**THE ASSOCIATION BETWEEN TELOMERE LENGTH**

**AND RISK OF BREAST CANCER**

**IN SINGAPORE CHINESE HEALTH STUDY**

by

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**ABSTRACT**

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**Background:**Telomeres are tandemly repeated sequences located at the distal ends of linear chromosomes, which play an important role in maintaining chromosome integrity and cell replication. Although numerous epidemiological studies have examined the association between telomere length and risk of breast cancer, the results are conflicting. In this study, we evaluated the association between telomere length and risk of breast cancer using the data from the Singapore Chinese Health Study, a prospective, population-based study. **Methods:**Study subjects were 14,306 women aged 45-74 years at enrollment in the Singapore Chinese Health Study. The subjects’ information of demographics, lifestyle, and reproductive history was collected at enrollment and blood sample collection. Telomere length was measured using the qPCR method. Hazard ratios (HRs) and 95% confidence intervals (95% CIs) for risk of breast cancer associated with telomere length in quintiles were calculated using Cox proportional hazard regression, with adjustment for age at blood collection, level of education, body mass index (BMI), number of live births (0, 1-2, 3-4, or 5+) and age at first live birth (nulliparous, <20, 21-25, 26-30, or 31+ years). **Results:** Breast cancer risk was increased significantly in association with longer telomere length. Women with the highest quintile of telomere length had a 47% higher risk of breast cancer (HR = 1.47, 95% CI = 0.90-2.38, *P*trend = 0.01) compared with the lowest quintile. The association is apparent in women that were overweight/obese (HR = 2.00, 95% CI 1.02 - 3.91, *P*trend = 0.01); women who had menarche less than 14 years old (HR = 1.56, 95% CI 0.84-2.89, *P*trend = 0.01); women who gave first live birth at or above 26 years old (HR = 1.74, 95% CI 0.86-3.54, *P*trend = 0.02); and women who had 3 or more children (HR = 1.64, 95% CI 0.83-3.23, *P*trend = 0.02). **Conclusion:** Longer telomere length is associated with increased risk of breast cancer in an Asian/Pacific Islander population. This finding could provide a significant benefit to public health, as it could serve as convincing evidence for telomere length measurement as a biomarker in breast cancer prevention and development.

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# Introduction

As the most common cancer for women in most countries around the world, breast cancer caused 534,000 deaths with 2.4 million incidence cases worldwide in 2015 (Fitzmaurice et al., 2017). It is estimated that breast cancer accounts for 1 in 3 new diagnosed cancers in American women, and over 40,000 American women will die from breast cancer in 2018 (Siegel, Miller, & Jemal, 2018). Though previous epidemiologic studies have established many risk factors of breast cancer, such as age, parity, reproductive factors and exogenous hormone use (Mina, Storniolo, Kipfer, Hunter, & Ludwig, 2016), with the upward trend of breast cancer incidence during recent years (Siegel et al., 2018), there is an urgent need to identify more modifiable risk factors or biomarkers to prevent more incidences of breast cancer.

## risk factors of Breast cancer

### Age

Older age is a crucial risk factor of breast cancer. As women grow old, the DNA damage in aging cells accumulates, which would increase the possibility of malignancy (López-Otín, Blasco, Partridge, Serrano, & Kroemer, 2013). The 10-year probability of developing breast cancer increases from 0.1% in 20 year olds to nearly 4% in 70 year olds (American Cancer Society, 2017). With longer life expectancy, lifetime risk of breast cancer has increased to 12% in recent years (American Cancer Society, 2017). Thus more and more women are under the threaten of developing breast cancer.

### Race

Race is another widely recognized risk factor of breast cancer. According to the National Center for Health Statistics, non-Hispanic white women have a 40% higher incidence rate than Asian/Pacific Islander women (American Cancer Society, 2017). Incidence rate in non-Hispanic black women is 126 per 100,000 and slightly less than the highest rate of 129 per 100,000 in non-Hispanic white women (American Cancer Society, 2017). Although Asian women historically tend to have the lowest incidence and mortality rates of breast cancer, there has been an increasing trend of breast cancer incidence among them between 2005 and 2014. Surprisingly, during the same time period, the breast cancer incidence rate remained stable among non-Hispanic white women (American Cancer Society, 2017).

### Body Mass Index

Numerous studies have shown that body mass index (BMI) has a significant impact on breast cancer risk that is dependent on menopausal status of women. For postmenopausal women, with a 4 kg/m2 increment of BMI, the risk of being diagnosed with breast cancer increases by 7% (van den Brandt et al., 2000). On the contrary, among premenopausal women, lower BMI is related to an increased risk of breast cancer (van den Brandt et al., 2000). When the association between BMI and breast cancer risk among postmenopausal women is adjusted for serum concentration of sex hormones such as estradiol, risk is reduced, which suggests that increased levels of estrogens may partly explain the higher risk of breast cancer in obese postmenopausal women (Endogenous Hormones Breast Cancer Collaborative Group, 2003).

### Reproductive Factors

Younger age at menarche, older age at first live birth, lower number of births, and older age at menopause are all related to a higher risk of breast cancer. This association could be largely owing to the time of estrogen exposure. The greater lifetime exposure to estrogen, the higher risk of developing breast cancer (Kelsey, Gammon, & John, 1993). Thus, younger age at menarche and older age at menopause are linked to more menstrual cycles and more cell proliferation in breast tissues, and ultimately leads to higher possibility of cancer occurrence. Each one year delay in menarche and menopause corresponds to a 5% decrease and 3% increase in breast cancer risk, respectively (Hunter et al., 1997; Trichopoulos, MacMahon, & Cole, 1972).

Comparing to nulliparous women, parous women have an overall 30% lower risk of breast cancer, whereas this association is not consistent among different age groups (Ewertz et al., 1990; Kelsey et al., 1993). For example, for women younger than 40 years old, full-term pregnancy might have an adverse effect on the risk of developing breast cancer. On the other hand, full term pregnancy acts as a protective factor for breast cancer diagnosis after 40 years old for both premenopausal and postmenopausal women (Kelsey et al., 1993).

Having first live birth at a younger age and more children also protect against breast cancer. The risk of developing breast cancer among women who have their first live birth after 35 years old is estimated to be 40% higher than those who have their first live birth earlier than 20 years old, and the risk reduces by 16% for every 2 more births (Ewertz et al., 1990). Breastfeeding could also reduce the risk of breast cancer, and this effect is greater with a longer time of breastfeeding (Beral, Bull, Doll, Peto, & Reeves, 2002).

### Exogenous Hormones

#### Oral Contraceptives Oral contraceptives (OC) use have been positively associated with breast cancer risk due to the presence of estrogen and progestin. However, according to the epidemiologic studies, the relationship is relatively weak for women who have stopped using OC within the past 10 years, and not significant for women who have stopped using them for 10 or more years (Collaborative Group on Hormonal Factors in Breast Cancer, 1996). The effect of OC use on breast cancer does not vary by the age started using and dosage of OC (Collaborative Group on Hormonal Factors in Breast Cancer, 1997a). A study also found no association between OC use and risk of breast cancer among women aged 35-64 years (Marchbanks et al., 2002). As OC is more usually used by younger women, it is likely that the effect of OC has already diminished by the time of breast cancer development in older age.

#### Menopausal Hormone Therapy Overall, longer duration of menopausal hormone therapy (MHT) is linked to increased risk of breast cancer. One more year usage of combined MHT which contains estrogen and progestin contributes to an 8% increase in the risk of breast cancer (Lee, Ross, & Pike, 2005). The effect is greater for women with lower BMI compared to women with higher BMI (Collaborative Group on Hormonal Factors in Breast Cancer, 1997). Most of the studies have shown that this positive association between MHT and risk of developing breast cancer exists only among current, long-term continuous users of combined MHT users, not in sequential scheduled users or those who have been on MHT for 5 years or longer after cessation of MHT use (Collaborative Group on Hormonal Factors in Breast Cancer, 1997; Lee et al., 2005). However, more studies are needed to address this question. Although the exact mechanism behind the association between MHT and risk of breast cancer is not clear, the enhancement of cell proliferation due to use of progestin and estrogen may play an important role in this process (Raafat, Hofseth, & Haslam, 2001).

### Lifestyle Factors

#### Alcohol Consumption and Smoking A reanalysis of 53 epidemiological studies including 153,582 women found a linear relationship between drinking alcohol and breast cancer risk. The relative risk of breast cancer increases by 7% per 10 g higher consumption of alcohol per day (Collaborative Group on Hormonal Factors in Breast Cancer, 2002). As alcohol drinking and cigarette smoking are highly correlated with each other, the study also stratified the participants by smoking status to eliminate potential confounding effects of cigarette smoking. However, the relationship remained the same between alcohol consumption and risk of breast cancer in both ever-smokers and never-smokers (Collaborative Group on Hormonal Factors in Breast Cancer, 2002).

Based on a literature review, tobacco smoking might not increase the risk of breast cancer, except for long-term duration of smoking and heavy smoking before first live birth (Terry & Rohan, 2002). The relationship is confounded by alcohol consumption, as most studies have not been successful in detecting a significant relationship between tobacco smoking and risk of breast cancer after adjustment for alcohol drinking status (Reynolds, 2013). Obviously, more investigations with larger sample sizes and validated assessment of exposure are required to unravel this association.

#### Physical Activity Numerous epidemiological studies have provided convincing evidence that physical activity is associated with a decreased risk of breast cancer, especially among women who are premenopausal and who have been diagnosed with estrogen and progesterone receptor-negative breast cancer. According to a meta-analysis of 31 studies, the overall relative risk of all types of breast cancer decreases 14% and 3% for women who have rigorous and moderate intensity of activity, respectively, regardless of their menopausal status (Wu, Zhang, & Kang, 2013). The mechanism behind this effect might related to the reduction in BMI, reduction in circulation of estrogens, improved effectiveness of insulin, which could inhibit tumor growth, and the stimulating effect of physical activity on the immune system (Montaruli, Patrini, Roveda, & Carandente, 2012).

### Family History and Genetic Factors

For women who have any relative with any type of breast cancer, the risk of developing breast cancer is nearly 2 times higher than women without any relative having breast cancer. The relative risk of developing breast cancer for women who have a first-degree relative with breast cancer is stronger than women who have a second-degree relative with breast cancer (Pharoah, Day, Duffy, Easton, & Ponder, 1997). Multiple studies have demonstrated that this relationship is related to inheritance of certain gene mutations, such as *BRCA1* and *BRCA2*. Mutations in these genes are associated with deficiencies in DNA repair potential, which may leads to cancer development (Chen, Silver, Cantor, Livingston, & Scully, 1999). There are also other genes related to high risks of breast cancer, including *TP53*, *PTEN*, *CDH1*, and *STK11*. Mutations in *TP53* cause Li-Fraumeni syndrome and those in *PTEN* result in Cowden syndrome. *CDH1* mutation carriers have an increased risk of breast cancer and diffuse gastric cancer and *STK11* mutations result in Peutz-Jeghers syndrome. All those syndromes mentioned above will significantly increase the risk of tumor development(Mina et al., 2016).

## telomere length and breast cancer

Telomeres are tandemly repeated sequences of TTAGGG located at the distal ends of linear chromosomes (Blasco, 2005). They play an essential role in preventing DNA double strand breaks, end-to-end chromosome fusions and degradation; therefore, maintaining the structural integrity of chromosomes and regulating cell replication (Aubert & Lansdorp, 2008). Owing to the incomplete DNA replication, the length of telomere decreases 50-200 bp during each cell division (Muraki, Nyhan, Han, & Murnane, 2012). Progressive telomere shortening often leads to genomic instability, and eventually results in apoptosis or cellular senescence (Campisi, 2001). Since telomere shortening controls the number of cell replications, this progress is considered a tumor suppressor mechanism (Blasco, 2005). However, 90% of human tumors have an up-regulated level of telomerase, a reverse transcriptase that increases the length of telomeres, which provides an opportunity for cancer cells to maintain telomere length and hence preserve active proliferation (Aubert & Lansdorp, 2008; Blasco, 2005).

Recently, a growing number of studies have focused on analyzing the association between telomere length measured in peripheral blood cells and the risk of cancers, and explored the potential possibility of using telomere length as a clinical biomarker of cancer risk. To date, there are 8 retrospective case-control studies with inconsistent results. Among them, 2 have shown longer telomere length was associated with increased risk of breast cancer (Gramatges, Telli, Balise, & Ford, 2010; Svenson et al., 2008); 4 studies have shown a non-significant association (Barwell et al., 2007; Shen et al., 2007, 2009; Zheng et al., 2010); and 2 have reported that telomere length was significantly shorter in breast cancer cases than non-cases (Levy et al., 1998; Pooley et al., 2010). However, case-control studies cannot provide information on the cause-effect relationship between telomere length and breast cancer risk, due to the reason that DNA samples for the measurement of telomere length were obtained from the cases after cancer diagnosis, and the progression of treatment for may have influenced the telomere length, which introduces potentially temporal or reverse causality bias. Results from prospective studies so far are also conflicting. Four studies including 2 nested case-control studies, 1 case-cohort study and 1 prospective cohort study all demonstrated nonsignificant association between telomere length and breast cancer status (De Vivo et al., 2009; Kim et al., 2011; Pooley et al., 2010; Willeit et al., 2010). One nested case-control study showed an increased risk of breast cancer for women in with either shortest or longest quintile of telomere length group comparing to the fourth quintile of telomere length group (Qu et al., 2013). The majority of prospective studies conducted so far are nested case-control studies. Many established risk factors of breast cancer, such as reproductive factors and use of exogenous hormones, were not matched for cases and controls or adjusted for in the statistical analyses (Mina et al., 2016), which reduces the power of the study. Only one prospective cohort study conducted in the Danish general population found a significant 9% reduction in breast cancer risk for each one kilobase decrement in telomere length (Weischer et al., 2013). As the percentage of women who were newly diagnosed with breast cancer among Asian/Pacific Islander population has been increasing in the past decades (Howlader et al., 2016), more attention should be paid to this population. Data from prospective cohort studies on telomere length and risk of breast cancer have been lacking in Asian populations. As more and more studies are focusing on exploring the relationship between telomeres and disease development, a better understanding of the association between telomere length and risk of breast cancer will have a significant public health impact on the risk assessment of breast cancer. For this work we conducted a prospective cohort analysis within the Singapore Chinese Health Study, and aimed to test the hypothesis that longer telomeres in pre-diagnostic peripheral blood leukocytes increases the risk of breast cancer.

# materials and methods

## Study population

Study subjects were participants in the Singapore Chinese Health Study, which is a population-based prospective cohort study aiming at investigating risk factors of cancer among Chinese in Singapore. The methodology and details of the study design and subject recruitment have been described elsewhere (Hankin et al., 2001). Briefly, between 1993 and 1998, 63,257 Chinese men and women aged 45 - 74 years of Cantonese or Hokkien dialect groups were interviewed in person by a trained interviewer using a structured questionnaire that collected information on demographics, such as age, height, weight and education level, potential risk factors including lifestyle factors, medical history, and family history of cancer including breast cancer. For women only, information related to menstrual and reproductive history, and use of OC pills and MHT was also collected. Beginning in April 1994, blood and urine samples were collected from a random 3% sample of cohort participants. In January 2000, we extended blood and urine collection to all surviving cohort participants who completed baseline interview. Of the 52,326 eligible subjects, 28,346 subjects (54% of eligible) donated blood samples by the end of 2005. During 1999 - 2004, we also conducted the first follow-up interview through telephone and 91% of female participants updated their smoking status, alcohol drinking status, menopausal status and MHT use status. The original cohort study was approved by the Institutional Review Boards of the National University of Singapore and the University of Pittsburgh.

## Assessment of breast cancer cases

Incident cases of all cancers, including breast cancer and all cases of deaths among cohort participants were identified through the annual record linkage analysis with the databases of the Singapore Cancer Registry and Birth and Death Registry, respectively (Chia, 2011). According to the latest record, only 56 (< 0.1%) of total 63,257 original cohort subjects were lost to follow-up due to migration out of Singapore. Breast cancer was coded according to the International Classification of Disease, 10th edition (ICD-10) including C50.0 - C50.9 codes. As of December 31, 2008, 210 women provided blood samples at baseline developed breast cancer with a mean follow-up period of 3.2 years.

## Laboratory methods

Genomic DNA was extracted from peripheral blood leukocytes sample using QIAamp 96 DNA Blood Kits (Qiagen, Valencia, CA) according to the manufacturer’s protocol. Relative telomere length was measured by comparing the ratio of telomere DNA content (T) relative to a single copy gene number for albumin (S) in experimental samples compared to standardized reference sample values using multiplexed quantitative polymerase chain reaction (PCR) method developed by Cawthon (Cawthon, 2009). This simple and rapid method allowed us to use smaller amounts of DNA to obtain relative telomere lengths in a very large population (Montpetit, 2015). PCR reaction was set up in the 96-well plate in the Bio-Rad MyiQ Single Color Real-Time PCR Detection System by aliquoting 15 $μ$L master mix and 10 $μ$L of experimental DNA sample into each reaction well. The reference standard DNA curve was made from a pooled sample of 77 participants from the Singapore Chinese Health Study identified in a previous study. The telomere length values of all the 77 samples were within 10% of the population mean. The reference samples were serial diluted in 4 concentrations with 8 replicates in every PCR plate together with experimental samples to provide relative quantitation. The experimental samples and reference sample maintain same relative quantity of single copy gene and telomeric repeat DNA by controlling the number of PCR cycles needed to generate a given amount of PCR product. All plates with experimental and reference samples were analyzed twice, and the average number of T/S value was reported, which is expected to correlated with the telomere length. The mean coefficient of variation, as a measure of reproducibility, of all technical sample duplicates for telomere length in the present study was 3.5%.


## Statistical analysis

Student’s t-test and $χ$2-test were performed to compare the distributions of selected characteristics and risk factors for continuous and categorical variables, respectively, between breast cancer cases and non-cases of the entire cohort. Analysis of covariance (ANCOVA) was used to examine the effect of age, BMI and other selected variables on telomere length.

The present study included 14,306 subjects after excluding 1,585 subjects with a history of cancer at the time of blood collection, 221 missing telomere length measurements due to assessment problems. Persons-years for each study participant at risk were calculated from the date of blood sample collection to the date of breast cancer diagnosis, death, migration out of Singapore, or December 31, 2008, which ever occurred first. Cox proportional hazard regression method was used to examine the association between relative telomere length in quintiles and the risk of developing breast cancer. The magnitude of the association was measured by hazard ratios (HRs) and corresponding 95% confidence intervals (CIs). Tests for linear trends were carried out by taking quintiles of telomere length as an ordinal variable in the Cox model. The proportional hazards assumption was assessed through the significance of the Pearson’s correlation coefficient of the relationship between the scaled Schoenfeld residuals of relative telomere length and survival time. No violations of assumption was found in our analyses.

According to the previous studies, the following factors that have been associated with breast cancer risk were including in the Cox regression models as covariates: age at blood collection, level of education, body mass index (BMI), number of live births (0, 1-2, 3-4, or 5+) and age at first live birth (nulliparous, <20, 21-25, 26-30, or 31+ years old). Since further adjustment for age at menarche (<13, 13-14, 15-16, or 17+ years), menopausal status (still menstruating or postmenopausal), age at menopause (<40, 40-44, 45-49, 50-54, or 55+ years), family history of breast cancer, use of oral contraceptives, alcohol drinking status (nondrinker/monthly drinkers, weekly drinkers, or daily drinkers), smoking status (never smokers, ex-smokers, or current smokers), physical activity (no, ½-3 hours/week, or 4+ hours/week) did not change the results, we did not additionally adjust for these variables. Interactions of telomere length and all covariates were also tested and none of them were significant. Thus, the interactions were not included in the final Cox regression model. Stratified analyses were conducted for women of different age at menarche (<14 vs $\geq $14 years old), age at first live birth ($<$26 vs $\geq 26$ years), number of children (<3 vs $\geq $3), and BMI ($<$23 vs $\geq $23 kg/$m^{2}$). The BMI cut-off point for overweight was considered 23 kg/$m^{2}$ as suggested in most Asian populations (Barba et al., 2004).

All statistical analyses were conducted using SAS 9.4 software package (SAS Institute, Cary, NC). All *P* values reported are two sided and *P*<0.05 was considered statistically significant.

# Results

## Basic characteristics of study population

A total of 210 (1.5%) patients developed breast cancer in our cohort. The mean $\pm $standard deviation (SD) age at blood draw for breast cancer cases and non-cases were 61.4 $\pm $ 7.9 years and 62.3 $\pm $7.7 years, respectively. There is no significant difference between mean age at blood draw or distribution of dialect groups among cases and non-cases. The mean time interval between blood sample collection and breast cancer diagnosis was 3.2 years (range 2 days - 12.2 years). Comparing to non-cases with a mean BMI of 23.3 (SD = 3.6), breast cancer cases had a significantly higher BMI with a mean of 23.9 (SD = 3.6). Table 1 shows the comparison of selected characteristics between breast cancer cases and non-cases. There were significant differences in the distributions of the level of education, BMI categories, number of live births, age at first live birth and MHT use among cases comparing with those among non-cases. Breast cancer cases were more likely to have higher level of education, higher BMI, older age at first live birth, fewer live births, and use MHT (Table 1). Breast cancer cases and non-cases had similar smoking status, drinking status, age at menarche, age at menopause, percentage of menopausal status, family history of breast cancer, and use of OC (Table 1).

Table 1. Distribution of selected demographic characteristics and risk factors of breast cancer cases and non-cases: The Singapore Chinese Health Study.

|  |  |  |  |
| --- | --- | --- | --- |
| Characteristics | Cases (%)N=210 | Non-cases (%)N=14,096 | *Pa* |
| Dialect group |  |  |  |
|  Cantonese | 55.7 | 52.5 | 0.36 |
|  Hokkien | 44.3 | 47.5 |
| Level of education |  |  |  |
|  No formal education | 23.8 | 32.1 | 0.04 |
|  Primary (1-6 years) | 47.6 | 42.1 |
|  Secondary school or higher | 28.6 | 25.8 |
| BMI(kg/m2) |  |  |  |
|  <20 | 11.4 | 17.1 | 0.03 |
|  20 to <24 | 42.4 | 45.7 |
|  24 to <28 | 32.9 | 27.1 |
|  28+ | 13.3 | 10.1 |
| Smoking status |  |  |  |
|  Never smokers | 91.0 | 91.3 | 0.12 |
|  Former/Current smokers | 9.0 | 8.8 |
| Drinking status |  |  |  |
|  Non-drinker | 89.1 | 88.4 | 0.78 |
|  Ever drinker | 11.0 | 11.6 |
| Number of live births |  |  |  |
|  None | 12.4 | 7.1 | 0.01 |
|  1-2 | 34.8 | 30.5 |
|  3-4 | 32.4 | 40.3 |
|  5+ | 20.5 | 22.1 |
| Age at first live birth (years) |  |  |  |
|  <21 | 14.3 | 17.0 | 0.03 |
|  21-25 | 33.8 | 37.5 |
|  26-30 | 26.7 | 27.7 |
|  31+ | 12.9 | 10.6 |
|  Nulliparous women | 12.4 | 7.1 |
| Age at menarche (years) |  |  |  |
|  <13 | 13.8 | 16.4 | 0.18 |
|  13-14 | 46.7 | 39.4 |
|  15-16 | 30.5 | 32.8 |
|  17+ | 9.1 | 11.4 |
| Age at menopause (years) |  |  |  |
|  Still have period | 10.5 | 10.7 | 0.67 |
|  <40 | 2.9 | 3.4 |
|  40-44 | 6.7 | 7.5 |
|  45-49 | 26.2 | 29.3 |
|  50-54 | 42.9 | 40.9 |
|  55+ | 11.0 | 8.2 |
| Menopausal status |  |  |  |
|  Still menstruating | 10.4 | 10.5 | 0.98 |
|  Postmenopausal | 89.6 | 89.5 |
| Use of OC |  |  |  |
|  Never users | 71.4 | 69.1 | 0.46 |
|  Ever used | 28.6 | 30.9 |
| MHT use |  |  |  |
|  Never users | 80.5 | 86.8 | 0.01 |
|  Ever used | 19.5 | 13.2 |
| Family history of breast cancer |  |  |  |
|  No | 98.1 | 98.3 | 0.80 |
|  Yes | 1.9 | 1.7 |
| Physical activity (weekly moderate activity) |  |  |  |
|  No  | 80.5 | 78.3 | 0.73 |
|  ½ - 3 hours/week | 11.9 | 13.7 |
|  4+ hours/week | 7.6 | 8.0 |

a2-sided *P*s were based on $χ^{2} $test for categorical variables.

**Table 1** Continued

## factors related to telomere length

The relationship between telomere length and selected factors are shown in Table 2. Overall, relative telomere length ranged from 0.30 to 2.86. Women in the longest telomere length group had a mean age of 59.8 (SD = 6.9), which was 5.4 years younger compared to women in the shortest group (SD = 7.9). There was a significant effect of the level of education, smoking status, number of live births, age at first live birth, age at menopause, menopausal status and use of OC on telomere length after controlling for age. Compared to women in the lowest quintile group of telomere length, women who had longer telomeres had a higher education level, were never smokers, had fewer number of children, were older at the first live birth and younger at menopause, still had menstrual period and were using OC. BMI was not significantly different across quintile levels of telomere length.

Table 2. Baseline characteristics of all participants by quintile levels of relative telomere length: The Singapore Chinese Health Study

|  |  |  |  |
| --- | --- | --- | --- |
| Characteristics | Total no. of subjects | Relative telomere length in quintile [median] and (range) | *P*values |
| 1st [0.78] (0.30-0.86) | 2nd [0.92] (0.86-0.97) | 3rd [1.02] (0.97-1.08) | 4th [1.14] (1.08-1.22) | 5th [1.33] (1.22-2.86) |
| Number of subjects | 14,306 | 2,861 | 2,861 | 2,861 | 2,861 | 2,862 |  |
| Age in years, mean (SD) | 14,306 | 65.2 (7.9) | 63.3 (7.7) | 62.0 (7.5) | 61.2 (7.2) | 59.8 (6.9) | <0.01 |
| Dialect groups, n (%) |  |  |  |  |  |  |  |
|  Cantonese  | 7,520 | 1,516(20.2) | 1,489(19.8) | 1,451(19.3) | 1,528(20.3) | 1,536(20.4) | 0.08 |
|  Hokkien | 6,786 | 1,345(19.8) | 1,372(20.2) | 1,410(20.8) | 1,333(19.6) | 1,326(19.5) |
| Level of education, n (%) |  |  |  |  |  |  |  |
| No formal education | 4,580 | 1,082 (23.6) | 982 (21.4) | 903 (19.7) | 829 (18.1) | 784 (17.1) |  |
| Primary school  | 6,035 | 1,200 (19.9) | 1,165(19.3) | 1,194 (19.8) | 1,259 (20.9) | 1,217 (20.2) | <0.01 |
| Secondary school or higher  | 3,691 | 579 (15.7) | 714 (19.3) | 764(20.7) | 773 (20.9) | 861 (23.3) |   |
| BMI (kg/m2), mean(SD)\*  | 14,306 | 23.3 (3.6) | 23.3 (3.6) | 23.4 (3.7) | 23.3(3.6) | 23.3 (3.5) | 0.60 |
| Smoking status, n (%)† |  |  |  |  |  |  |  |
| Never smoker  | 13,054 | 2,538(19.4) | 2,584(19.7) | 2,621(20.1) | 2,645(20.3) | 2,666(20.4) |  |
| Former smoker | 570 | 162(28.4) | 119(20.9) | 103(18.1) | 102(17.9) | 84(14.7) | <0.01 |
| Current smoker | 682 | 161(23.6) | 158(23.2) | 137(20.1) | 114(16.7) | 112(16.4) |  |
| Drinking status, n (%) |  |  |  |  |  |  |  |
|  Non-drinker | 12,646 | 2,131(16.6) | 2,375(18.8) | 2,496(19.7) | 2,757(21.8) | 2,887(22.8) | 0.15 |
|  Ever drinker | 1,499 | 214(14.28) | 262(17.5) | 299(20.0) | 346(23.1) | 278(25.22) |
| Number of live birth, n(%) |  |  |  |  |  |  |  |
|  None | 1,028 | 203(19.7) | 199(19.4) | 203(19.7) | 206(20.0) | 217(21.1) | <0.01 |
|  1-2 | 4,372 | 772(17.7) | 810(18.5) | 894(20.4) | 906(20.7) | 990(22.6) |
|  3-4 | 5,748 | 1,084(18.9) | 1,156(20.1) | 1,155(20.0) | 1,184(20.6) | 1,169(20.3) |
|  5+ | 3,185 | 802(25.2) | 696(21.9) | 609(19.1) | 565(17.7) | 486(15.3) |
| Age at first live birth, n (%) |  |  |  |  |  |  |  |
|  <20**Table 2** Continued | 2,420 | 552(22.8) | 504(20.8) | 460(19.0) | 466(19.3) | 438(18.1) | <0.01 |
|  21-25 | 5,357 | 1,075(20.1) | 1,089(20.3) | 1,095(20.4) | 1,059(19.8) | 1,039(19.4) |
|  26-30 | 3,962 | 738(18.6) | 779(19.7) | 806(20.3) | 812(20.5) | 827(20.9) |
|  31+ | 1,526 | 292(19.1) | 288(18.9) | 293(19.2) | 315(20.6) | 338(22.1) |
|  Nulliporous | 1,028 | 203(19.7) | 199(19.4) | 203(19.7) | 206(20.0) | 217(21.1) |
| Age at menarche, n (%) |  |  |  |  |  |  |  |
|  <13 | 3,536 | 480(13.6) | 611(17.3) | 704(20.0) | 820(23.2) | 921(26.1) | 0.54 |
|  13-14 | 5,238 | 890(17.0) | 964(18.4) | 1,031(19.7) | 1,128(21.5) | 1,225(23.4) |
|  15-16 | 4,101 | 746(18.2) | 793(19.3) | 811(19.8) | 867(21.1) | 884(21.6) |
|  17+ | 1,430 | 263(18.4) | 298(20.8) | 277(19.4) | 320(22.4) | 272(19.0) |
| Menopausal status, n (%) |  |  |  |  |  |  |  |
|  Still menstruating | 1,474 | 150(10.2) | 238(16.2) | 292(19.8) | 329(22.3) | 465(31.6) | 0.03 |
|  Postmenopausal | 12,671 | 2,195(17.3) | 2,399(18.9) | 2,503(19.8) | 2,774(21.9) | 2,800(22.1) |
| Age at menopause, n (%) |  |  |  |  |  |  |  |
|  Still have period | 4,688 | 623(13.3) | 821(17.5) | 989(21.1) | 1,064(22.7) | 1,191(25.4) | <0.01 |
|  <40 | 353 | 77(21.8) | 66(18.7) | 66(18.7) | 79(22.4) | 65(18.4) |
|  40-44 | 884 | 190(21.5) | 161(18.2) | 180(20.4) | 165(18.7) | 188(21.3) |
|  45-49 | 3,091 | 696(22.5) | 630(20.4) | 621(20.1) | 563(18.2) | 581(18.8) |
|  50-54 | 4,576 | 1,085(23.7) | 1,027(22.4) | 877(19.2) | 858(18.8) | 729(15.9) |
|  55+ | 693 | 187(27.0) | 152(21.9) | 1,28(18.5) | 125(18.0) | 101(14.6) |
| Use of OC, n (%) |  |  |  |  |  |  |  |
|  Never users | 9,888 | 2,082(21.1) | 1,989(20.1) | 1,948(19.7) | 1,929(19.5) | 1,940(19.6) | 0.00 |
|  Ever used | 4,416 | 779(17.6) | 872(19.7) | 913(20.7) | 932(21.1) | 920(20.8) |
| Use of MHT, n (%) |  |  |  |  |  |  |  |
|  Never users | 10,579 | 1,916(18.1) | 2,040(19.3) | 2,082(19.7) | 2,287(21.6) | 2,254(21.3) | 0.19 |
|  Ever used | 3,727 | 463(12.4) | 626(16.8) | 741(19.9) | 848(22.8) | 1,049(28.2) |
| Family history of breast cancer, n (%) |  |  |  |  |  |  |  |
|  No | 1,4066 | 2,357(16.8) | 2,614(18.6) | 2,774(19.7) | 3,085(21.9) | 3,236(23.0) | 0.18 |
|  Yes | 240 | 22(9.2) | 52(21.7) | 49(20.4) | 50(20.8) | 67(27.9) |
| Physical activity (weekly moderate activity), n (%) |  |  |  |  |  |  |  |
|  No  | 11,212 | 1,836(16.4) | 2,077(18.5) | 2,226(19.9) | 2,487(22.2) | 2,586(23.1) | 0.71 |
|  ½ - 3 hours/week | 1,958 | 338(17.3) | 371(19.0) | 376(19.2) | 410(20.9) | 463(23.7) |
|  4+ hours/week | 1,136 | 205(18.1) | 218(19.2) | 221(19.5) | 238(21.0) | 254(22.4) |

\* Adjusted for age at sample collection.

**Table 2** Continued

† Among former and current smokers only.

## Telomere length and risk of breast cancer

Table 3 shows the associations between telomere length and risk of breast cancer. Relative telomere length was significantly associated with increased risk of breast cancer. After controlling for age, BMI, level of education, number of live birth and age at first live birth, the risk of developing breast cancer for women in the longest telomere length group (5th quintile) was 47% higher than women in the lowest telomere length group (1st quintile) (*P*trend = 0.01). Women in the second longest group (4th quintile) of telomere length had the highest risk of breast cancer among all quintile groups, and there was a similar pattern in women had menarche younger than 14 years old or had 3 or more children. When results were stratified by multiple variables as shown in Table 4, the positive association between telomere length and breast cancer risk was only significant in women who were overweight or obese (HR = 2.00, 95% CI 1.02 - 3.91, *P*trend = 0.01); women who had menarche less than 14 years old (HR = 1.56, 95% CI 0.84 - 2.89, *P*trend = 0.01); women who gave first live birth 26 years old or older (HR = 1.74, 95% CI 0.86 - 3.54, *P*trend = 0.02); and women who had 3 or more children (HR = 1.64, 95% CI 0.83 - 3.23, *P*trend = 0.02) (Table 4), although the interaction tests were not significant between those variables and telomere length (data not shown). Further stratifications by smoking status, drinking status, menopausal status, physical activity, use of OC and use of MHT were not significant due to the small number of breast cancer cases in each category. When the analysis was restricted to women who had at least 2 years of follow-up period, there were 144 breast cancer cases left. In this reduced dataset, no significant association between telomere length and risk of breast cancer was detected in repeated analysis (*P*trend = 0.10).

Table 3. Hazard ratio (HR) of breast cancer in relation to relative telomere length in all subjects: The Singapore Chinese Health Study.

|  |  |  |  |
| --- | --- | --- | --- |
| Relative telomere length in quintile | Person-years | No. of cases | Adjusted HR (95% CI)\* |
| All subjects |  |  |  |
|  1st (shortest) | 17,964 | 29 | 1.00 |
|  2nd | 18,186 | 35 | 1.06 (0.62, 1.80) |
|  3rd | 18,288 | 48 | 1.36 (0.83, 2.24) |
|  4th | 18,487 | 52 | 1.70 (1.05, 2.73) |
|  5th (longest) | 19,097 | 46 | 1.47 (0.90, 2.38) |
| *P* trend |  |  | 0.01 |
| \*Adjusted for age at blood collection, BMI, level of education, number of live births, and age at first live birth.  |

Table 4. Hazard ratio (HR) of breast cancer in relation to relative telomere length in all subjects by stratified by median age, BMI, age at menarche, age at first live birth, number of children and length of follow-up: The Singapore Chinese Health Study.

|  |  |  |  |
| --- | --- | --- | --- |
| Relative telomere length in quintile | Person-years | No. of cases | Adjusted HR (95% CI)\* |
| Age < 62 (median age)  |  |  |  |
|  1st (shortest) | 5,145 | 7 | 1.00 |
|  2nd | 7,523 | 14 | 1.34 (0.54, 3.34) |
|  3rd | 9,309 | 22 | 1.70 (0.72, 3.98) |
|  4th | 11,210 | 37 | 2.38 (1.05, 5.34) |
|  5th (longest) | 14,367 | 37 | 1.86 (0.82, 4.16) |
| *P* trend (median age) |  |  | 0.05 |
| Age $\geq $62 |  |  |  |
|  1st (shortest) | 9,773 | 18 | 1.00 |
|  2nd | 9,382 | 16 | 0.96(0.49, 1.88) |
|  3rd | 8,849 | 20 | 1.25(0.66, 2.37) |
|  4th | 8,840 | 21 | 1.28(0.68, 2.41) |
|  5th (longest) | 7,625 | 18 | 1.30(0.67, 2.51) |
| *P* trend |  |  | 0.27 |
| BMI<23 |  |  |  |
|  1st (shortest) | 7,219 | 13 | 1.00 |
|  2nd | 8,417 | 11 | 0.73 (0.32, 1.62) |
|  3rd | 8,740 | 17 | 1.08 (0.52, 2.24) |
|  4th | 9,610 | 19 | 1.09 (0.53, 2.23) |
|  5th (longest) | 11,070 | 20 | 1.01 (0.49, 2.06) |
| *P* trend |  |  | 0.64 |
| BMI $\geq $23 |  |  |  |
|  1st (shortest) | 7,699 | 12 | 1.00 |
|  2nd | 8,488 | 19 | 1.44(0.70, 2.98) |
|  3rd | 9,418 | 25 | 1.68(0.84, 3.35) |
|  4th | 10,439 | 39 | 2.34(1.22, 4.50) |
|  5th (longest)**Table 4** Continued | 10,922 | 35 | 2.00(1.02, 3.91) |
| *P* trend |  |  | 0.01 |
| Age at menarche $<$14 years |  |  |  |
|  1st (shortest) | 7,725 | 15 | 1.00 |
|  2nd | 9,038 | 14 | 0.84(0.40, 1.73) |
|  3rd | 9,968 | 21 | 1.14(0.58, 2.22) |
|  4th | 11,440 | 40 | 1.89(1.04, 3.46) |
|  5th (longest) | 13,147 | 37 | 1.56(0.84, 2.89) |
| *P* trend |  |  | 0.01 |
| Age at menarche $\geq $14 years |  |  |  |
|  1st (shortest) | 7,193 | 10 | 1.00 |
|  2nd | 7,868 | 16 | 1.45(0.66, 3.21) |
|  3rd | 8,189 | 21 | 1.76(0.83, 3.72) |
|  4th | 8,609 | 18 | 1.40(0.64, 3.06) |
|  5th (longest) | 8,834 | 18 | 1.34(0.61, 2.96) |
| *P* trend |  |  | 0.65 |
| Age at first live birth < 26 years |  |  |  |
|  1st (shortest) | 8,409 | 13 | 1.00 |
|  2nd | 9,404 | 15 | 1.03(0.48, 2.17) |
|  3rd | 9,978 | 21 | 1.35(0.67, 2.71) |
|  4th | 10,571 | 32 | 1.90(0.99, 3.65) |
|  5th (longest) | 11,520 | 20 | 1.09(0.53, 2.22) |
| *P* trend |  |  | 0.32 |
| Age at first live birth $\geq $26 years |  |  |  |
|  1st (shortest) | 5,418 | 11 | 1.00 |
|  2nd | 6,421 | 7 | 1.02(0.50, 1.41) |
|  3rd | 6,929 | 17 | 1.21(0.56, 2.60) |
|  4th | 8,028 | 18 | 1.13(0.53, 2.43) |
|  5th (longest) | 8,908 | 30 | 1.74(0.86, 3.54) |
| *P* trend**Table 4** Continued |  |  | 0.02 |
| Number of children < 3 |  |  |  |
|  1st (shortest) | 3,944 | 11 | 1.00 |
|  2nd | 4,780 | 7 | 0.53(0.20, 1.36) |
|  3rd | 5,543 | 17 | 1.10(0.51, 2.36) |
|  4th | 6,257 | 14 | 0.79(0.35, 1.76) |
|  5th (longest) | 7,577 | 24 | 1.10(0.53, 2.30) |
| *P* trend |  |  | 0.46 |
| Number of children $\geq $3 |  |  |  |
|  1st (shortest) | 9,891 | 13 | 1.00 |
|  2nd | 11,056 | 15 | 1.06(0.50, 2.23) |
|  3rd | 11,395 | 21 | 1.45(0.72, 2.90) |
|  4th | 12,369 | 36 | 2.28(1.20, 4.33) |
|  5th (longest) | 12,869 | 26 | 1.64(0.83, 3.23) |
| *P* trend |  |  | 0.02 |
| Length of follow-up < 2 years |  |  |  |
|  1st (shortest) | 67 | 8 | 1.00 |
|  2nd | 67 | 8 | 1.14(0.41, 3.11) |
|  3rd | 64 | 13 | 1.24(0.49, 3.12) |
|  4th | 87 | 20 | 1.99(0.83, 4.74) |
|  5th (longest) | 75 | 17 | 1.43(0.59, 3.48) |
| *P* trend |  |  | 0.23 |
| Length of follow-up $\geq $2 years |  |  |  |
|  1st (shortest) | 14,851 | 17 | 1.00 |
|  2nd | 16,838 | 22 | 1.14(0.61, 2.16) |
|  3rd | 18,093 | 29 | 1.38(0.76, 2.52) |
|  4th | 19,961 | 38 | 1.61(0.90, 2.88) |
|  5th (longest) | 21,916 | 38 | 1.45(0.81, 2.61) |
| *P* trend |  |  | 0.10 |
| \*Adjusted for age at blood collection, BMI, level of education, number of live births, and age a first live birth where appropriate. |

# discussion

Based on this prospective population-based cohort study of 14,306 women in the Singapore Chinses Health Study, we detected a significant positive association between telomere length and risk of breast cancer among certain groups of women. Compared to the lowest quintile group of telomere length, extreme long telomeres were associated with 47% increased risk of developing breast cancer after adjustment for potential confounding variables. This association was modified by BMI, age at menarche, age at first live birth, and number of live births. However, when the results were restricted to the subjects with 2 years or longer follow-up period, the association was no longer significant.

Previous studies have reported inconsistent results on the relationship between telomere length and risk of breast cancer. The main reason for this inconsistency may due to the study design and the number of breast cancer cases. Most of earlier studies were case-control studies. As telomere length was measured after breast cancer diagnosis, it could be influenced by cancer development and the treatment progress. In addition, selection bias can exist during the process of recruiting cases and controls. For prospective cohort studies conducted so far, 2 nested case-control studies found a nonsignificant association between telomere length and risk of breast cancer (De Vivo et al., 2009; Willeit et al., 2010). Due to the limited number of matched variables between cases and controls, the power of nested case-control studies was reduced. A recent prospective cohort study based on 47,102 Danish participants reported that shorter telomere length was significantly associated with lower breast cancer risk (Weischer et al., 2013). The study had 574 breast cancer cases and used the same qPCR method as us to measured telomere length in peripheral blood cells. Our results corroborate by the findings from this study supporting the hypothesis that longer telomere length might be a risk factor of breast cancer.

The mechanism behind the association between telomere length and breast cancer risk is still unclear. Telomere attrition is a complex process related to many interacting factors. Cells with longer telomeres would have a larger number of cell divisions, which increases the probability of acquiring mutations that may promote carcinogenesis (Blasco, 2005). However, longer telomeres could also protect the stability of chromosomes, which may be part of the reason why the risk of developing breast cancer for the longest telomere length group is slightly lower than the second-longest telomere length group. In addition, there are studies showing that longer telomere length at baseline is correlated to a higher shortening rate of telomere length (Aviv et al., 2009; Nordfjäll et al., 2009). It is possible that in our study, women who had longer telomeres at blood draw progressed to shorter telomeres during older age: a period of high incidence of breast cancer. Shorter telomeres in senescent tissues could result in unstable genomic structure, which eventually leads to genetic mutations and cancer.

Our study measured telomere length in peripheral blood leukocytes instead of telomere length in breast tissue. Telomeres in leukocytes are less than synovial, fibroblast and buccal tissues due to a higher rate of proliferation. However, many studies have demonstrated significant correlations of telomere lengths between peripheral blood leukocytes and fibroblast (r2 = 0.80) (Friedrich et al., 2000), buccal cells (r = 0.74) (Gadalla, Cawthon, Giri, Alter, & Savage, 2010), and skin (r2 = 0.71) (Friedrich et al., 2000). Although the relationship between telomeres in blood cells and breast tissues has not been revealed, the existing findings so far provide evidence for taking blood cells as surrogates for breast tissues in the measurement of telomere length.

Our study found that a positive association between telomere length in peripheral blood cells and risk of breast cancer was only significant in women who were overweight/obese; had menarche less than 14 years old; gave first live birth at or above 26 years old; and those who had 3 or more children. This might be due to a relatively lower number of cases in the stratified groups. Higher BMI in adulthood has been found to be associated with a larger decrease in telomere length later in life (Kim et al., 2009). In this situation, larger BMI and longer telomere length at baseline could act together to accelerate the telomere attrition, suggesting that they may have a synergistic effect on breast cancer development. Thus, for women who had higher BMI, the association between telomere length and breast cancer risk is stronger than women who had lower BMI. In addition, telomere length shortening is affected by oxidative stress, which could be attenuated by the antioxidant effect of estrogen (Aviv, 2002). Therefore, longer estrogen exposure due to a younger age at menarche or an older age at first live birth can be a protective factor for telomere attrition, and subsequently result in longer telomere lengths. This notion is also consistent with our results of factors related to telomere length. Moreover, longer exposure to estrogen is also related to a higher risk of breast cancer (Clemons & Goss, 2001). There can be a combined effect of endogenous hormones and telomere length on risk of breast cancer.

The major strengths of our study were the prospective cohort study design and relatively large samples. Since telomere length was measured before cancer diagnosis, potential bias caused by breast cancer treatment and cancer progression should have been at minimal. In addition, the large sample size (14,306 subjects) and adjustment for multiple related variables increased the reliability of the results. However, several limitations also exist in this study. First, our study only included data up to Dec 31, 2008. Updated data on this cohort is needed to validate these findings. As some of the cancer diagnoses were close to the time of blood draw, the causal relationship cannot be established convincingly. Second, telomere length was measured through peripheral blood cells instead of breast tissues in our study. Although previous studies have demonstrated a correlation between telomere length in peripheral blood cells and somatic tissues including muscle, fat and skin (Daniali et al., 2013), there could be potential bias when using telomere length in peripheral blood for analysis. Third, even though multiple variables were controlled for in the models, unmeasured or residual confounding factors could still exist.

In conclusion, our study showed that longer telomere length is significantly associated with higher risk of breast cancer in certain groups of Asian women. This finding has public health significance because it provides supportive evidence for utilizing telomere length as a biomarker for prevention and development of breast cancer. Additional studies are needed to explore the effect of hormone factors on the relationship between telomere length and risk of breast cancer.

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