Introduction

Breast cancer is the most common malignancy in women (1). In the USA, breast cancer incidence rates have been rising slowly for the past two decades, and breast cancer is the second leading cause of cancer-related death in women (2,3). However, there is currently no accurate method to predict who is most probably to develop breast cancer. If confirmed by future studies, chromosome 9p telomere length has the potential to be incorporated into the current prediction models to significantly enhance breast cancer risk prediction.

Materials and methods

Study population

Breast cancer cases (n = 153) were recruited at the Georgetown University Hospital clinics (Lombardi Comprehensive Cancer Center’s Division of Medical Oncology, Department of Surgery and the Betty Lou Ourisman Breast Cancer Clinic). The inclusion criteria for cases included a diagnosis of breast cancer within the prior 6 months, women who have not been treated yet with chemotherapy and radiation treatment or had active infection or immunologic disorder that needed to be treated with antibiotics or immunosuppressive medication within the prior 1 month. From 2006 through 2008, a total of 228 newly diagnosed breast cancer patients were identified to be eligible and 153 (67%) participated in our study. The common reasons for non-participation were too busy or not interested (24%), overwhelmed by cancer diagnosis (5%) and not responsive to phone call or e-mail contact (4%).

Controls (n = 159) were randomly selected from healthy women who visited the mammography screening clinic at Georgetown University Hospital; frequency matched to cases by age (two year interval), race and state of residency (DC, Maryland or Virginia). Other inclusion and exclusion criteria for controls were the same as for cases. Additionally, women who had breast biopsy or were pregnant or breast-feeding were not eligible. The overall participation rate among the eligible women was 57% for controls.

Abbreviations: CI, confidence interval; FITC, fluorescein isothiocyanate; OR, odds ratio; Rb, retinoblastoma; RFL, relative telomere length.

Reference

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Background: Telomere dysfunction is involved in the development of breast cancer and very short telomeres are frequent genetic alterations in breast tumors. However, the influence of telomere lengths of specific chromosomal arms on the breast cancer risk is unknown. Methods: We conducted a case–control study of breast cancer to examine the associations of the telomere length on chromosome 9 short arms (9p) and long arms (9q) with risk of breast cancer. Chromosome 9 arm-specific telomere lengths were measured by quantitative fluorescent in situ hybridization using cultured blood lymphocytes. Results: Telomere length on chromosome 9p was significantly shorter in breast cancer patients than in control subjects (P < 0.001). Using the 50th percentile value in controls as a cut point, women who have short 9p telomeres had an increased risk of breast cancer [adjusted odds ratio (OR) = 2.6; 95% confidence interval (CI) = 1.5–4.3]. When the 9p telomere length was divided into quartiles, a significant inverse dose–response relationship between 9p telomere length and breast cancer risk was observed (P< 0.001), with a quartile ORs of 3.0 (95% CI = 1.2–7.5), 3.9 (95% CI = 1.6–9.5) and 6.6 (95% CI = 2.8–15.9) for third, second and first quartile, respectively, when compared with women in the fourth quartile. Conclusions: Short telomere length on chromosome 9p is strongly associated with the risk of breast cancer. If confirmed by future studies, chromosome 9p telomere length has the potential to be incorporated into the current prediction models to significantly enhance breast cancer risk prediction.
Ten cases were excluded due to no histological confirmation of breast cancer diagnosis (5). Thirteen cases were excluded due to no case–control analysis is 140 cases and 159 controls. Statistical analysis

The final sample size for case–control analysis is 140 cases and 159 controls. Thirteen cases were excluded due to no histological confirmation of breast cancer diagnosis (n = 8) and blood culture failure (n = 5). Student’s t-test was used to compare the means of chromosome 9 telomere lengths between cases and controls because the normality test indicated that these variables were symmetrically distributed. Chi-square tests were used to compare the distribution of categorical variables between cases and controls. Pearson correlation was used to examine the correlations between chromosome 9 telomere lengths and age.

We examined the associations between chromosome 9 telomere lengths and the risk of breast cancer, using unconditional logistic regression. Telomere lengths were dichotomized as short/long using the 50th or 75th percentile values in the controls as a cut point. Telomere lengths were also categorized according to the quartiles in controls. Odds ratios (ORs) were adjusted for age, race, smoking status, alcohol use, education, history of cancer in first- and second-degree relatives, menopausal status and physical activity in the teens. P-values were two sided and considered statistically significant if P < 0.05. All analyses were performed using SAS software, version 9 (SAS Institute, Cary, NC).

Results

Characteristics of study population

Table I lists the characteristics of the study subjects. The mean age is 52.7 years for cases and 53.3 years for controls. There are no significant case–control differences in the distributions of race, menopausal status, tobacco smoking, alcohol use, education levels, family history of cancer and hormone replacement therapy use. The mean body mass index was similar between cases and controls. The mean total telomere length was not significantly different between cases and controls. Controls were significantly more probably to be physically active in both the teens and in the past year compared with cases. There were no significant differences in the distribution of the levels of household income between cases and controls among those who reported household income. However, 31% of the cases and 16% of the controls did not report household income (Table I).

Chromosome 9 telomere lengths by case–control status

Table II presents the case–control comparisons of the means of the four chromosome 9 telomeres (9p-short, 9p-long, 9q-long and 9q-short) to chromosome 9q-long. The mean RTL of the 9p-short was significantly shorter in cases than in controls (mean = 0.515%, P < 0.001). The mean RTL of the 9p-long was also significantly shorter in cases than in controls (mean = 1.184%, P = 0.017). However, the mean RTLS of 9q-short and 9q-long were not significantly different between cases and controls. When the case–control comparison was stratified by menopausal status, tobacco smoking status or physical activity in teens, we observed similar patterns of case–control differences across all subgroups of women.

RTL of chromosome 9p-short telomere and breast cancer risk

Table III shows the distributions of breast cancer patients and control subjects according to the RTL of 9p-short telomere. Using the 50th percentile value in controls as a cut point, women who had shorter 9p-short telomeres had significantly increased risk of breast cancer compared with women who had longer 9p-short telomeres [adjusted OR = 2.6; 95% confidence interval (CI) = 1.5–4.3] in the overall study population. When stratified by menopausal status, the ORs were 2.8 (95% CI = 1.3–6.2) and 2.4 (95% CI = 1.2–4.9) for pre- and postmenopausal women, respectively. When the RTL data were categorized into quartiles, a significant inverse dose–response relationship was observed (P = 0.001), and the lowest-versus-highest quartile OR was 6.6 (95% CI = 2.8–15.9) for all women. The ORs were 6.2 (95% CI = 1.8–21.3) and 7.5 (95% CI = 2.1–26.8) for pre-and postmenopausal women, respectively.

RTL of chromosome 9q-long telomere and breast cancer risk

Table IV shows the distributions of breast cancer patients and control subjects according to the RTL of 9q-long telomere. Using the 50th value in controls as a cut point, women who had shorter 9q-long telomere had marginally significant increased risk of breast cancer compared with women who had longer 9q-long telomere (OR = 1.6, 95% CI = 1.0 to 2.7) in the overall study population. When the RTL
data were categorized into quartiles, a significant inverse dose–response relationship was observed ($P_{\text{trend}} = 0.020$), and the lowest-versus-highest quartile OR was 2.2 (95% CI = 1.1–4.4) for all women. The OR was 3.0 (95% CI = 1.1–8.7) and 1.6 (95% CI = 0.6–4.3) for pre- and postmenopausal women, respectively.

Table V presents the joint effects of 9p-short and 9p-long RTL on breast cancer risk. The data suggested additive effects. However, no statistical significant interactions between 9p-short and 9p-long RTL were detected in all subjects ($P = 0.108$), premenopausal women ($P = 0.113$) or postmenopausal women ($P = 0.415$) when the interactions were formally tested in logistic models.

**RTL of chromosome 9q-short and 9q-longtelomeres and breast cancer risk**

Using the 50th percentile value in controls as a cut point, when women who had shorter 9q-short telomere were compared with women who had longer 9q-short telomere, the OR was 1.0 (95% CI = 0.6–1.7) for all women. When stratified by menopausal status, the ORs were 1.4 (95% CI = 0.7–2.7) and 0.8 (95% CI = 0.4–1.8) for pre- and postmenopausal women, respectively. When the data were categorized into quartiles, no significant dose–response relationship or a statistically significant difference by comparing the lowest to highest quartile was observed.

Similarly, when we used the 50th percentile value in controls as a cut point, women who had shorter 9q-long telomeres compared with longer 9q-long telomeres had an adjusted OR of 1.3 (95% CI = 0.8–2.0) overall. When stratified by menopausal status, the ORs were 1.6 (95% CI = 0.8–3.0) and 1.0 (95% CI = 0.5–2.1) for pre- and postmenopausal women, respectively. When the data were categorized into quartiles, no significant dose–response relationship or a statistically significant difference by comparing the lowest to highest quartile was observed.

### Table I. Distribution of characteristics of study population

<table>
<thead>
<tr>
<th>Host factors</th>
<th>Cases (N = 140)</th>
<th>Controls (N = 159)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD)</td>
<td>52.71 (10.61)</td>
<td>53.25 (9.92)</td>
<td>0.656</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whites</td>
<td>104 (74.2)</td>
<td>118 (74.2)</td>
<td></td>
</tr>
<tr>
<td>Blacks</td>
<td>28 (20.0)</td>
<td>34 (21.4)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>8 (5.7)</td>
<td>7 (4.4)</td>
<td>0.850</td>
</tr>
<tr>
<td>Menopausal status, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopausal status</td>
<td>56 (42.8)</td>
<td>67 (42.4)</td>
<td></td>
</tr>
<tr>
<td>Postmenopausal status</td>
<td>75 (57.3)</td>
<td>91 (57.6)</td>
<td>0.953</td>
</tr>
<tr>
<td>Tobacco smoking, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>81 (60.0)</td>
<td>93 (58.9)</td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>54 (40.0)</td>
<td>65 (41.1)</td>
<td>0.843</td>
</tr>
<tr>
<td>Alcohol use, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>15 (11.5)</td>
<td>12 (7.6)</td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>116 (88.6)</td>
<td>147 (92.5)</td>
<td>0.225</td>
</tr>
<tr>
<td>Physical activity in teens, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>55 (39.3)</td>
<td>31 (19.5)</td>
<td></td>
</tr>
<tr>
<td>Yes$^a$</td>
<td>85 (60.7)</td>
<td>128 (80.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Physical activity last year, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>59 (42.1)</td>
<td>36 (22.6)</td>
<td></td>
</tr>
<tr>
<td>Yes$^a$</td>
<td>81 (57.9)</td>
<td>123 (77.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Education, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\leq$4 years college</td>
<td>78 (58.2)</td>
<td>82 (51.6)</td>
<td></td>
</tr>
<tr>
<td>$&gt;4$ years college</td>
<td>56 (41.8)</td>
<td>77 (48.4)</td>
<td>0.256</td>
</tr>
<tr>
<td>Family income, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\leq$10K</td>
<td>46 (32.9)</td>
<td>56 (35.2)</td>
<td></td>
</tr>
<tr>
<td>$&gt;10$K</td>
<td>51 (36.4)</td>
<td>77 (48.4)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>43 (30.7)</td>
<td>26 (16.4)</td>
<td>0.010</td>
</tr>
<tr>
<td>Family history of cancer$^b$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>50 (39.7)</td>
<td>73 (46.5)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>76 (60.3)</td>
<td>84 (53.5)</td>
<td>0.250</td>
</tr>
<tr>
<td>HRT, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>86 (65.7)</td>
<td>94 (59.1)</td>
<td></td>
</tr>
<tr>
<td>Yes$^a$</td>
<td>45 (34.4)</td>
<td>65 (40.9)</td>
<td>0.254</td>
</tr>
<tr>
<td>BMI, mean (SD)</td>
<td>27.14 (6.30)</td>
<td>27.28 (6.64)</td>
<td>0.862</td>
</tr>
<tr>
<td>Total telomere length$^c$, mean (SD)</td>
<td>4.36 (0.99)</td>
<td>4.58 (1.01)</td>
<td>0.069</td>
</tr>
</tbody>
</table>

Bold numbers are statistically significant. BMI, body mass index; HRT, hormone replacement therapy.

$^a$Defined as physical activity at least once a week for at least 20 min at a time that either made the woman sweat or increased heart rate.

$^b$Defined as any cancer in the first degree blood relatives.

$^c$The unit of total telomere length is fluorescent intensity units in million (MFIU).

### Table II. Case–control comparison of mean chromosome 9 telomere lengths, by host factors

| conceivable relationship was observed ($P_{\text{trend}} = 0.020$), and the lowest-versus-highest quartile OR was 2.2 (95% CI = 1.1–4.4) for all women. The OR was 3.0 (95% CI = 1.1–8.7) and 1.6 (95% CI = 0.6–4.3) for pre- and postmenopausal women, respectively.

Table V presents the joint effects of 9p-short and 9p-long RTL on breast cancer risk. The data suggested additive effects. However, no statistical significant interactions between 9p-short and 9p-long RTL were detected in all subjects ($P = 0.108$), premenopausal women ($P = 0.113$) or postmenopausal women ($P = 0.415$) when the interactions were formally tested in logistic models.

**RTL of chromosome 9q-short and 9q-longtelomeres and breast cancer risk**

Using the 50th percentile value in controls as a cut point, when women who had shorter 9q-short telomere were compared with women who had longer 9q-short telomere, the OR was 1.0 (95% CI = 0.6–1.7) for all women. When stratified by menopausal status, the ORs were 1.4 (95% CI = 0.7–2.7) and 0.8 (95% CI = 0.4–1.8) for pre- and postmenopausal women, respectively. When the data were categorized into quartiles, no significant dose–response relationship or a statistically significant difference by comparing the lowest to highest quartile was observed.

Similarly, when we used the 50th percentile value in controls as a cut point, women who had shorter 9q-long telomeres compared with longer 9q-long telomeres had an adjusted OR of 1.3 (95% CI = 0.8–2.0) overall. When stratified by menopausal status, the ORs were 1.6 (95% CI = 0.8–3.0) and 1.0 (95% CI = 0.5–2.1) for pre- and postmenopausal women, respectively. When the data were categorized into quartiles, no significant dose–response relationship or a statistically significant difference by comparing the lowest to highest quartile was observed.

**Correlations of chromosome 9 telomere lengths and age**

Among control subjects, we observed a weak inverse correlation between 9p-short telomere length and age [Pearson correlation coefficient ($r = -0.162$, $P = 0.042$)]. No significant correlations were seen between age and the other three chromosome 9 telomeres. There were significant correlations between the allelic 9p telomere lengths ($r = 0.431$, $P < 0.001$) and between the allelic 9q telomere lengths ($r = 0.428$, $P < 0.001$). There were no significant correlations between 9p telomeres and 9q telomere lengths, except for a weak correlation between 9p-short and 9q-short ($r = 0.204$, $P = 0.010$).

Among cases, there were no significant correlations between chromosome 9 telomere lengths and age. There were significant correlations between the allelic 9p telomeres ($r = 0.525$, $P < 0.001$) and between the allelic 9q telomeres ($r = 0.326$, $P < 0.001$).
By quartiles

<table>
<thead>
<tr>
<th>Compared group</th>
<th>Cases/controls</th>
<th>OR^a (95% CI) P</th>
<th>OR^b (95% CI) P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1 (0.494–0.583)</td>
<td>64/38</td>
<td>7.51 (3.25–17.37) &lt;0.001</td>
<td>6.62 (2.75–15.94) &lt;0.001</td>
</tr>
</tbody>
</table>

Postmenopausal women

<table>
<thead>
<tr>
<th>Compared group</th>
<th>Cases/controls</th>
<th>OR^a (95% CI) P</th>
<th>OR^b (95% CI) P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1 (0.494–0.583)</td>
<td>28/15</td>
<td>6.99 (2.14–22.83) &lt;0.001</td>
<td>6.16 (1.78–21.32) 0.002</td>
</tr>
</tbody>
</table>

By quartiles

<table>
<thead>
<tr>
<th>Compared group</th>
<th>Cases/controls</th>
<th>OR^a (95% CI) P</th>
<th>OR^b (95% CI) P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q4 (≥0.680)</td>
<td>5/18</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Q3 (0.583–0.680)</td>
<td>12/20</td>
<td>2.29 (0.67–7.85)</td>
<td>2.16 (0.60–7.83)</td>
</tr>
<tr>
<td>Q2 (0.494–0.583)</td>
<td>11/14</td>
<td>2.90 (0.81–10.35)</td>
<td>2.87 (0.76–10.81)</td>
</tr>
<tr>
<td>Q1 (≤0.494)</td>
<td>28/15</td>
<td>6.99 (2.14–22.83) &lt;0.001</td>
<td>6.16 (1.78–21.32) 0.002</td>
</tr>
</tbody>
</table>

Discussion

Our results showed that, after adjustment for known breast cancer risk factors, shorter telomeres on chromosome 9p-short (allelic shorter version) were strongly associated with an increased risk of breast cancer. Our results are corroborated by previous reports showing that telomere dysfunction is involved in the development of breast cancer (6,7). For example, marked shortening of chromosome telomeres were reported in grade II and III breast cancers (35). Very short telomeres are also frequent genetic alterations in premalignant lesions (6,36), suggesting that telomere dysfunction is an early event in breast carcinogenesis.

A study of genetically engineered mice provided evidence that the average telomere length in a cell may be less important for cancer risk than short telomeres on specific chromosome arms because chromosome fusions associated with telomere dysfunction occur preferentially on chromosome arms with the shortest telomeres (37). In humans, there are 23 pairs of chromosomes and 92 telomeres, and chromosome-specific telomere lengths are highly polymorphic between chromosomal arms (38–40). One potential implication of this chromosome arm-specific telomere length variation is that chromosome arms bearing the shortest telomeres may predispose to the chromosome aberrations and therefore have an impact on the evolution of tumors. This concept is supported by several studies demonstrating that chromosome arms with the shortest telomeres were more often found in the telomere fusions leading to chromosome instability (37,41). Our observation that shortest 9p telomere (shorter 9p-short telomeres) was strongly associated with breast cancer risk supports this concept. To the best of our knowledge, our study is the first to report that shorter telomere on chromosome 9p is strongly associated with breast cancer risk. If confirmed by other studies, chromosome 9p telomere length, in combination with other risk biomarkers, could potentially improve breast cancer risk assessment for individual women. Based on these promising findings, it is reasonable to anticipate that lengths of other arm-specific telomeres might be associated with breast cancer risk. Thus, future studies should aim to identify such telomeres.

Our results also indicated that telomere lengths on chromosome 9q were not associated with breast cancer risk. We observed no significant correlations between 9p and 9q telomere lengths in controls, except a weak correlation between 9p-short and 9q-long, suggesting telomere lengths on 9p or 9q are independent events. It is well documented that frequent chromosomal abnormalities in early stage breast cancers only involves a handful of chromosome arms, including gains of 1q, 8q, 17q and 20q and losses of 8p, 9p, 16q and 17p (5,8,9). Therefore, it is not surprising to find that chromosome 9q telomeres were not associated with breast cancer risk in our study because chromosome 9q aberration is not a common abnormality in breast tumors. In this study, we also found that there was no significant difference in mean overall (cell total) telomere length between cases and controls (Table I) (31). It would be expected that overall telomere length would not be informative due to the inclusion of large number of ‘irrelevant’ telomeres in the measurement. This may in part explain the statistically significant but weak correlations between 9p-short and 9q-short (r = 0.256, P = 0.002) and between 9p-long and 9q-long (r = 0.186, P = 0.028).

Table III. Logistic regression analysis examining the association of chromosome 9p-short telomere length and breast cancer risk

<table>
<thead>
<tr>
<th>9p-short RTL (%)</th>
<th>Cases/controls</th>
<th>OR^a (95% CI) P</th>
<th>OR^b (95% CI) P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>130/118</td>
<td>5.08 (2.35–10.96) &lt;0.001</td>
<td>4.43 (1.98–9.88) &lt;0.001</td>
</tr>
<tr>
<td>By quartiles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q4 (≥0.680)</td>
<td>10/41</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Q3 (0.583–0.680)</td>
<td>31/40</td>
<td>3.43 (1.44–8.19)</td>
<td>3.00 (1.20–7.45)</td>
</tr>
<tr>
<td>Q2 (0.494–0.583)</td>
<td>35/40</td>
<td>3.93 (1.66–9.29)</td>
<td>3.87 (1.58–9.49)</td>
</tr>
<tr>
<td>Q1 (≤0.494)</td>
<td>64/38</td>
<td>7.51 (3.25–17.37) &lt;0.001</td>
<td>6.62 (2.75–15.94) &lt;0.001</td>
</tr>
</tbody>
</table>

Bold numbers are statistically significant.

^aOR adjusted for age as continuous, race, smoking status (never/ever), alcohol use (never/ever), education, family history of cancer (no/yes), hormone replacement therapy (no/yes), history of pregnancy (no/yes), menopausal status (when appropriate) and physical activity in teens (no/yes).

^bP for trend.
All subjects
≥Median (≥1.211) 54/81 1.00 0.037 1.00 1.63 (0.99–2.67) 0.056
<Median (<1.211) 86/78 1.65 (1.03–2.64) 0.037 1.00 1.00 1.00 1.00
Long (≥1.343) 24/40 1.00 0.118 1.56 (0.83–2.79) 0.170
Short (<1.343) 116/119 1.56 (0.89–2.796) 0.118 1.56 (0.83–2.79) 0.170

By quartiles
Q4 (≥1.343) 24/40 1.00 0.008 2.19 (1.09–4.39) 0.020
Q3 (1.211–1.343) 30/41 1.18 (0.59–2.36) 1.12 (0.54–2.36)
Q2 (1.122–1.211) 35/39 1.47 (0.73–2.94) 1.27 (0.60–2.66)
Q1 (≤1.122) 51/39 2.11 (1.09–4.07) 0.018 2.10 (1.09–4.39) 0.020

Premenopausal women
≥Median (≥1.211) 21/36 1.00 0.070 1.84 (0.84–4.00) 0.126
<Median (<1.211) 35/31 1.97 (0.95–4.12) 1.84 (0.74–4.60) 0.192
Long (≥1.343) 10/20 1.00 1.00 1.00
Short (<1.343) 46/47 2.00 (0.84–4.77) 0.117 1.84 (0.74–4.60) 0.192

By quartiles
Q4 (≥1.343) 10/20 1.00 1.00
Q3 (1.211–1.343) 11/16 1.41 (0.48–4.19) 1.32 (0.42–4.19)
Q2 (1.122–1.211) 12/16 1.52 (0.52–4.47) 1.18 (0.36–3.81)
Q1 (≤1.122) 23/15 3.18 (1.16–8.74) 0.025 3.03 (1.06–8.70) 0.042

Postmenopausal women
≥Median (≥1.211) 31/44 1.00 1.00
<Median (<1.211) 44/47 1.35 (0.72–2.52) 0.345 1.45 (0.75–2.80) 0.275
Long (≥1.343) 13/20 1.00 1.00
Short (<1.343) 62/71 1.36 (0.62–2.96) 0.446 1.26 (0.55–2.88) 0.586

By quartiles
Q4 (≥1.343) 13/20 1.00 1.00
Q3 (1.211–1.343) 18/24 1.15 (0.45–2.93) 0.096 1.35 (0.34–3.6) 0.275
Q2 (1.122–1.211) 25/24 1.61 (0.66–4.76) 0.270 1.64 (0.63–4.26) 0.233
Q1 (≤1.122) 25/24 1.61 (0.66–4.76) 0.270 1.64 (0.63–4.26) 0.233

Bold numbers are statistically significant.
*OR adjusted for age as continuous and race.
**OR adjusted for age as continuous, race, smoking status (never/ever), alcohol use (never/ever), education, family history of cancer (no/yes), hormone replacement therapy (no/yes), history of pregnancy (no/yes), menopausal status (when appropriate) and physical activity in teens (no/yes).
*P for trend.

The case–control differences reported here are unlikely to be ascribed to assay bias because the samples were processed and measured blinded to case–control status. Measurements of four chromosome 9-arm telomere lengths were made simultaneously from the same cells captured by the fluorescent imaging system. While there were significant case–control differences for chromosome 9p telomere lengths, the mean telomere lengths of chromosome 9q and cell total were very similar between cases and controls. During the study, a systematic quality control plan was implemented to ensure the consistent efficiency of fluorescent in situ hybridization (the coefficient of variation of the total telomere lengths among 20 repeats of the control cell = 12.4%). Co-hybridization of a FITC-labeled chromosome 9-specific probe (green) was used as an internal control for hybridization efficiency, and only slides that showed bright green chromosome 9 signals were accepted. The samples that failed to meet these quality control standards were rejected and repeated (3% of the samples). Additionally, relative telomere lengths of chromosome 9 arms were defined as the ratio of chromosome 9 telomere lengths to the total telomere length, thus minimizing the assay variation between the individual samples. Bias in telomere length measurement is thus unlikely.

Given that this is a case–control study, a theoretical concern is that telomere length in leukocytes is affected by case status (reverse causality). Data by previous studies and by us indicated that the mean overall telomere length of blood leukocytes in breast cancer patients was not significantly shorter than in healthy women controls (31–34), suggesting there is no significant shortening of blood leukocyte telomere length associated with having breast cancer. Although previous studies (42,43) suggested that chemotherapy and/or radiotherapy can induce telomere shortening in leukocytes, all the blood samples in our study were drawn before any chemotherapy and radiotherapy.
treatments. Thus, reverse causality is not a plausible explanation for our results. Recall bias might influence information about self-reported breast cancer risk factors in a case–control study where the data were collected after the diagnosis of cancer. We compared chromosome 9 telomere lengths in control subjects by numerous variables from the questionnaire and did not find any significant differences in the mean chromosome 9 telomere lengths between subgroups defined by race, age, smoking status, alcohol drinking, menopausal status, physical activity in the teens, hormone replacement therapy, history of pregnancy, family history of cancer, education and income (data not shown). Cases and controls were closely matched by age, and age was included as continuous variable in all the logistic models for adjustment. Thus, age would not confound the telomere results.

In conclusion, our data provide the first evidence that short telomere on chromosome 9p is strongly associated with breast cancer risk. If confirmed in future studies, chromosome 9p telomere length could be incorporated into the current prediction models to significantly enhance breast cancer risk prediction. Better risk assessment would improve the efficiency of both population-based preventive programs, such as screening mammography, as well as individual-based preventive strategies such as chemoprevention by targeting women who are at the greatest risk for breast cancer.

Supplementary material
Supplementary Figure 1 can be found at http://carcin.oxfordjournals.org/

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References

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