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Telomere Length, Telomere-Related Genes, and Breast Cancer Risk: The Breast Cancer Health Disparities Study

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Abstract

Telomeres are involved in maintaining genomic stability. Previous studies have linked both telomere length (TL) and telomere-related genes with cancer. We evaluated associations between telomere-related genes, TL, and breast cancer risk in an admixed population of US non-Hispanic white (1,481 cases, 1,586 controls) and U.S. Hispanic and Mexican women (2,111 cases, 2,597 controls) from the Breast Cancer Health Disparities Study. TL was assessed in 1,500 women based on their genetic ancestry. TL-related genes assessed were *MEN1, MRE11A, RECQL5, TEP1, TERC, TERF2, TERT, TNKS*, and *TNKS2*. Longer TL was associated with increased breast cancer risk [odds ratio (OR) 1.87, 95% confidence interval (CI) 1.38, 2.55], with the highest risk (OR 3.11, 95% CI 1.74, 5.67 p interaction 0.02) among women with high Indigenous American ancestry. Several TL-related single nucleotide polymorphisms had modest association with breast cancer risk overall, including *TEP1* rs93886 (OR 0.82, 95% CI 0.70,0.95); *TERF2* rs3785074 (OR 1.13, 95% CI 1.03,1.24); *TERT* rs4246742 (OR 0.85, 95% CI 0.77,0.93); *TERT* rs10069690 (OR 1.13, 95% CI 1.03,1.24); *TERT* rs2242652 (OR 1.51, 95% CI 1.11,2.04); and *TNKS* rs6990300 (OR 0.89, 95% CI 0.81,0.97). Several differences in association were detected by hormone receptor status of tumors. Most notable were associations with *TERT* rs2736118 (OR_{adj} 6.18, 95% CI 2.90, 13.19) with estrogen receptor negative/progesterone receptor positive

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(ER−/PR+) tumors and *TERT* rs2735940 (OR_{adj} 0.73, 95% CI 0.59, 0.91) with ER−/PR− tumors. These data provide support for an association between TL and TL-related genes and risk of breast cancer. The association may be modified by hormone receptor status and genetic ancestry.

Introduction

Telomeres cap the ends of linear chromosomes and play a role in maintaining genomic stability (Mirabello et al., 2010a,b). They prevent chromosomes from shortening during DNA replication, by precluding chromosome ends from being recognized as double strand breaks (DSBs) that are targeted for repair, resulting in the improper joining of chromosome ends (Cassidy et al., 2010). Telomeres consist of tandem $(TTAGGG)_n$ nucleotide repeats that shorten with aging (Lin et al., 2010) and are necessary for the maintenance of chromosome length because DNA polymerase is unable to replicate the 3^I ends of chromosomes. Telomere length (TL) has been linked to a number of diseases, including cardiovascular diseases, diabetes, osteosarcoma, and multiple types of cancer (Mirabello et al., 2009; Njajou et al., 2009; Mirabello et al., 2010a,b; Pooley et al., 2010; Hoen et al., 2011; Mirabello et al., 2011; O'Donovan et al., 2011). Recent studies have suggested an association between TL and breast cancer risk, however, relatively few studies examined the association between genetic variants in telomere-related genes and breast cancer susceptibility (Savage et al., 2007; Martinez-Delgado et al., 2011; Shen et al., 2012).

TL is maintained by a number of telomere-related factors. One such factor is the telomerase enzyme. Telomerase is composed of telomerase reverse transcriptase (*TERT*) and telomere RNA component (*TERC*). *TERT* uses *TERC* as a template for the synthesis of single stranded DNA within the telomere, thereby preventing the chromosome from shortening during chromosomal replication. However, telomerase activity is usually absent in differentiated cells presumably causing telomeres to shorten with age; when TL reaches a critical limit the cell undergoes senescence (Cassidy et al., 2010; Mirabello et al., 2010a,b; Shammas, 2011; Wolkowitz et al., 2011).

In addition to *TERT* and *TERC* activity, recent studies have begun to examine the effects of single nucleotide polymorphisms (SNPs) in other telomere-related genes. Multiple endocrine neoplasia type I (*MEN1*), RECQ Protein-Like 5 (*RECQL5*), Meiotic Recombination 11 Homolog A (*MRE11A*), TRF1-interacting ankyrin-related ADP-ribose polymerase (*TNKS*), TRF1-interacting ankyrin-related ADP-ribose polymerase 2 (*TNKS2*), Telomerase-associated protein 1 (*TEP1*), and Telomeric repeat-binding factor 2 (*TERF2*) have been reported as being associated with TL (Chiang et al., 2006; Mirabello et al., 2010a,b). *MEN1* is associated with the *TERT* promoter. *RECQL5* is a helicase associated with protecting genomic integrity. *MRE11A* modulates t-loop formation; t-loops prevent telomeres from being recognized as DSBs and being targeted for repair. *TNKS* is a polymerase believed to positively regulate TL. *TNKS2* inhibits telomere elongation. *TEP1* and *TERF2* are components of the ribonucleoprotein complex responsible for telomerase activity (Chiang et al., 2006).

Some reports have linked these genes to breast cancer. *TERT* (Savage et al., 2007; Shen et al., 2012), *TEP1* (Salhab et al., 2008), *TERF2* (Varadi et al., 2009; Shen et al., 2012), *RECQL5* (Islam et al., 2012), *TNKS2* (Varadi et al., 2009), and *MEN1* (Imachi et al., 2010) have been shown to be associated with breast cancer susceptibility and/or survival. Although *MRE11A* and *TNKS* have been shown to interact with *BRCA1* and *BRCA2*, evaluation of specific SNPs within these genes has been inconclusive regarding their effect on breast cancer risk overall (Gelmini et al., 2004; McCabe et al., 2009; Loizidou et al., 2010; Rebbeck et al., 2011).

In this study, we examined associations between breast cancer risk and TL and telomere-related SNPs. We evaluated SNPs in *TERT* and candidate SNPs in *TERC*, MEN1, *RECQL5, MRE11A, TEP1, TERF2, TNKS*, and *TNKS2* which have previously been associated with TL. We added to the previous work by examining TL and telomere-related genes in an admixed population of non-Hispanic white (NHW), Hispanic and Native American women from the U.S and Mexico. Breast cancer risk has been shown to vary significantly in these racial and ethnic groups, with higher incidence rates in NHW than Hispanic and Native American women. We also evaluated breast cancer associations by menopausal status and estrogen receptor (ER) and progesterone receptor (PR) tumor status.

Methods

The Breast Cancer Health Disparities Study includes participants from three population-based case–control studies: the 4-Corner's Breast Cancer Study, the Mexico Breast Cancer Study, and the San Francisco Bay Area Breast Cancer Study (Slattery et al., 2012). All participants signed informed written consent prior to participation and each study was approved by their Institutional Review Board for Human Subjects.

The 4-Corner's Breast Cancer Study participants were NHW, Hispanic, or Native American women living in nonreservation areas in the states of Arizona, Colorado, New Mexico, or Utah at the time of diagnosis or selection (Slattery et al., 2007). The present analyses included female cases between 25 and 79 years of age with a histologically confirmed diagnosis of in situ or invasive breast cancer between October 1999 and May 2004. Controls were selected to match the age and ethnicity of the cases. A total of 1,833 cases and 2,057 controls with interview data collected by in-person interview and DNA were included.

Participants from the Mexico Breast Cancer Study were between 28 and 74 years of age, living in Monterrey, Veracruz and Mexico City for the past five years (Seinost et al., 2000). Eligible cases were diagnosed with either a new histologically confirmed in situ or invasive breast cancer between January 2004 and December 2007 at 12 participating hospitals from three main health care systems in Mexico. Controls were randomly selected from the catchment area of the 12 participating hospitals using a probabilistic multistage design. Interview data and DNA were available for 816 cases and 994 controls.

The San Francisco Bay Area Breast Cancer Study included women aged 35 to 79 years from the San Francisco Bay Area diagnosed with a first primary histologically confirmed invasive breast cancer; controls were identified by random-digit dialing and frequency-matched to cases based on the expected race/ethnicity and 5-year age distribution (John et al., 2003, 2005). This analysis included subjects with data collected by in-person interview and DNA (cases diagnosed between April 1997 and April 2002 and their matched controls) including 1,105 cases and 1,318 controls.

Data Harmonization

Data were harmonized across all study centers and questionnaires (Slattery et al., 2012). Adjustment variables used in the analyses included body mass index (BMI) calculated as weight (kg) divided by height squared [meters squared (m^2)] which was based on measured height (or self-reported height if the measurement was declined) and self-reported weight during the referent year. The referent year was defined as the year prior to diagnosis for cases or selection for controls. Parity was defined as the number of full-term pregnancies, age at first birth was defined as age at first live birth or still birth, race/ethnicity in U.S. studies was based on self-report (all women in Mexico were classified as Hispanic since race/ethnicity was not asked), and education was based on the highest level of reported education. Women were classified as either premenopausal or postmenopausal based on

responses to questions on menstrual history. Women who reported still having periods during the referent year were classified as premenopausal. Center-specific definitions were used to define postmenopausal women. Within each study, women were classified as postmenopausal if they were taking menopausal hormone therapy and still having periods and were at or above the 95th percentile of age for race/ethnicity of those who reported having a natural menopause (i.e., ≥12 months since their last period). This age was 58 years for NHW and 56 for Hispanics from the 4-Corner's Breast Cancer Study, 54 for the Mexico Breast Cancer Study, and 55 for NHW and 56 for Hispanics from the San Francisco Bay Area Breast Cancer Study. Average alcohol intake (grams per day) consumed over the lifetime was available for all but about 600 cases and controls from California. For those women, we used alcohol consumption during the referent year as an adjustment variable. Physical activity was harmonized as hours per week of vigorous intensity activity performed during the referent year and analyzed using center-specific cut-points to accommodate the level of inquiry of each study questionnaire

Genetic Data

DNA was derived from either whole blood or mouthwash samples obtained from study participants. A total of 7,287 blood-derived and 634 mouthwash-derived samples were examined. Candidate SNPs were selected based on previous data that suggested an association with TL, except for *TERT* and *TNKS* where a tagSNP approach was used to identify SNPs. TagSNPs were selected based on linkage disequilibrium (LD) blocks defined using a Caucasian LD map and an $r^2 = 0.8$; minor allele frequency >0.1; range = −1,500 bps from the initiation codon to +1,500 bps from the termination codon; and 1 SNP/LD bin. Genetic admixture data were obtained by evaluation of 104 Ancestral Informative Markers (AIMs) to distinguish European and Indigenous American (IA) ancestry in the study population (Slattery et al., 2012). All markers were genotyped using a multiplexed bead array assay format based on GoldenGate chemistry (Illumina, San Diego, California). A genotyping call rate of 99.93% was attained (99.65% for whole genome amplified samples). We included 132 internal replicates that were blinded representing 1.6% of the sample set. The duplicate concordance rate was 99.996% as determined by 193,297 matching genotypes among sample pairs. Table 1 shows SNPs assessed in this study. Online Supporting Information Table S1 details the LD between SNPs within a gene.

TL

TL was measured using a multiplexed quantitative PCR (qPCR) method previously described by Cawthon (2009). This method modified earlier qPCR methods in which telomere signals were measured separately from single copy gene (scg) signals in order to produce a *T/S* ratio corresponding to TL. The multiplexed PCR analysis uses a single dye and measures both the telomere signals and scg signals in the same well. This is achieved by using CG clamps to stabilize the scg giving it a higher melting point. The telomere amplification signal is collected early in the thermal cycling, while the scg amplification signal is still at baseline; and the scg amplification signal is collected at later cycles at a high temperature that completely melts the telomere amplification product, sending its fluorescent signal to baseline. This design allows a single qPCR to determine the *T/S* ratio. DNA from blood was used to measure TL on a subset of the entire study population that was selected to include 250 cases and 250 controls from each of the three genetic admixture groups evaluated in this study. Thirty-two individuals were excluded from analysis because of failed sample or results being outside of the boundaries of the standard curve.

Tumor Characteristics

Cancer registries in Utah, Colorado, Arizona, New Mexico, and California provided information on stage at diagnosis, ER and PR status. Information on ER and PR status was

available for 1,019 (69%) NHW cases and 977 (75%) Hispanic cases. Information on tumor characteristics was not available for cases from Mexico.

Statistical Methods

The program STRUCTURE was used to compute individual ancestry for each study participant assuming two founding populations (Pritchard et al., 2000; Falush et al., 2003; Slattery et al., 2012). A two-population structure model best fit the population and was used in analysis. Genetic ancestry was used as a continuous variable to adjust for possible confounding. Genes were assessed for their association with breast cancer risk by strata of genetic ancestry, ER/PR status, and menopausal status. For these later analyses, genetic ancestry was categorized based on the distribution of percent IA ancestry in the control population. Three strata, 0–28%, 29–70%, and 71–100%, were used to evaluate potential differences in association by level of IA ancestry. All statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). Logistic regression models were used to estimate the age and study center-adjusted odds ratios (OR) for breast cancer risk associated with SNPs. Additionally, we adjusted for potential confounding variables of BMI, parity, age at first birth, hours of vigorous-intensity physical activity, alcohol consumption, and genetic admixture when not stratifying by it. Associations with SNPs were assessed assuming a co-dominant model. Based on the initial assessment, SNPs which appeared to have a dominant or recessive mode of inheritance were evaluated with those inheritance models in subsequent analyses. Interactions between genetic variants, genetic ancestry and menopausal status were assessed using *P* values from a likelihood-ratio test comparing a full model that included an ordinal interaction term with a reduced model without an interaction term.

P values corresponding to Wilcoxon rank-sum tests are presented alongside TL medians and interquartile ranges, stratified by self-reported race/ethnicity and genetic admixture group. Associations with breast cancer risk and TL categorized into quartiles for the overall population and for the three genetic admixture groups were estimated using ORs from logistic regression models. Interaction between TL and genetic ancestry was assessed using *P* values from a 1-df likelihood-ratio test (as previously mentioned). Generalized linear models (GLMs) were used to estimate the log transformed TL means by genotype among the control population. Logistic regression and GLM analyses were adjusted for age, study center, genetic admixture, and BMI.

As *TERT* and *TNKs* were evaluated using a tagSNP approach we adjusted for multiple comparisons. We used *P* values based on 1-df Wald test statistics for adjustment of multiple comparisons taking into account tagSNPs within the gene using the step-down Bonferroni correction (i.e., Holm method) based on the effective number of independent SNPs as determined using the SNP spectral decomposition method proposed by Nyholt (Suarez et al., 2007) and modified by Li and Ji (2005).

Results

Among controls, 31.5% of NHWs and 40.7% of Hispanics were premenopausal (Table 2). Among cases, ER+/PR+ was the most common tumor phenotype for both NHW and Hispanic women. ER−/PR− tumors were observed in 18.4% of NHW women and 23.4% of Hispanic women. TL in cases was generally longer than that observed for controls. This trend in TL was observed for all self-reported race/ethnicity groups and for all strata of genetic ancestry. TL was significantly inversely associated with age for all women combined and for women in each ancestry group ($P < 0.001$).

The association between TL and breast cancer risk for all women and by genetic ancestry is shown in Table 3. Longer TL was associated with increased breast cancer risk (OR 1.87, 95% CI 1.38, 2.55). The TL and breast cancer association was stronger in women with greater IA ancestry, with ORs of 3.11 (95% CI 1.74, 5.67) for women with IA ancestry >70%, 2.15 (95% CI 1.22, 3.82) for those with IA ancestry 29–70%, and 1.22 (95% CI 0.75, 1.96) for women with IA ancestry <29%; the difference in associations between TL and breast cancer risk between admixture groups was statistically significant (*P* interaction = 0.02). Associations between TL and breast cancer risk did not differ by menopausal status (data not shown).

Assessment of the relationship between SNPs and TL among controls showed some associations (see online Supporting Information Table S2 for TL by genotype and by menopausal status). *TERT* rs2736118 was associated with TL overall (*P* = 0.0007), with longer TL in individuals with the GG genotype after adjusting for age, BMI, genetic ancestry, and study; this association was strongest for postmenopausal women (*P* = 0.003). *TNKS* rs10509637 also was associated with TL among controls after adjustment, where individuals with the GG genotype had significantly longer TL relative to those carrying other genotypes (*P* = 0.019). Among premenopausal women the variant genotypes of *MRE11A* rs12270338 and rs13447720 were associated with longer TL (both *P* = 0.028) and the variant genotype of *TERT* rs2736100 was marginally associated with longer TL (*P* = 0.055). Among postmenopausal women the variant genotypes of *TNKS* rs11249943 and *TNKS* rs11991621 were associated with shorter TL (*P* = 0.04 and 0.054 respectively).

Five *TERT* SNPs: rs4246742, rs10069690, rs2242652, rs2736100, and rs2853676, were associated with overall breast cancer risk, although most associations were modest (Table 4). The variant allele in *TERT* rs4246742 was inversely associated with breast cancer risk among all women (OR 0.85, 95% CI 0.77, 0.93). Positive associations were found for *TERT* rs10069690 (OR 1.13, 95% CI 1.03, 1.24), *TERT* rs2242652 (OR 1.51, 95% CI 1.11, 2.04), and *TERT* rs2853676 (OR 1.23, *95*% CI 1.00, 1.51). The variant allele in *TERT* rs2242652 exhibited a slightly stronger association among postmenopausal women (OR 1.86, 95% CI 1.27, 2.74) and *TERT* rs2736100 was associated with risk only in postmenopausal women (OR 1.20, 95% CI 1.01, 1.42).

Associations with variants in *TEP1* (rs938886), *TERF2* (rs3785074), and *TNKS* (rs6990097 and rs6990300) are shown in Table 4. *TEP1* rs938886 was inversely associated with breast cancer risk (OR 0.82, 95% CI 0.70, 0.95), whereas *TERF2* rs3785074 was positively associated with risk (OR 1.13, 95% CI 1.03, 1.24). For both *TNKS* SNPs we found inverse associations, however, for *TNKS* rs6990097 the association was statistically significant for premenopausal women only (OR 0.83, 95% CI 0.71, 0.98). For TNKS rs6990300, the OR was 0.89 (95% CI 0.81, 0.97) for all women combined.

We also examined associations with breast cancer risk within genetic admixture groups (data in online Supporting Information Table 3). Some associations were significantly different across the admixture groups. *MEN1* rs670358 showed slightly greater risk for the AA genotype among women with greater European ancestry (OR 1.39, 95% CI 0.73, 2.64), whereas those with more IA ancestry were at a nonsignificant reduced risk (OR 0.93, 95% CI 0.65, 1.35) (*P* interaction 0.03). Likewise *MRE11A* rs13447720 showed a slightly greater nonsignificant risk for those with higher IA ancestry (OR 1.21, 95% CI 0.85, 1.74), whereas for women with higher European ancestry the OR was 0.94 (95% CI 0.82, 1.07) (*P* interaction 0.04). Other SNPs showed significant associations within strata of admixture, although no significant interactions between admixture groups were noted. Noteworthy associations were observed for *TEP1* rs938886 CC versus GG/GC genotypes for intermediate and high IA ancestry groups (OR 0.79, 95% CI 0.63, 0.98; and OR 0.73, 95%

CI 0.52, 1.05, respectively), *TERT* rs4246742 dominant model for higher European ancestry (OR 0.79, 95% CI 0.68, 0.91) and higher IA ancestry (OR 0.76, 95% CI 0.58, 0.99), and *TNKS* rs6990300 dominant model for those with the highest level of European ancestry (OR 0.86,95% CI 0.75, 0.98) or IA ancestry (OR 0.73, 95% CI 0.54, 0.98).

We observed differences in risk by menopausal status and IA ancestry (Table 5). Among premenopausal women we observed a significant interaction with *TERC* rs12696394, with a slightly increased risk among those with more European ancestry (OR 1.50, 95% CI 0.95, 2.32) and a reduced risk among those with the most IA ancestry (OR 0.59, 95% CI 0.27, 1.11) (p interaction 0.03).

Multiple SNPs were found to be associated with joint ER/PR status of breast cancer tumors (Table 6). *RECQL5* rs821052, *TERT* rs2736118, *TERT* rs2736100, *TERT* rs4246742, *TERT* rs10069690, *TERT* rs2735940, *TNKS* rs6990097, *TNKS* rs10903314, and *TNKS* rs17150478 were all associated with ER/PR status. *TERT* rs10069690 was found to be associated with ER+/PR+ tumors (OR 1.17, 95% CI 1.03, 1.34). *RECQL5* rs821052 was associated with ER+/PR− tumors (OR 1.46, 95% CI 1.08, 1.98) and *TERT* rs2736118 with ER−/PR+ tumors (OR 6.18, 95% CI 2.90, 13.19). *TERT* rs4246742 (OR 0.80, 95% CI 0.64, 0.99), *TERT* rs2736100 (OR 0.79 95% CI 0.63, 0.98), *TERT* rs27359400 (OR 0.73 95% CI 0.59, 0.91), *TNKS* rs6990097 (OR 0.78, 95% CI 0.63, 0.98), *TNKS* rs10903314 (OR 0.79, 95% CI 0.64, 0.99), and *TNKS* rs17150478 (OR 0.76, 95% CI 0.61, 0.96) were all associated with ER−/PR− tumors only. Although data for TL and ER/PR status of breast cancer tumors were only available for Utah and thus we had limited power to detect an association, we did not see an association between TL and ER/PR status.

Discussion

In this study, we found that longer TL was associated with increased breast cancer risk. The magnitude of this association was dependent on level of IA ancestry, with risk being highest among those with more IA ancestry. We also observed multiple associations between SNPs in telomere biology-related genes and breast cancer. SNPs in *TERT, TEP1, TERF2*, and *TNKS* were positively associated with risk. Additionally, we found that SNPs in *MEN1* and *MRE11A* had significantly different associations with breast cancer risk across admixture groups. *TEP1, TERT, TERF2*, and *TNKS* were observed to have significant associations within strata of admixture groups, although no interaction between specific groups was observed. Several SNPs were also found to be associated with ER/PR tumor status, with the majority of these SNPs showing associations with ER−/PR− tumors.

Earlier reports on the relationship between TL and breast cancer have been mixed. Some studies failed to observe an association between TL and sporadic breast cancer risk, or between TL and hereditary breast cancer (Zheng et al., 2010; Kim et al., 2011; Yanowsky et al., 2012). One study reported an association between shorter TL and breast cancer risk in families carrying mutations in *BRCA1* or *BRCA2* but not among families without these inherited mutations (Martinez-Delgado et al., 2011). Consistent with our results, other studies found that longer TL is associated with both sporadic and familial breast cancer (Svenson et al., 2008; Gramatges et al., 2010). Meta-analyses of TL data and cancer further support the inconsistency of data on associations between TL and cancer, although they have summarized that shortened TL in most often associated with increased cancer risk (Ma et al., 2011; Wentzensen et al., 2011). Importantly, we found the association with TL was strongest among those women with greater IA ancestry. This is the first report on TL and breast cancer risk evaluating IA ancestry, which appears to be an important modifier of risk in this study. Further evaluation of factors that influence TL among those with more IA ancestry may shed additional light on this association.

TL in both cancer tissue and in blood leukocytes has been associated with cancer (Meeker, 2006; Han et al., 2009; Lan et al., 2009; Mirabello et al., 2010a,b; Nan et al., 2011; Cui et al., 2012; Pellatt et al., 2012), although it appears that the direction of association between TL and cancer may be cancer-specific. For instance shorter leukocyte telomeres appear to be associated with risk of colon, ovarian, and prostate cancer, whereas longer telomeres have been associated with breast, melanoma, and hepatocellular cancer, melanoma, and non-Hodgkin's lymphoma. It has been proposed that TL may influence cancer by maintaining genomic stability. Genetic instability has been linked to a variety of cancers, including breast cancer (Miki, 2012; Sens-Abuazar et al., 2012). Telomeres maintain genomic stability and integrity by ensuring that DNA is not lost during the replication of chromosomes and by protecting the ends of chromosomes from being recognized by repair enzymes as DSBs which are targeted for repair. Telomeres are also responsible for preventing the improper joining of chromosomes. When telomeres reach a critical length, the cell undergoes apoptosis or senescence. However, alterations that result in telomeres lengthening could prevent cells from undergoing senescence or apoptosis and allows cells to continuously regenerate, which could ultimately result in cancer development. In this scenario, increased number of cell cycles associated with longer telomeres could result in greater mutation potential. Some studies have suggested that both long and short TL may alter risk of colorectal cancer (Cui et al., 2012; Pellatt et al., 2012), implying that mechanisms that involve both genomic instability and increased mutation potential are viable. Data also have found increased telomerase activity, often associated with survival in multiple types of cancer tissue (Meeker and Argani, 2004; Bautista et al., 2007; Lu et al., 2011), further suggesting a role for telomere-related factors in the etiology and progression of cancer. Leukocyte TL may indicate susceptibility to cancer risk, that is defined in the biology of the tumors themselves. Studies have shown that an individual's TL is tissue independent and that TL in blood is a surrogate for relative TL in other tissues (Friedrich et al., 2000).

Previous data found that *TERT, TERC, MEN1, MRE11A, RECQL5, TNKS, TNKS2, TEP1* and *TERF2* are all associated with TL (Chiang et al., 2006; Varadi et al., 2009; Mirabello et al., 2010a,b; Jones et al., 2012). We were only able to confirm an association between TL and *TERT* rs2736118 and *TNKS* rs10509637 when evaluating associations among controls. We did not observe unique differences in TL by genotype and by admixture group. Our inability to confirm previously reported associations could stem from the younger age range of our population, whereas most other studies included women who were predominately over age 60 years. We did observe differences in TL for *TERT, MRE11A*, and *TNKS* by menopausal status after adjustment for age, lending some support for this hypothesis. We also adjusted for potential confounding factors, such as BMI, which could alter associations. It is possible that other lifestyle factors that are associated with TL could differ between our study population and those previously reported and influence associations between TL-related SNPs and TL.

Variants in *TERT* (Savage et al., 2007; Shen et al., 2012), *TEP1* (Salhab et al., 2008), *TERF2* (Savage et al., 2007; Varadi et al., 2009), *RECQL5* (Islam et al., 2012), *TNKS2* (Varadi et al., 2009), *MEN1* (Imachi et al., 2010) have previously been associated with breast cancer, whereas associations between variants in *MRE11A*, and *TNKS* and breast cancer are inconclusive. We replicated earlier reports of associations between *TERT, TEP1*, and *TERF2* and breast cancer. Additionally, we found an association between *TNKS* and breast cancer risk. However, we failed to replicate earlier findings for *RECQL5, TNKS2*, and *MEN1.*

The associations between these SNPs and breast cancer have genetic plausibility. *TERT* is a functional unit of the telomerase enzyme, which is responsible for the maintenance of TL. Telomerase activity is usually absent from differentiated cells; however, it has been

previously observed in multiple types of cancer cells, including breast cancer (Bautista et al., 2007; Hofer et al., 2011; Mohajeri et al., 2011; Jones et al., 2012). We found that multiple *TERT* SNPs are directly associated with breast cancer risk, indicating that these polymorphisms might be involved in the activation of telomerase. However, one *TERT* SNP (rs4246742) showed an inverse association with breast cancer, suggesting that this SNP might inhibit telomerase expression in differentiated cells. *TEP1* is another component of the telomerase ribonucleoprotein complex; it is responsible for catalyzing the addition of new telomeres to chromosomes. We found that *TEP1* rs938886 is inversely associated with breast cancer, suggesting that this SNP might inhibit telomerase activity in differentiated cells. *TERF2* plays a role in the prevention of end to end ligation of chromosomes by telomeres. This allows *TERF2* to protect the integrity of the telomere, and therefore, genomic stability. Polymorphisms within this gene could lead to genomic instability, which has been linked to breast cancer (Miki, 2012; Sens-Abuazar et al., 2012). *TNKS* enhances the access of telomerase to telomeres (Gao et al., 2011), thereby playing a role in telomere maintenance. We found an inverse association with two *TNKS* SNPs (rs6990097 and rs6990300), suggesting that certain polymorphisms can inhibit its ability to increase the access of telomerase to telomeres and therefore prevents telomerase from maintaining chromosomes in differentiated somatic cells. However, the associations observed between SNPs and TL may stem from non-telomere related mechanisms (Epel et al., 2004; Wolkowitz et al., 2011; Ghosh et al., 2012). For instance, Ghosh has demonstrated that telomerase expression is associated with signaling via NFκB, a regulator of inflammation (Ghosh et al., 2012). Others have documented that hTERT can enhance cell proliferation, decrease apoptosis, regulate DNA damage response, and alter cell proliferation lifespan (Mukherjee et al., 2011). These functions of hTERT provide mechanisms for telomere-related genes to alter cancer risk that is non-telomere related.

To our knowledge this is the first study to examine the effects of telomere-biology related SNPs on breast cancer within genetic admixture groups. We have previously reported higher risk of breast cancer associated with more Europeans ancestry and a lower risk associated with more IA ancestry (Akman et al., 2009; Lurje et al., 2009; Slattery et al., 2012). Moreover, these earlier studies have found heterogeneity in the magnitude and the direction of genetic associations with breast cancer among Hispanic women. We found a few modest associations with breast cancer risk when evaluating telomere-related genes by genetic admixture groups. *MEN1* rs670358 was associated with a slightly greater risk in NHW women which is consistent with earlier findings of increased breast cancer risk among women of higher European ancestry. We also found that *MRE11A* rs1344720 was associated with increased risk among individuals with higher IA ancestry. Other associations were observed for overall breast cancer risk; however, no significant interactions between admixture groups were noted for these SNPs.

Seven SNPs were associated with ER/PR status, and of these SNPs five were associated with the ER−/PR− subtype. This is the first study to suggest that the ER−/PR− tumor subtype might be influenced by TL. This is significant because previous studies have found that women with ER−/PR− tumors have lower survival rates (Onitilo et al., 2009). We had limited power to assess TL with ER/PR status.

Our study has several strengths, including the large admixed population, which allowed us to evaluate breast cancer risk among Hispanic women with a wide range of IA American ancestry. Additionally, we evaluated associations with breast cancer risk overall as well as by tumor phenotype while adjusting for potentially confounding variables. Although the study was population-based, not all eligible women participated. However, it is unlikely that genotypes of telomere-related genes influenced participation.

In addressing our findings, it is important to consider the timing of the blood draw for the case-control studies in which we examined TL. Blood was drawn from the Mexico samples prior to treatment; in Utah blood was drawn after treatment. Associations between TL and breast cancer were strongest among those with higher Native American ancestry, that is, Mexico. This finding would suggest that differences in associations are not from treatment as has been hypothesized by Pooley as one possible explanation for differences in association observed between their cohort and case–control study participants (Pooley et al., 2010). The lack of association between treatment and TL is supported by Pooley that shows no association between any one type of treatment and TL (Pooley's online Supporting Information). Differences in association between prospective and retrospective studies could stem from changes in lifestyle factors that influence TL between the time that blood was drawn and TL assessment since lifestyle factors have been associated with TL (Mirabello et al., 2009; Puterman et al., 2010; Pellatt et al., 2012).

In summary, our study strongly suggests that both TL and telomere-related genes influence breast cancer risk in NHW and Hispanic women. The association with TL appears to be modified by IA ancestry. ER/PR tumor status seems to be an important modifier of the associations with telomere-related genes and breast cancer risk. Further exploration of factors that may be associated with both breast cancer and TL among admixed populations such as this may provide further insight into breast cancer etiology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Description of Genes Included in Study

				MAF		FDR HWE P	
Gene	**Chromosome Location**	**dbSNP ID**	**Major/Minor Allele**	**NHW**	**HISP/NA**	**NHW**	**HISP/NA**
MEN1	11q13.1	rs670358[a]	G/A	0.09	0.34	0.97	0.00
MRE11A	11q21	rs12270338	C/A	0.21	0.12	0.96	0.50
		rs13447720	A/G	0.22	0.12	0.97	0.37
RECQL5	17q25.1	rs820152[b]	T/C	0.38	0.27	0.96	0.49
TEP1	14q11.2	rs938886	G/C	0.20	0.37	0.89	0.01
TERC	3q26	rs12696304	C/G	0.27	0.53	0.83	0.002
TERF2	16q22.1	rs3785074	A/G	0.26	0.18	0.96	0.66
TERT	5p15.33	rs2736118	A/G	0.26	0.15	1.00	0.42
		rs4246742	A/T	0.17	0.29	1.00	0.79
		rs10069690	C/T	0.25	0.18	0.96	0.82
		rs2242652	C/T	0.19	0.14	0.65	0.79
		rs2736100	T/G	0.50	0.36	0.96	0.85
		rs2853676	G/A	0.26	0.19	0.96	0.64
		rs2735940	C/T	0.50	0.36	0.97	0.93
TNKS	8p23.1	rs11991621	C/T	0.19	0.08	0.82	0.72
		rs6990097	T/C	0.27	0.18	0.93	0.47
		rs12549064	A/C	0.18	0.09	0.86	0.59
		rs10903314	C/T	0.26	0.17	0.96	0.34
		rs6990300	A/G	0.32	0.42	0.97	0.73
		rs11249943	A/C	0.20	0.09	0.93	0.81
		rs17150478	A/G	0.20	0.18	1.00	0.76
TNKS2	10q23.3	rs10509637	A/G	0.15	0.09	0.96	0.65

[a]This SNP is located within the *CDC42BPG* gene.

[b]This SNP is located in *MYO15B* gene.

Table 2

Description of Study Population by Self-Reported Race/Ethnicity[a]

	Non-Hispanic white (NHW)					Hispanic				
	Controls		Cases			Controls		Cases		
	N	%	*N*	%	*P* value[b]	*N*	%	*N*	%	*P* value
Study site										
4 Corner's study	1321	83.3	1227	82.8	NA	723	27.8	597	28.3	NA
Mexico	0	0.0	0	0.0		994	38.3	816	38.7	
California	264	16.7	254	17.2		880	33.9	698	33.1	
Age (years)										
<40	116	7.3	89	6.0	NA	311	12.0	200	9.5	NA
40–49	408	25.7	409	27.6		831	32.0	713	33.8	
50–59	409	25.8	413	27.9		756	29.1	617	29.2	
60–69	349	22.0	361	24.4		526	20.3	430	20.4	
70+	303	19.1	209	14.1		173	6.7	151	7.2	
Menopausal status										
Premenopausal	494	31.5	489	33.5	NA	1027	40.7	836	40.9	
Postmenopausal	1075	68.5	970	66.5		1499	59.3	1210	59.1	
Estimated indigenous American (IA) ancestry[c]										
Low IA (0–28%)	1577	99.5	1472	99.4	NA	278	10.7	275	13.0	NA
Intermediate IA (29–70%)	7	0.4	7	0.5		1686	64.9	1393	66.0	
High 1A (71–100%)	1	0.1	2	0.1		633	24.4	443	21.0	
ER/PR status[d]										
ER+/PR+			695	68.2	NA			605	61.9	NA
ER+/PR−			121	11.9				115	11.8	
ER−/PR+			15	1.5				28	2.9	
ER−/PR−			188	18.4				229	23.4	
Telomere Length		Median (Q1,Q3)		Median (Q1,Q3)			Median (Q1,Q3)		Median (Q1,Q3)	
Overall	232	1.26 (1.08,1.51)	237	1.30 (1.10,1.55)	0.418	494	1.27 (1.11,1.43)	500	1.35 (1.17,1.53)	<0.00l
Low IA (0–28%)	231	1.25 (1.08,1.51)	236	1.30 (1.10,1.55)	0.396	16	1.27 (1.04,1.57)	8	1.27 (1.19,1.53)	0.540

	Non-Hispanic white (NHW)					Hispanic				
	Controls		Cases			Controls		Cases		
	N	%	*N*	%	*P* value[b]	*N*	%	*N*	%	*P* value
Intermediate IA (29–70%)	1	1.26	1	1.05	NA	241	1.29 (1.08,1.49)	246	1.35 (1.18,1.52)	0.049
High 1A (71–100%)	0	NA	0	NA	NA	237	1.26 (1.12,1.40)	246	1.35 (1.17,1.53)	<0.001

[a] AII women from Mexico are classified as Hispanic since race/ethnicity was not asked.

[b] P value for Wilcoxon rank-sum test.

[c] Assessment of indigenous American ancestry (IA) is based on 104 AIMs markers.

[d] Tumor information unavailable for the Mexico study site.

Table 3

Associations Between TL and Breast Cancer Risk Overall and by PERCENT Indigenous American (IA) Ancestry

	Overall				IA Ancestry											
					Low (0–28%)				Intermediate (29–70%)				High (71–100%)			
Quartile	Controls	Cases	OR[a]	(95% CI)	Controls	Cases	OR	(95% CI)	Controls	Cases	OR	(95% CI)	Controls	Cases	OR	(95% CI)
1	183	148	1.00		68	64	1.00		62	44	1.00		53	40	1.00	
2	186	150	0.96	(0.70,1.31)	58	50	0.90	(0.54,1.50)	52	46	1.20	(0.66,2.17)	76	54	0.98	(0.57,1.68)
3	180	192	1.27	(0.94,1.73)	44	50	1.24	(0.73,2.12)	65	77	1.60	(0.93,2.77)	71	65	1.22	(0.71,2.11)
4	171	238	1.87	(1.38,2.55)	74	80	1.22	(0.75,1.96)	62	76	2.15	(1.22,3.82)	35	82	3.11	(1.74,5.67)

[a]Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age, study center, genetic admixture, and BMI in the referent year; P interaction between ancestry groups was 0.02.

Table 4

Associations Between Telomere-Associated SNPs for All Breast Cancers Combined and by Menopausal Status

	All women				Premenopausal women				Postmenopausal wonen				
	Controls *N*	Cases *N*	OR[b]	(95% CI)	Controls *N*	Cases *N*	OR	(95% CI)	Controls *N*	Cases *N*	OR	(95% CI)	Interaction[a] *P* value
TEPI (rs938886)													
GG/GC	3645	3221	1.00		1295	1181	1.00		2270	1963	1.00		0.38
CC	453	311	**0.82**	**(0.70, 0.95)**	182	119	**0.75**	**(0.59, 0.97)**	266	182	0.82	(0.67, 1.01)	
TERF2 (rs3785074)													
AA	2561	2088	1.00		932	781	1.00		1577	1256	1.00		0.74
AG/GG	1542	1447	**1.13**	**(1.03, 1.24)**	547	521	1.09	(0.93, 1.28)	962	890	**1.16**	**(1.03, 1.30)**	
TERT (rs4246742)													
AA	2323	2177	1.00		798	782	1.00		1479	1343	1.00		0.38
AT/TT	1766	1349	**0.85**	**(0.77, 0.93)**	677	515	*0.82*	(*0.70, 0.96*)	1050	799	**0.85**	**(0.76, 0.96)**	
TERT (rs10069690)													
CC	2568	2093	1.00		956	777	1.00		1558	1262	1.00		0.36
CT/TT	1518	1429	**1.13**	**(1.03, 1.24)**	520	523	*1.20*	(*1.02, 1.40*)	968	873	1.11	(0.98, 1.25)	
TERT (rs2242652)													
CC/CT	3680	3098	1.00		1290	1143	1.00		2326	1893	1.00		0.13
TT	78	102	**1.51**	**(1.11, 2.04)**	30	33	1.08	(0.65, 1.80)	45	68	**1.86**	**(1.27, 2.74)**	
TERT (rs2736100)													
TT	1450	1179	1.00		558	467	1.00		863	686	1.00		0.47
TG	1934	1674	1.03	(0.93, 1.15)	676	606	1.00	(0.85, 1.19)	1219	1023	1.05	(0.92, 1.20)	
GG	714	681	1.10	(0.97, 1.26)	244	228	0.99	(0.79, 1.24)	454	437	*1.20*	(*1.01, 1.42*)	
TERT (rs2853676)													
GG/GA	3916	3327	1.00		1419	1231	1.00		2417	2015	1.00		0.98
AA	186	206	*1.23*	(*1.00, 1.51*)	59	69	1.19	(0.83, 1.71)	122	131	1.26	(0.98, 1.63)	
TNKS (rs699097)													
TT	2560	2211	1.00		906	831	1.00		1608	1325	1.00		0.05
TC/CC	1538	1320	0.96	(0.87, 1.05)	570	*470*	*0.83*	(*0.71, 0.98*)	929	819	1.06	(0.94, 1.20)	
TNKS (rs6990100)													
AA	1545	1462	1.00		520	549	1.00		992	876	1.00		0.03

	All women				Premenopausal women				Postmenopausal wonen				
	Controls *N*	**Cases *N***	**OR[b]**	**(95% CI)**	**Controls *N***	**Cases *N***	**OR**	**(95% CI)**	**Controls *N***	**Cases *N***	**OR**	**(95% CI)**	**Interaction[a] *P* value**
AG/GG	2557	2073	**0.89**	**(0.81, 0.97)**	959	753	**0.76**	**(0.65, 0.89)**	1546	1270	0.94	(0.84, 1.06)	

[a] *P* value for difference by menopausal status.

[b] OR and 95% CI adjusted for age, study, BMI in referent year, vigorous activity in referent year, parity, age at first birth, alcohol consumption, and genetic admixture; Bold text indicates significant at the 0.05 level after adjustment for multiple comparisons; italics indicates adjusted p value between 0.05 and 0.15.

Table 5

Associations by Admixture Among Premenopausal and Postmenopausal Women Where Significant Differences Between Admixture Groups Were Observed

	Low IA ancestry (0–23%)				Intermediate IA ancestry (29–70%)				High IA ancestry (71–100%)				2-way	
	Controls *N*	Cases *N*	OR	(95% CI)	Controls *N*	Cases *N*	OR	(95% CI)	Controls *N*	Cases *N*	OR	(95% CI)	Interaction *P* value	Holms *P* value
Premenopausal women														
MRE11A (rs12270338)														
CC	361	387	1.00		494	380	1.00		235	163	1.00		0.03	0.03
CA/AA	204	189	0.85	(0.67, 1.09)	147	149	1.30	(0.99, 1.71)	37	32	1.23	(0.71, 2.15)		
MREI IA (rs13447720)														
AA	356	372	1.00		505	385	1.00		236	163	1.00		0.04	0.04
AG/GG	209	204	0.92	(0.72, 1.18)	137	146	**1.38**	**(1.05, 1.82)**	36	32	1.27	(0.73, 2.23)		
TERC (rs12696304)														
CC	285	276	1.00		158	134	1.00		22	24	1.00		0.03	0.03
CG	234	235	1.02	(0.80, 1.31)	312	263	1.01	(0.75, 1.35)	122	86	0.63	(0.31, 1.28)		
GG	41	58	1.50	(0.96, 2.32)	169	132	0.97	(0.69, 1.36)	127	84	0.55	(0.27, 1.11)		
TNKS (rs1199162I)														
CC	375	408	1.00		527	447	1.00		251	169	1.00		0.03	0.06
CT/TT	190	168	0.80	(0.62, 1.04)	113	82	0.87	(0.63, 1.20)	21	26	1.57	(0.80, 3.10)		
TNKS (rs699097)														
TT	296	336	1.00		411	364	1.00		199	131	1.00		0.05	0.06
TC/CC	269	239	**0.77**	**(0.61, 0.98)**	228	167	0.80	(0.62, 1.03)	73	64	1.25	(0.80, 1.96)		
TNKS (rs12549064)	2													
AA	369	411	1.00		528	433	1.00		240	163	1.00		0.01	0.03
AC/CC	196	165	**0.75**	**(0.58, 0.97)**	113	98	1.05	(0.77, 1.43)	32	32	1.38	(0.77, 2.47)		
INKS (rs17150478)														
AA	363	410	1.00		412	354	1.00		192	121	1.00		0.003	0.01
AG/GG	202	166	**0.73**	**(0.57, 0.94)**	230	175	0.89	(0.70, 1.15)	80	74	*1.59*	(*1.04, 2.42*)		
Post-Menopausal Women														
MEN1 (rs670358)														
GG	994	880	1.00		462	402	1.00		86	55	1.00		0.02	0.02
GA	222	221	1.13	(0.92, 1.40)	411	325	0.87	(0.71, 1.06)	163	118	1.24	(0.80, 1.92)		
AA	10	15	1.63	(0.72, 3.67)	94	73	0.82	(0.58, 1.16)	97	55	0.86	(0.52, 1.42)		

Adjusted for age, study, BMI in referent year, vigorous activity in referent year, parity, age at first birth and alcohol consumption; Bold text indicates significant at the 0.05 level after adjustment for multiple comparisons; italics indicates significant between 0.05 and 0.15

Table 6

Associations Between ER and PR Status of Tumors and Telomere-Related Genes

	Controls *N*	Cases *N*	ER+PR+		Cases *N*	ER+/PR−		Cases *N*	ER−/PR+		Cases *N*	ER−/PR−	
			OR[a]	(95% CI)		OR	(95% CI)		OR	(95% CI)		OR	(95% CI)
RECQL5 (rs821052)													
TT	1270	503	1.00		69	1.00		18	1.00		143	1.00	
TC/CC	1611	648	1.00	(0.87, 1.15)	131	**1.46**	**(1.08, 1.98)**	17	0.76	(0.39, 1.49)	198	1.09	(0.87, 1.37)
TERT (rs2736118)													
AA	1909	787	1.00		138	1.00		9	1.00		236	1.00	
AG/GG	1207	505	0.98	(0.85, 1.12)	97	1.11	(0.84, 1.46)	31	**6.18**	**(2.90, 13.19)**	174	1.21	(0.97, 1.49)
TERT (rs2736100)													
TT	986	371	1.00		70	1.00		9	1.00		154	1.00	
TG/GG	2134	921	1.12	(0.97, 1.29)	165	1.09	(0.81, 1.47)	32	1.90	(0.89, 4.04)	256	*0.79*	(0.63, 0.98)
TERT (rs2735940)													
CC	958	379	1.00		62	1.00		11	1.00		157	1.00	
CT/TT	2159	912	1.03	(0.89, 1.19)	173	1.23	(0.91, 1.67)	30	1.38	(0.68, 2.80)	254	**0.73**	**(0.59, 0.91)**
TNKS (rs6990097)													
TT	1866	781	1.00		147	1.00		26	1.00		268	1.00	
TC/CC	1254	510	0.95	(0.83, 1.08)	87	0.88	(0.66, 1.16)	15	0.85	(0.44, 1.62)	142	*0.78*	(*0.63, 0.98*)
TNKS (rs10903314)													
CC	1900	804	1.00		152	1.00		28	1.00		273	1.00	
CT/TT	1224	488	0.93	(0.81, 1.06)	83	0.84	(0.64, 1.11)	13	0.73	(0.37, 1.42)	138	*0.79*	(*0.64, 0.99*)
TNKS (rsl7150478)													
AA	2102	902	1.00		157	1.00		28	1.00		298	1.00	
AG/GG	1021	388	0.89	(0.77, 1.02)	78	1.03	(0.78, 1.37)	13	0.94	(0.48, 1.83)	111	*0.76*	(*0.61, 0.96*)

[a]OR and 95% CI adjusted for age, study, BMI in referent year, vigorous activity in referent year, parity, age at first birth, genetic admixture, and alcohol consumption; Bold text indicates significant at the 0.05 level after adjustment for multiple comparisons; italics indicates adjusted P value between 0.05 and 0.10.