. Ets2 binding site single nucleotide polymorphism at the hTERT gene promoter--effect on telomerase expression and telomere length maintenance in non-small cell lung cancer. Eur J Cancer. 2006; 42:1466–1474. [PubMed: 16737810]

41. Kondo T, Oue N, Yoshida K, Mitani Y, Naka K, Nakayama H, Yasui W. Expression of POT1 is associated with tumor stage and telomere length in gastric carcinoma. Cancer Res. 2004; 64:523–529. [PubMed: 14744765]

42. Elkak A, Mokbel R, Wilson C, Jiang WG, Newbold RF, Mokbel K. hTERT mRNA expression is associated with a poor clinical outcome in human breast cancer. Anticancer Res. 2006; 26:4901–4904. [PubMed: 17214359]

43. Lin J, Jin R, Zhang B, Yang PX, Chen H, Bai YX, Xie Y, Huang C, Huang J. Characterization of a novel effect of hPinX1 on hTERT nucleolar localization. Biochem Biophys Res Commun. 2007; 353:946–952. [PubMed: 17198684]

Table 1

Age-adjusted hazard ratio (HR) and 95% confidence interval (CI) for longer telomere length and all-cause or breast cancer-specific mortality after 8 years of follow-up among a population-based cohort of women diagnosed with breast cancer in 1996–1997, Long Island Breast Cancer Study Project

characteristics	Telomere length* (T/S ratio)	No. of cases	All-cause mortality		Breast cancer-specific mortality	
			Death	HR (95%CI)	Death	HR (95%CI)
All subjects	< 0.73	510	89	1.00 (Ref.)	53	1.00 (Ref.)
	≥ 0.73	516	103	1.10 (0.83–1.46)	54	1.01 (0.69–1.47)
Menopausal status						
Premenopausal	< 0.73	183	24	1.00 (Ref.)	22	1.00 (Ref.)
	≥ 0.73	145	19	1.01 (0.55–1.84)	13	0.75 (0.38–1.50)
Postmenopausal	< 0.73	315	62	1.00 (Ref.)	28	1.00 (Ref.)
	≥ 0.73	359	82	1.16 (0.83–1.62)	41	1.30 (0.81–2.11)
Cancer type**						
Invasive	< 0.73	418	83	1.00 (Ref.)	50	1.00 (Ref.)
	≥ 0.73	430	96	1.07 (0.80–1.44)	52	1.01 (0.69–1.50)
Tumor size**						
≥ 2cm	< 0.73	97	25	1.00 (Ref.)	21	1.00 (Ref.)
	≥ 0.73	96	21	0.69 (0.38–1.25)	13	0.53 (0.26–1.06)
ER status						
Positive	< 0.73	254	40	1.00 (Ref.)	25	1.00 (Ref.)
	≥ 0.73	253	50	1.11 (0.73–1.69)	26	1.05 (0.60–1.83)
Negative	< 0.73	72	23	1.00 (Ref.)	16	1.00 (Ref.)
	≥ 0.73	86	26	0.87 (0.50–1.52)	15	0.74 (0.37–1.50)
PR status						
Positive	< 0.73	254	40	1.00 (Ref.)	25	1.00 (Ref.)
	≥ 0.73	253	50	1.11 (0.73–1.69)	26	1.05 (0.60–1.83)
Negative	< 0.73	72	23	1.00 (Ref.)	16	1.00 (Ref.)
	≥ 0.73	86	26	0.87 (0.50–1.52)	15	0.74 (0.37–1.50)
HER-2/neu status						
Positive	< 0.73	325	67	1.00 (Ref.)	41	1.00 (Ref.)
	≥ 0.73	344	65	0.85 (0.61–1.20)	37	0.83 (0.53–1.29)

characteristics	Telomere length* (T/S ratio)	No. of cases	All-cause mortality		Breast cancer-specific mortality	
			Death	HR (95%CI)	Death	HR (95%CI)
Negative	< 0.73	185	22	1.00 (Ref.)	12	1.00 (Ref.)
	≥ 0.73	172	38	**1.90 (1.12–3.22)**	17	1.62 (0.77–3.39)
Chemotherapy**						
Prior to	< 0.73	453	81	1.00 (Ref.)	49	1.00 (Ref.)
	≥ 0.73	436	84	1.04 (0.77–1.41)	43	0.91 (0.60–1.37)

*Median;

**Sample sizes were less than 5 for select subgroups (breast cancer cases with *in situ* tumor stage, tumors <2 cm, and blood draw occurred after chemotherapy had been initiated) yielding unstable results, thus these data are not shown.

Table 2

Age-adjusted hazard ratios (HRs) and 95% confidence interval (CIs) for selected genetic variants of telomere-pathway genes and all-cause or breast cancer-specific mortality after 8 years of follow-up among a population-based cohort of women diagnosed with breast cancer in 1996–1997, Long Island Breast Cancer Study Project

Gene rs#	Genotype	All-cause mortality		Breast cancer-specific mortality	
		Death (%)	HR (95%CI)	Death (%)	HR (95%CI)
PINX1-33	CC	58 (14.83)	1.00 (Ref.)	35 (8.95)	1.00 (Ref.)
rs2277130	CG/GG	122 (21.33)	**1.45 (1.06–1.98)**	67 (11.71)	1.36 (0.90–2.04)
POT1-18	AA	82 (17.23)	1.00 (Ref.)	41 (8.61)	1.00 (Ref.)
rs1034794	AT/TT	106 (20.46)	1.15 (0.86–1.53)	64 (12.36)	**1.48 (1.00–2.19)**
TERF2-03	GG	136 (20.51)	1.00 (Ref.)	78 (11.76)	1.00 (Ref.)
rs35439397	GA/AA	49 (15.22)	**0.72 (0.52–0.99)**	26 (8.07)	0.67 (0.43–1.05)
TERT-14	CC	75 (23.96)	1.00 (Ref.)	45 (14.38)	1.00 (Ref.)
rs2853677	CT/TT	108 (16.17)	**0.64 (0.48–0.86)**	57 (8.53)	**0.57 (0.39–0.84)**
TERT-67	TT	112 (22.27)	1.00 (Ref.)	62 (12.33)	1.00 (Ref.)
rs2853669	TC/CC	84 (15.82)	**0.71 (0.54–0.94)**	49 (9.23)	0.73 (0.50–1.06)

Table 3

Number of unfavorable alleles in telomere maintenance genes and all-cause or breast cancer-specific mortality after 8 years of follow-up among a population-based cohort of women diagnosed with breast cancer in 1996–1997, Long Island Breast Cancer Study Project.

No of Unfavorable alleles/genotypes*	Telomere length (T/S ratio)**	No. of cases	All-cause mortality		Breast cancer-specific mortality	
			Deaths	HR (95% CI) #	Deaths	HR(95% CI) #
0–1	1.09	163	20	1.00	8	1.00
2–3	1.03	520	90	1.36 (0.84–2.21)	54	2.15 (1.02–4.51)
4–5	1.02	218	61	2.24 (1.35–3.72)	35	3.53 (1.64–7.62)

*Carrying *PINX1-33 G*-allele, *POT1-18 T*-allele, *TERF2-03 GG*, *TERT-14 CC*, and *TERT-67 TT* genotypes;

**Means

#Adjusted for age at diagnosis as continuous, HR = 1.54 (95%CI: 1.22–1.96) for all-cause mortality, P_{trend} = 0.0004; HR = 1.78 (95%CI: 1.30–2.45) for breast cancer-specific mortality, P_{trend} = 0.0004