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Association between genetic risk score for telomere length and risk of breast cancer

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Abstract

Purpose—While leukocyte telomere length (TL) has been associated with breast cancer risk, limited information is available regarding the role of genetically-determined TL on breast cancer risk. We investigated whether aggregated TL-associated variants are associated with the risk of breast cancer in 2,865 breast cancer cases and 2,285 controls from the Shanghai Breast Cancer Genetics Study.

Methods—Six genetic variants, identified through a genome-wide association study (GWAS) of TL in European-ancestry participants, were included in the study. A separate sample [$n = 1,536$, from the Shanghai Women's Health Study (SWHS), for whom information on both phenotypical leukocyte TL and genetic information was collected] was used to evaluate the association of six variants with TL in Asians. Three genetic risk scores (GRSs), based on the number of alleles associated with shorter TL that each individual carries for the six variants, were derived for the study: un-weighted, internally weighted (from the SWHS), and externally weighted (from the European-ancestry GWAS study), and evaluated for their association with breast cancer risk by applying logistic regression analysis.

Results—Both internally and externally weighted GRSs were significantly associated with a decreased risk of breast cancer (OR 0.83, 95 % CI 0.72–0.95 and OR 0.84, 95 % CI 0.74–0.96, respectively, for tertile 3 vs. tertile 1). Non-genetic risk factors for breast cancer (i.e., age, years of menstruation/reproduction, oral contraceptive usage, and BMI) did not modify the association between GRSs and the risk of breast cancer.

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Compliance with ethical standards

Conflict of interest None declared.

Conclusion—Our results suggest that short TL, determined by genetic factors, may be associated with a reduced susceptibility to breast cancer.

Keywords

Telomere length-associated variants; Reduced risk; Breast cancer

Introduction

Breast cancer is one of the most common malignancies among women worldwide [1–3]. It is thought to be caused by a complex assortment of factors, including genetic influences [1, 2].

Telomeres and telomerase (an enzyme that maintains telomere length) preserve genomic stability by protecting chromosome ends from degradation, fusion, and irregular recombination [4, 5]. In human somatic cells, telomeres are approximately 10–15 kb. They gradually shorten over time, by approximately 30–200 bp after each cycle of mitotic division [6]. Incomplete replication of linear DNA molecules and gradual shortening of telomere length lead to regulated cell senescence and apoptosis or cell death, a process that acts as a key mechanism in cancer development [7, 8]. While earlier observational studies reported that a shorter TL in white blood cells (WBC) was associated with an increased risk of various cancers [9–12], cumulative evidence has discovered that a longer TL is also problematic, as it was found to be associated with increased risk of lung cancer [13–15], pancreatic cancer [16], renal cancer [17], and breast cancer [18].

TL shortens with aging, but the degree of shortening shows extensive inter-individual variability and significant heritability (i.e., estimated 44–88 %) [19–21]. Recently, a genome-wide association study (GWAS) by Codd et al. [22] identified seven loci robustly associated with peripheral WBC TL. These seven loci were located on or nearby the following genes: *TERC* (rs10936599), *TERT* (rs2736100), *NAFI* (rs7675998), *OBFC1* (rs9420907), *ZNF208* (rs8105767), *RTEL1* (rs755017), and *ACY2* (rs11125529). While these variants were believed to explain only a small proportion of the total variation in TL (0.08–0.36 %), the age-related shortening per variant risk allele was equal to 1.9–3.9 years of attrition in the telomere/single copy gene (*T/S*) ratio (or 58–117 base-pair in TL per risk allele) [22]. Codd et al. [22] further performed a Mendelian randomization analysis using a genetic risk score (GRS) based on these variants and confirmed an association between shorter mean peripheral WBC TL and increased risk of coronary artery disease. In addition, a recent study using a GRS of these seven loci in a consortium study among Asian female never smokers by Machiela et al. [23] showed that genetic variants related to a longer TL are associated with an increased risk of lung cancer.

Five of the seven genes (*TERC*, *TERT*, *NAFI*, *OBFC1*, and *RTEL*) that the GWAS loci are on or nearby were known to be involved in the biologic function(s) of telomeres. *TERC* (or telomerase RNA component) is an RNA gene responsible for telomere replication (i.e., reverse transcription) [24]. *TERT*, on the other hand, encodes the reverse transcriptase component of the telomerase and is necessary for the maintenance of TL, cellular immortality, and chromosomal stability [25, 26]. *NAFI* (or nuclear assembly factor 1 ribonucleoprotein) was recently found to be required for accumulation of all types of box

N/ACA RNPs, including telomerase, which functions in RNA modification [27]. *OBFC1* (or oligonucleotide/oligosaccharide-binding fold containing 1) was found to be involved in telomere elongation, one important mechanism of TL regulation [28]. *RTEL1* (or regulator of telomere elongation helicase 1) is responsible for encoding a DNA helicase which functions in the stability, protection, and elongation of telomeres and interacts with proteins in the shelterin complex to protect telomeres during DNA replication [29]. The relevance of other two genes (*ZNF* and *ACYP2*) to TL is unclear. It is, however, known that several zinc finger proteins, including *ZNF208*, regulate gene transcription via DNA binding [30]. *ACYP2* gene encodes a muscle-specific acylphosphate and is associated with stress-induced apoptosis in rat muscle [31].

GRSs have been increasingly used as instrumental variables in Mendelian randomization to assess the cumulative effect of genetic variants identified from GWAS and to evaluate possible causal links [32, 33]. Research on the role of genetic variants associated with TL and breast cancer is, however, very limited. Three studies have reported on the association of the *TERT* gene variants (i.e., rs7726159, rs2736108, rs2736109, and rs3816659) and *POT1* gene variant (i.e., rs33964002) [15–17], and one has reported on the *TERT* gene variants (i.e., rs2853677 and rs2853669) and *TERF2* gene variant (i.e., rs35439397) [18] with breast cancer risk. Recently, Pooley et al. [34] found that the *NAF1*, *RTEL1*, *ACYP2*, and *TERT* variants were individually associated with telomere length but that only the *TERT* variant was associated with an increased risk of hormonal-related cancers, including breast cancer, in the Collaborative Oncology Gene-environment Study (COGS).

To our knowledge, only one study [35] has used a GRS as an aggregate measure to evaluate the relationship between TL-associated genetic variants and breast cancer risk in European descendants. Such a study, however, has not been conducted in Asian women. We report here just such an effort using resources from ongoing cohort studies of breast cancer among women in Shanghai, China.

Methods

Study population

In the current analysis of the associations of TL-related single nucleotide polymorphisms (SNPs) and breast cancer risk, a total of 2,865 breast cancer cases and 2,285 controls from the population-based, case–control Shanghai Breast Cancer Study (SBCS), Shanghai Breast Cancer Survival Study (SBCSS), and the ongoing population-based cohort Shanghai Women’s Health Study (SWHS) were included. A separate subsample of 1,536 participants from the SWHS, from whom both phenotypically measured leukocyte TL and genetic information were available, was used to evaluate the association of these genetic variants with TL. Study designs and methods of these studies have been described previously [36–38]. Briefly, recruitment for the SBCS occurred between August 1996 and March 1998 (1,491 cases; 1,556 controls) (stage I—SBCS-I; response rate of 91.1 % for cases and 90.3 % for controls) and again between April 2002 and February 2005 (1,932 cases; 1,857 controls) (stage II—SBCS-II; response rate: 83.7 % for cases and 70.4 % for controls). Recruitment for the SBCSS (5,042 study participants, response rate: 80.1 %) occurred between April 2002 and December 2006, and recruitment for the SWHS (74,941 study

participants, response rate: 92.7 %) occurred between January 1997 and September 2000. Breast cancer cases were identified via the Shanghai Cancer Registry, and controls were randomly selected using the Shanghai Resident Registry or from participants of the SWHS. An in-person interview was used to obtain both general demographic and breast cancer-related information, including age at menarche, age at menopause, age at first live birth, number of live births, oral contraceptive use, hormone replacement therapy (HRT) use, and family history of breast cancer. Trained personnel also took anthropometric measurements from all study participants, including weight, height, and circumferences of waist and hips. Blood or buccal cell samples were collected and made available for 81.8 % of cases and 84.2 % of controls from the SBCS-I, 97.1 % of cases and 96.8 % of controls from the SBCS-II, and 96.0 % of cases from the SBCSS [39]. Also, 75.8 % of SWHS participants donated blood samples [36]. Participants of these studies provided written informed consent, and the Institutional Review Boards of all participating institutions approved the study protocols.

Laboratory methods, TL measurement, and genotyping

Laboratory protocols for the DNA extraction and genotyping methods have been described in detail elsewhere [37, 39, 40]. Briefly, genomic DNA was extracted from either buffy coats or buccal cells using a QIAamp DNA kit (Qiagen, Valencia, California) following the manufacturer's protocols. A total of 1,536 subjects from the SWHS had both these types of genotyping data. Genotyping for the SBCS, SBCSS, and SWHS was performed using Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, California) [37, 39]. GWAS data were imputed using Minimac with 1000 Genomes Project phase I data as reference. Dosage data for the genetic variants included in this study were extracted from the imputed data. SNP rs9420907 showed an imputation quality score RSQ of 0.68 and MAF of 0.01 and thus was not included in the present study. All other 6 SNPs (rs10936599, rs2736100, rs7675998, rs8105767, rs755017, and rs11125529) showed very high imputation quality with RSQ>0.9 and were included in the data analyses.

Relative leukocyte TL was measured using a monochrome multiplex quantitative polymerase chain reaction (PCR) as described previously by Cawthon [41], with minor modifications [40]. Briefly, a TL assay was performed in a 15- μ L PCR consisting of 1 $\times$ QuantiFast SYBR Green PCR Master Mix (Qiagen). A multistep thermal cycling procedure was carried out on a Bio-Rad (Hercules, California) CFX384 Real-Time System. The relative TL was determined using Bio-Rad CFX manager version 1.6 software using a 2-step relative quantification (i.e., ratio values of T/S were extracted and then were log-transformed to improve normality).

Statistical analysis

Means and standard errors were calculated for continuous variables, while counts and proportions were computed for categorical variables. Linear regression was conducted to estimate associations of each SNP with relative TL among the 1,536 SWHS subjects with adjustment for age and batch of TL assays. The beta (β) coefficient for each SNP with relative TL was used for constructing a GRS. We used logistic regression to determine the associations with breast cancer for 6 individual genetic variants, adjusting for age at

interview. Three GRSs were constructed to measure the cumulative effect of these six genetic variants [41]: an un-weighted GRS, a weighted GRS using an external weight (weighted on the beta estimate from the previous TL GWAS [22], and a weighted GRS using an internal weight (weighted on the beta estimate between SNP and TL in the present study) as described by the following equation:

$$GRS = \sum_{i=1}^n \beta_i SNP_i$$

where n is the number of SNPs and β_i is the weight for the allele of SNP $_i$. As described above, dosage data (0–2) for each SNP were extracted from imputed data. Each SNP was coded on the allele associated with shorter TL based on previous GWAS. We calculated the un-weighted GRS by summing the number of alleles associated with shorter TL of each individual for the six variants included in the study, with $\beta_i = 1$. The β_i for the externally weighted GRS was obtained from a previous GWAS study by Codd et al. [22], while β_i for the internally weighted GRS came from our current study. The associations between GRS and breast cancer were evaluated using a logistic regression model in which the GRS was treated first as a continuous scale and then as a tertile scale with adjustment for age. The reason we chose a tertile scale was that this scale is less susceptible to long-tailed distribution and outliers than means. [42] The correlation between the three GRSs was extremely high: $r^2 = 0.88$ (un-weighted GRS vs. internally weighted GRS); 0.97 (un-weighted GRS vs. externally weighted GRS); and 0.91 (internally weighted GRS vs. externally weighted GRS). Results on the association between both GRSs (internally and externally weighted scores) with telomere length and breast cancer risk were similar (Table 3). Since both allele frequencies and patterns of linkage disequilibrium between Asians and Europeans differ from each other, we believe that the internally weighted GRS derived from Asian women is more relevant to our study population than the externally weighted GRS derived from European descendants. We thus focused our main analyses primarily on the internally weighted GRS. We used generalized linear models, adjusted for age at interview, to examine the association between the internally weighted GRS and different risk factors for breast cancer (i.e., age at interview, age at menarche, years of menstruation/reproduction, age at menopause, age at first live birth, number of live births, and BMI) among cases and controls, separately. Similar analyses on breast cancer were performed using logistic models for the following variables: postmenopausal status, oral contraceptive usage, physical activity, ever-smoking status, ever-drinking status, family history of breast cancer, hormone replacement therapy (HRT) use, estrogen receptor (ER) group—cases only, and progesterone receptor (PR) group—cases only. We further conducted analyses evaluating the association between the internally weighted GRS and breast cancer stratified by the following variables: age at interview (<40, 40–49, 50–59, and 60), age at diagnosis (categorized scale: <40, 40–49, 50–59, and 60; and binary scale: <50/ 50), menopausal status (no/yes), age at menarche (<14, 14–17, >17), years of menstruation/reproduction (tertile scale where tertile 1: <28.46; tertile 2: 28.46–32.70; tertile 3: > 32.70), age at menopause (<48, 48–54, 55), age at first live birth (<30, 30), number of live births (0, 1–2, 3), oral contraceptive usage (no/yes), BMI (<25, 25), physical activity (no/yes), ever smoking (no/yes), ever drinking (no/yes), family history of breast cancer (no/yes), HRT usage (no/yes), ER group (negative/positive),

and PR group (negative/positive). Interactions between the GRS and these selected risk factors were evaluated using likelihood ratio tests to compare nested models that included only main effects, with models that included both main effects and respective interaction terms. Because age is an important factor affecting TL, all stratified logistic regression models were adjusted for age at interview, except that of age at interview itself. To further examine the potential nonlinear association between GRS and breast cancer, we performed logistic regression with restricted cubic splines with three knots placed at the 5th, 50th, and 95th percentiles of GRS. All statistical analyses were conducted using SAS, version 9.4 (SAS Institute, Inc., Cary, NC). All tests were two-sided, and $p < 0.05$ was considered statistically significant.

Results

Table 1 presents the distribution of socio-demographic characteristics and major risk factors for breast cancer cases and controls in the current analysis. Cases were slightly but significantly older than controls (mean $\pm$ SD: 50.7 ± 9.1 for cases vs. 49.6 ± 8.4 for controls). Controls had fewer years of menstruation/reproduction (mean $\pm$ SD: 29.7 ± 5.2 vs. 30.8 ± 5.1) and a younger age at menopause (47.8 ± 4.8 vs. 48.7 ± 4.4) than cases. Controls also had a lower prevalence of family history of breast cancer (2.8 vs. 4.5 %) and HRT usage (2.4 vs. 3.6 %) than cases. Cases and controls were comparable in terms of menopausal status, number of live births, oral contraceptive use, physical activity, and ever-smoking status.

Table 2 shows the association between six individual SNPs with TL (in 1,536 SWHS participants) and with breast cancer (in 5,150 women). All SNPs had beta coefficients in the same direction as those reported by Codd et al. [22], while only the *TERT* variant (rs2736100) was significantly associated with shorter TL ($\beta(\pm\text{SE}) = -0.016 \pm 0.008$; $p = 0.035$). None of these SNPs was significantly associated with breast cancer risk.

Table 3 reports the associations between three GRSs with TL and with breast cancer risk, using both continuous and tertile scales. All three GRSs were significantly associated with shorter TL ($p = 0.002$, 0.0004 , and 0.001 for un-weighted GRS, internally weighted GRS, and externally weighted GRS, respectively). The risk of breast cancer was significantly decreased in tertile 3 in comparison with tertile 1 in all sets of GRSs, i.e., un-weighted GRSs (OR 0.81, 95 % CI 0.71–0.93), internally weighted GRSs (OR 0.83, 95 % CI 0.72–0.95), or externally weighted GRSs (OR 0.84, 95 % CI 0.74–0.96). The association of the internally weighted GRS with breast cancer risk appears to be linear (Fig. 1). Similar association patterns were observed for other two GRSs (data not shown).

We found no significant associations between major non-genetic risk factors for breast cancer and the internally weighted GRS (Table 4) or the two other GRSs (data not shown). A high internally weighted GRS is consistently associated with decreased risk of breast cancer across almost all subgroups as defined by known factors for breast cancer, with a significant association primarily seen for the upper tertile of GRS. No significant multiplicative interaction was seen between GRSs and any of the selected risk factors (all p value > 0.05) (Supplemental Table 1).

Discussion

In this analysis of 2,865 breast cancer cases and 2,285 controls from Shanghai, China, we evaluated the association between six TL-associated variants and the derived GRSs as aggregated measures of these variants with breast cancer risk. Individually, only one SNP was significantly associated with WBC TL and none of these SNPs was significantly associated with breast cancer risk. The GRSs derived from these six SNPs, however, were all significantly associated with both leukocyte TL and breast cancer risk, regardless of the weight used. We did not find that the GRSs were associated with non-genetic risk factors for breast cancer, and their associations with breast cancer were not modified by these non-genetic risk factors.

To our knowledge, our study is the first to demonstrate an association between a GRS, an instrumental variable based on TL-associated variants identified from a GWAS study, and breast cancer risk in Asian women. Recently, Zhang et al. [35] reported a Mendelian randomization analysis on TL and risk of common cancers (i.e., breast, lung, colorectal, ovarian and prostate cancer, including subtypes) using data of 51,725 cases and 62,035 controls from the Genetic Associations and Mechanisms in Oncology (GAME-ON) consortium. They [35] found that long TL GRS was significantly associated with increased risk of lung adenocarcinoma (OR 2.87, 95 % CI 2.20–3.74; $p = 6.3 \times 10^{-15}$); however, it was not associated with breast cancer (OR 1.02, 95 % CI 0.86–1.21; $p = 0.82$). The clear difference between our study and Zhang's [35] is the ethnicity of study populations; our study was conducted in Chinese women, a low-risk population for breast cancer, while the population of Zhang's study [35] was European descendants, a high-risk population for breast cancer. A genetic determinant with a small effect size would be difficult to identify in a population that has overwhelming non-genetic influences, even with a large sample size. On the other hand, it is also possible that the positive finding from our study is due to chance, particularly given that we found in our earlier study [40] a bidirectional association, i.e., the risk of breast cancer was increased among women with either short or long leukocyte TL (OR 1.35, 95 % CI 0.90–2.04, 1.39, 95 % CI 0.95–2.04; 1.79, 95 % CI 1.17–2.75; and 2.39, 95 % CI 1.45–3.92, respectively, for the 5th, 3rd, 2nd, and 1st quintiles vs. 4th quintile). However, our finding that variants associated with a shorter TL are associated with decreased risk of breast cancer is consistent with two other previous reports [18, 43].

While short leukocyte TL is known to be related to many non-genetic breast cancer risk factors, such as aging, smoking, and obesity, the biologic mechanism linking long TL with breast cancer risk is also plausible. Long telomeres could be associated with high telomerase activation, which may predispose cells to delayed senescence and increase the probability of genetic alterations/abnormalities [40]. Recent evidence also suggests that excessively long telomeres may influence chromosomal instability, a cancer hallmark [44]. It is noteworthy that longer TL has also been shown to be associated with increased risk of lung cancer [13–15], pancreatic cancer [16], and renal cancer [17]. Cumulative evidence has also shown the association of long TL GRS and increased risk of different cancers in both Asian and European populations, including lung cancer in Asian women [23] and in European descendants [35], melanoma in European descendants [45], non-Hodgkin lymphoma in European descendants [46], and glioma in European descendants [47]. Because phenotypic

TL is determined by both genetic and non-genetic factors, future studies with a large sample size are recommended to take both factors into consideration.

A few recent studies have reported associations between individual SNPs on the *TERT* gene (i.e., rs7726159, rs2736108, rs2736109, and rs3816659 variants) or *POT1* gene (i.e., rs33964002 variant) and breast cancer risk [34, 48, 49], or between SNPs on the *TERT* gene (i.e., rs2853677 and rs2853669 variants) or *TERF2* gene (i.e., rs35439397 variant) and breast cancer survival [50]. The lack of an association for six individual TL-associated variants or the weaker association for the *TERT* gene (rs2736100) with TL in our study of Asian women, compared with those of European ancestry, is not surprising because the sample size for our study is not sufficiently large to detect weak associations related to individual SNPs. In addition, the difference in allele distribution might also contribute to our inability to detect the association between individual TL-associated variants and breast cancer risk. For example, the allele frequencies of the *TERC* gene (rs10936599) and *RTEL1* gene (rs755017) in the Codd et al. [22] study were 0.252 and 0.869, while their respective frequencies in our study were 0.535 and 0.543. Also, most of the GWAS-identified SNPs are likely to be SNPs that tag the underlying causal SNPs. Tagging SNPs may vary by the specific populations under study. Future studies with larger sample sizes are needed to identify the causal SNPs for telomere length to help refine the assessment for TL-associated breast cancer risk.

A noticeable strength of our study is the use of a GRS of six TL-associated variants in determining the association of TL with breast cancer. This avoids the reverse causation bias [33] from which a case-control study design inherently suffers. Moreover, using a GRS as a proxy for TL also eliminates confounding from non-genetic TL-influencing factors, since, as demonstrated in our study, GRS is not associated with these factors. Our study's other strength is our comprehensive evaluation of gene-environmental interactions. However, our study is limited by its relatively small sample size, and the fact that it is based on a GRS that explains only a small proportion of the TL variance. Also, the case group in our study (mean $\pm$ SD: 50.7 $\pm$ 9.1) is slightly older than controls (49.6 $\pm$ 8.4). However, the incidence of breast cancer is low in our study population, ranging from 50.2 to 53.4/100,000 between the ages of 45–75. Thus, the less than 1-year difference in mean age between cases and controls is unlikely to have caused major bias. If a detection bias does exist, it would likely bias the result toward the null.

In summary, we found that a genetically determined short TL was associated with a decreased risk of breast cancer in Asian women. This association did not vary by known non-genetic risk factors for breast cancer. This finding suggests that TL may be associated with women's susceptibility to developing breast cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
Association between internally weighted GRS and breast cancer

Table 1

Socio-demographic characteristics of study participants

	Cases (<i>n</i> = 2,865)	Controls (<i>n</i> = 2,285)	<i>p</i> value
Age at interview (mean ± SD)	50.7 ± 9.1	49.6 ± 8.4	<0.0001
<40	215 (7.5)	195 (8.5)	
40–49	1,294 (45.2)	1,066 (46.6)	0.0007
50–59	786 (27.4)	668 (29.2)	
60	570 (19.9)	356 (15.6)	
Age at menarche (mean ± SD)	14.5 ± 1.7	14.7 ± 1.8	<0.0001
<14	890 (31.1)	615 (26.9)	
14–17	1,631 (56.9)	1,319 (57.7)	0.0001
>17	344 (12.0)	351 (15.4)	
Age at first live birth (mean ± SD)	26.6 ± 4.0	26.2 ± 3.8	<0.0001
<30	2,332 (81.4)	1,945 (85.1)	0.0004
30	533 (18.6)	340 (14.9)	
Number of live births			
0	72 (2.6)	45 (2.0)	
1–2	2,401 (85.8)	1,940 (86.4)	0.41
3	325 (11.6)	260 (11.6)	
Years of menstruation/reproduction (mean ± SD)	30.8 ± 5.1	29.7 ± 5.2	<0.0001
Tertile 1 (<28.46)	670 (30.0)	835 (36.6)	
Tertile 2 (28.46–32.70)	719 (32.2)	787 (34.5)	<0.0001
Tertile 3 (>32.70)	847 (37.9)	657 (28.8)	
Oral contraceptive usage			
No	1,796 (80.2)	1,811 (79.3)	0.44
Yes	444 (19.8)	474 (20.7)	
Menopausal status			
No	1,641 (57.4)	1,336 (58.6)	0.38
Yes	1,220 (42.6)	945 (41.3)	
Age at menopause ^a (mean ± SD)	48.7 ± 4.4	47.8 ± 4.8	<0.0001
<48	391 (32.0)	360 (38.1)	
48–54	770 (63.1)	542 (57.3)	0.01
55	59 (4.8)	43 (4.5)	
HRT usage			
No	2,751 (96.2)	2,217 (97.0)	
Yes	102 (3.6)	56 (2.4)	0.03
Perimenopause	8 (0.3)	12 (0.5)	
Family history of breast cancer			
No	2,736 (95.5)	2,220 (97.2)	0.002
Yes	129 (4.5)	65 (2.8)	
BMI (mean ± SD)	23.9 ± 3.4	23.4 ± 3.3	<0.0001
BMI <25	1,889 (65.9)	1,635 (71.5)	<0.0001

	Cases (<i>n</i> = 2,865)	Controls (<i>n</i> = 2,285)	<i>p</i> value
BMI 25	976 (34.1)	650 (28.4)	
Physical activity			
No	1,935 (67.6)	1,582 (69.3)	0.19
Yes	929 (32.4)	702 (30.7)	
Ever smoked			
No	2,793 (97.5)	2,218 (97.1)	0.36
Yes	72 (2.5)	67 (2.9)	
Ever drank alcohol			
No	2,757 (96.2)	2,167 (95.0)	0.03
Yes	108 (3.8)	114 (5.0)	
ER group ^a			
ER (–)	832 (29.0)		
ER (+)	1,555 (54.3)		
Unknown	478 (16.7)		
PR group ^a			
PR (–)	909 (31.7)		
PR (+)	1,471 (51.3)		
Unknown	485 (16.9)		

BMI body mass index, *HRT* hormone replacement therapy, *SD* standard deviation

^aCases only

Table 2

Association between individual SNPs with telomere length and breast cancer

SNP	Chr	Position (hg19)	On or Nearby Gene	Effect allele	Ref. allele	Effect allele freq. in Codd et al. [22]	Genetic variants and telomere length in Codd et al. [22]			Genetic variants and telomere length in Asian women ^a			Genetic variants and breast cancer in Asian women ^b					
							β -Coef.	SE	p value	β -Coef.	SE	p value	Effect allele freq	β -Coef.	OR	95 % CI	p value	
rs10936599	3	169,492,101	<i>TERC</i>	T	C	0.252	-0.097	0.008	2.54×10^{-31}	-0.013	0.008	0.085	0.545	-0.028	0.97	0.90	1.05	0.494
rs2736100	5	1,286,516	<i>TERT</i>	A	C	0.514	-0.078	0.009	4.38×10^{-19}	-0.016	0.008	0.035	0.590	-0.071	0.93	0.86	1.01	0.077
rs7675998	4	164,007,820	<i>NAF1</i>	A	G	0.217	-0.074	0.009	4.35×10^{-16}	-0.015	0.010	0.134	0.175	0.016	1.11	0.92	1.13	0.763
rs8105767	19	22,215,441	<i>ZNF208</i>	A	G	0.709	-0.048	0.008	1.11×10^{-9}	-0.005	0.008	0.586	0.705	-0.052	0.95	0.87	1.03	0.235
rs755017	20	62,421,622	<i>RTEL1</i>	A	G	0.869	-0.062	0.011	6.71×10^{-9}	-0.003	0.008	0.736	0.548	-0.016	0.98	0.91	1.07	0.701
rs11125529	2	54,475,866	<i>ACYP2</i>	C	A	0.858	-0.056	0.010	4.48×10^{-8}	-0.016	0.009	0.093	0.805	-0.072	0.93	0.84	1.03	0.155

Bold values indicate $p < 0.05$

SE standard error

^a Model adjusted for age and batch

^b Model adjusted for age at interview

Table 3
Association between genetic risk score (GRS) with telomere length and breast cancer

	Telomere length ^a			Breast cancer ^b		
	β -Coef.	SE	p value	OR	95 % CI	p value
Un-weighted						
Continuous scale	-0.010	0.003	0.002	0.96	0.93	1.00 0.028
Tertile scale						
Tertile 1				Ref.		
Tertile 2				0.92	0.80	1.05 0.221
Tertile 3				0.81	0.71	0.93 0.003
Internally weighted						
Continuous scale	-0.009	0.003	0.0004	0.96	0.93	1.00 0.029
Tertile scale						
Tertile 1				Ref.		
Tertile 2				0.93	0.81	1.06 0.267
Tertile 3				0.83	0.72	0.95 0.007
Externally weighted						
Continuous scale	-0.009	0.003	0.001	0.96	0.93	1.00 0.040
Tertile scale						
Tertile 1				Ref.		
Tertile 2				1.00	0.87	1.14 0.999
Tertile 3				0.84	0.74	0.96 0.013

Bold values indicate $p < 0.05$

CI confidence interval, OR odds ratio, SE standard error

^aModel adjusted for age and assay plates

^bModel adjusted for age

Table 4
Association between internally weighted genetic risk score and risk factors for breast cancer

	Cases			Controls		
	β -Coef.	SE	p value	β -Coef.	SE	p value
Linear regression						
Age at interview	-0.158	0.103	0.124	-0.143	0.108	0.184
Age at menarche	-0.006	0.019	0.748	0.011	0.022	0.617
Years of menstruation/reproduction	-0.076	0.057	0.185	0.010	0.057	0.861
Age at menopause	-0.005	0.074	0.948	0.038	0.093	0.685
Age at first live birth	-0.009	0.043	0.841	-0.064	0.046	0.163
Number of live births	0.008	0.008	0.334	0.012	0.009	0.169
BMI	-0.009	0.037	0.816	-0.005	0.041	0.908
Logistic regression						
Postmenopause	0.022	0.043	0.619	-0.024	0.046	0.604
OC use	0.031	0.032	0.340	0.026	0.032	0.416
Physical activity	-0.031	0.025	0.220	-0.027	0.029	0.346
Ever smoked	-0.007	0.072	0.923	0.021	0.076	0.784
Ever drank	0.024	0.059	0.680	-0.035	0.058	0.544
Family BC history	-0.071	0.034	0.038	-0.013	0.048	0.782
HRT use	0.029	0.039	0.454	-0.003	0.052	0.955
ER group (+)	0.038	0.026	0.137			
PR group (+)	-0.004	0.025	0.886			

BMI/body mass index, *HRT*/hormone replacement therapy, *SE*/standard error

Model adjusted for age at interview