Leukocyte telomere length, breast cancer risk in the offspring: The relations with father's age at birth

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ABSTRACT

Recent studies have reported that leukocyte telomere length (LTL) is longer in offspring of older fathers. Longer telomeres might increase cancer risk. We examined the relation of father’s age at the birth of the offspring (FAB) with LTL in the offspring in 2177 participants of the Family Heart Study and the probability of developing breast cancer in 1405 women from the Framingham Heart Study (offspring cohort). For each year of increase in FAB (adjusted for mother’s age at birth), LTLs in the daughters and sons were longer by 19.4 bp and 12.2 bp, respectively (p < 0.0001). Daughters of older fathers were less likely to stay free of breast cancer compared to daughters of younger fathers in empirical (p = 0.014) and Cox regression analyses (p = 0.0012) adjusted for relevant covariates. We conclude that older fathers endow their offspring with a longer LTL and their daughters with increased susceptibility to breast cancer. These independent observations cannot provide evidence for a causal relationship, mediated by telomere length, between FAB and increased breast cancer risk in daughters. However, with couples delaying having children in today’s society, studies exploring the LTL association with increased breast cancer risk in daughters of older fathers might be timely and relevant.

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1. Introduction

Telomere length is highly variable among different species of non-human primates, but it is distinctly short in human beings in relationship to their comparatively long lifespan (Gardner et al., 2007; Kakuo et al., 1999; Steinert et al., 2002). Humans also have shorter telomeres than smaller mammals, such as rodents (Hemann and Greider, 2000), in which species with a larger body shorter telomeres than smaller mammals, such as rodents.

Multiple mutations, which require manifold cell replications, are necessary for the development of cancer. But runaway cell replication entails progressive telomere shortening, which would bring about replicative senescence due to critically short telomeres. On a rare occasion, when malignant transformation does occur, it takes place only when the telomere barrier is breeched by activation of telomerase (or an alternate telomere maintenance pathway) (Finkel et al., 2007; Stewart and Weinberg, 2006). This activation does not elongate telomeres; rather it prevents further telomere shortening. Thus, most cancers display short telomeres and robust telomerase activity (Calcagnile and Gisselsson, 2007; Kim et al., 1994).

Many researchers have attached substantial weight to the short telomeres in cancerous tissues and the fact that patients with dyskeratosis congenita, who display very short telomeres and ‘genomic instability’, are highly susceptible to cancer (Alter et al., 2009). But the genomic instability caused by exceedingly short telomeres in patients with dyskeratosis congenita hardly represents circumstances in the general population, except perhaps for telomere length in leukocytes of some of the elderly. Yet numerous investigations have been undertaken with a view that leukocyte telomere length (LTL) might be short in patients with various forms of cancer, including breast cancer (e.g., Barwell et al., 2007; De Vivo et al., 2009; Shen et al., 2007, 2009; Svenson et al., 2008). Findings of these studies have been mixed, with reports showing no.
association between LTL and breast cancer (Barwell et al., 2007; De Vivo et al., 2009; Shen et al., 2009, 2007) or longer LTL in breast cancer patients with poor outcome whose blood was collected prior to receiving chemotherapy or hormonal therapy (Svenson et al., 2008). Moreover, many of these studies were confounded by factors such as anti-cancer therapy (Schröder et al., 2001; Shen et al., 2009) and poor reproducibility of methods used to measure telomere length (Shen et al., 2007).

The present study draws on two converging lines of inquiry that link breast cancer and LTL with father's age at birth (FAB) and by implication, paternal age at conception. Several studies have reported that offspring of older fathers have relatively long LTL (De Meyer et al., 2007; Kimura et al., 2008; Njajou et al., 2007; Unrny et al., 2005), and the consensus is that daughters of older fathers display increased risk of developing breast cancer (Weiss-Salz et al., 2007; Xue and Michels, 2007). We hypothesize, therefore, that if LTL explains, in part, the connection between FAB and breast cancer risk in the offspring, the age-dependent trajectory of this risk would be shifted towards younger ages in daughters conceived by older fathers (long telomere length in individuals with older fathers may also place these individuals at higher risk of other adult cancers, e.g., hematologic malignancies, Lu et al., 2010). We proceeded in two phases to test this hypothesis. First, we examined in detail the FAB effect on the offspring’s LTL in the NHLBI-Family Heart Study because this cohort has a wealth of LTL data. Second, we explored the effect of FAB on the risk of breast cancer in women participating in the Offspring Study of the Framingham Heart Study (FHSoffs).

2. Materials and methods

2.1. Cohorts

The first phase of the study focused on the NHLBI-Family Heart Study, a multicenter investigation of the genetic and epidemiologic basis of cardiovascular disease (Higgins et al., 1996). The present investigation includes only white participants with available LTL data and known parental ages, whose blood samples were collected between January 2002 and January 2004 (Table 1). We note that there were relatively few African Americans in the NHLBI-Family Heart Study with known parental age and LTL data. Moreover, the second phase of the study focused on the FHSoffs, who are almost exclusively white. For this reason, our data are limited to white participants.

The Offspring Cohort of the Framingham Heart Study started in 1971 and, by 1975, a sample of 5124 offspring of participants in the original Framingham cohort (and spouses of the offspring) had been enrolled in the study (2641 females, 2483 males; ages at entry: 5–70). Participants have completed examinations with follow-ups at intervals of four to six years and have been followed for morbidity (cardiovascular diseases and cancer) and mortality. The occurrence of diseases (including cancer) and mortality has been followed through continuous surveillance of hospital admissions, death registries, clinical exams, and other sources, so that all respective events were included in the study. Cancer sites have been coded using the International Classification of Diseases for Oncology (ICD-O-3) codes. More details on the design and selection criteria of the FHSoffs can be found elsewhere (Kannel et al., 1979).

For this study, data on six FHSoffs exams were available, with a follow-up period (since the dates of the first exam) of about 26 years. As we were interested in parental ages at birth which were available only for offspring (not their spouses), we focused only on daughters of participants of the original Framingham cohort in this study. FAB (mean 33.1 years; range 16–63 years) was computed for 1410 women as the average age in the sample and 0 otherwise. Model 2 contained both FABdichot and MABdichot, which equals 1 if FAB is above the average age in the sample and 0 otherwise. Model 2 contained both FABdichot and MABdichot, which equals 1 if FAB is above the average age in the sample and 0 otherwise. Model 3 was used to adjust Model 2 for effects of mean BMI and smoking status (1 if an individual smoked cigarettes in at least one exam and 0 otherwise) of daughters. Model 4 contained one covariate MARichoch. Note that all four models are applied to a sample of 827 offspring for whom measurements of all covariates (i.e., those included in Model 3) are available. Probabilities of staying free of breast cancer for daughters from upper and lower halves of distribution of FAB (i.e., for strata defined by FABrichoch) adjusted for parental and daughters’ education, MABdichot, BMI and smoking status were evaluated from the Cox regression model (Model 3). Respective survival curves were calculated at the mean values of covariates (parental and daughters’ education, MARichoch, BMI, and smoking) in corresponding strata. In all models, age at the first exam was used to define left truncation (delayed entry) and individuals with ages at onset of breast cancer younger than ages at the first exam were excluded from the analyses.

Statistical analyses were performed using MATLAB (© MathWorks Inc.) and SAS/STAT (© SAS Institute Inc.) software packages. $P$-values for the regression parameters in Model 3 were calculated using the Wald chi-square statistic with respect to a chi-square distribution with one degree of freedom using SAS/STAT PROC PHREG. The log-rank test $p$-value for the null hypothesis about the equality of the empirical survival curves in the strata and the estimates of the survival curves in these strata shown in Fig. 1 were calculated using SAS/STAT PROC LIFETEST.

3. Results

3.1. The relation between father’s age at birth and leucocyte telomere length in the offspring in the NHLBI-Family Heart Study

Table 2 provides data about the FAB effect on LTL in adult offspring (age range in the cohort 31–86 years) based on the most recent data from our ongoing study in the NHLBI-Family Heart

![Table 1](https://example.com/table1.png)

**Table 1**

<table>
<thead>
<tr>
<th>Offspring</th>
<th>N</th>
<th>Offspring’s age</th>
<th>FAB</th>
<th>MAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daughters</td>
<td>1182</td>
<td>55.6 ± 12.7 (31–84)</td>
<td>31.5 ± 6.9 (18–56)</td>
<td>28.4 ± 6.1 (16–53)</td>
</tr>
<tr>
<td>Sons</td>
<td>995</td>
<td>54.7 ± 12.9 (31–86)</td>
<td>31.4 ± 7.3 (17–69)</td>
<td>28.2 ± 6.2 (17–46)</td>
</tr>
</tbody>
</table>

FAB: Father’s age at the offspring’s birth; MAB: Mother’s age at the offspring’s birth.
Regression of the offspring’s LTL on parents’ ages at the offspring’s birth in the Table 2

daughters (Kimura et al., 2008).

more extensive than that we published before on the NHLBI-individuals (daughters) from upper and lower halves of distribution of FAB Fig. 1.

the figure shows values of probability (\(y\)-axis) in the lower and upper halves are 24 and 23, respectively. Note that the figure shows values of probability (y-axis) from 0.8 to 1 (not from 0 to 1) to better visualize the differences between the curves.

Study. This dataset of 995 sons and 1182 daughters is considerably more extensive than that we published before on the NHLBI-Family Heart Study, which consisted of 335 sons and 492 daughters (Kimura et al., 2008).

Model A provides an account of the offspring’s age on LTL. It shows that based on cross-sectional analysis, age-dependent LTL shortening was 21.5 base pairs (bp)/year for daughters and 23.1 bp/year for sons. Model B demonstrates the profound effect of FAB on the offspring’s LTL. For each year of increase in FAB, LTLs in the daughters and sons were longer by 15.5 bp and 13.7 bp, respectively. Model C shows the joint effect of FAB and MAB. For each year of increase in FAB, LTLs were longer by 19.4 bp for daughters and 12.2 bp for sons. Though when modeled independently of FAB, MAB also impacted LTL in the offspring (model D), this phenomenon was attributed to the strong correlation between FAB and MAB (\(r = 0.83\), \(p < 0.0001\)). When FAB and MAB were jointly considered (model C), only FAB contributed significantly to the offspring’s LTL.

3.2. The relation between father’s age at conception and breast cancer in the Offspring Study of the Framingham Heart Study

While the NHLBI-Family Study has a wealth of LTL data, we capitalized on the longitudinal nature of the FHS offspring to explore the role of FAB effect on breast cancer in the daughters. In this cohort, as per the NHLBI-Family Heart Study, FAB and MAB were highly correlated (\(r = 0.78\), \(p < 0.0001\)).

Fig. 1 displays empirical probabilities of staying free of breast cancer for daughters from upper and lower halves of distribution of FAB evaluated from the FHS sample. The log-rank test showed a significant difference between the curves (\(p = 0.0144\)) in that daughters of older fathers (i.e., those from the upper half of the

![Fig. 1. Probabilities of staying free of breast cancer (Kaplan–Meier estimates) for individuals (daughters) from upper and lower halves of distribution of FAB evaluated from the empirical data (the FHS sample). Numbers of individuals at risk at different ages x in two strata (lower half, \(N_{lower}\); and upper half, \(N_{upper}\)); \(N_{lower}(25) = 703\), \(N_{upper}(25) = 702\), \(N_{lower}(50) = 610\), \(N_{upper}(50) = 388\), \(N_{lower}(75) = 62\), \(N_{upper}(75) = 103\). Total numbers of cases (breast cancer incidence) in the lower and upper halves are 24 and 23, respectively. Note that the number shows values of probability (y-axis) from 0.8 to 1 (not from 0 to 1) to better visualize the differences between the curves.](image)

![Table 2](image)

Regression of the offspring’s LTL on parents’ ages at the offspring’s birth in the Family Heart Study—995 sons and 1182 daughters.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>(b^2) (bp/yr)</th>
<th>95% CI (bp/yr)</th>
<th>(p)-Value</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Daughter’s age</td>
<td>-21.5 to -24.5</td>
<td>(&lt;.0001)</td>
<td>17.1</td>
</tr>
<tr>
<td>B</td>
<td>Daughter’s age</td>
<td>-21.9 to -24.9</td>
<td>(&lt;.0001)</td>
<td>19.3</td>
</tr>
<tr>
<td>C</td>
<td>FAB</td>
<td>15.5</td>
<td>10.1 to 21.0</td>
<td>(&lt;.0001)</td>
</tr>
<tr>
<td>D</td>
<td>Daughter’s age</td>
<td>-22.1 to -25.0</td>
<td>(&lt;.0001)</td>
<td>19.4</td>
</tr>
<tr>
<td>E</td>
<td>FAB</td>
<td>19.4</td>
<td>9.3 to 29.5</td>
<td>.0002</td>
</tr>
<tr>
<td>F</td>
<td>MAB</td>
<td>-4.9</td>
<td>-16.6 to 6.8</td>
<td>.41</td>
</tr>
<tr>
<td>G</td>
<td>Daughter’s age</td>
<td>-21.4 to -24.3</td>
<td>(&lt;.0001)</td>
<td>18.2</td>
</tr>
<tr>
<td>H</td>
<td>MAB</td>
<td>13.4</td>
<td>7.1 to 19.8</td>
<td>(&lt;.0001)</td>
</tr>
<tr>
<td>A</td>
<td>Son’s age</td>
<td>-23.1 to -25.8</td>
<td>(&lt;.0001)</td>
<td>18.6</td>
</tr>
<tr>
<td>B</td>
<td>Son’s age</td>
<td>-23.0 to -25.5</td>
<td>(&lt;.0001)</td>
<td>21.3</td>
</tr>
<tr>
<td>C</td>
<td>FAB</td>
<td>13.7</td>
<td>9.4 to 18.1</td>
<td>(&lt;.0001)</td>
</tr>
<tr>
<td>D</td>
<td>Son’s age</td>
<td>-22.9 to -25.4</td>
<td>(&lt;.0001)</td>
<td>21.3</td>
</tr>
<tr>
<td>E</td>
<td>MAB</td>
<td>2.0</td>
<td>-9.2 to 13.2</td>
<td>.73</td>
</tr>
<tr>
<td>F</td>
<td>Son’s age</td>
<td>-22.5 to -25.1</td>
<td>(&lt;.0001)</td>
<td>20.8</td>
</tr>
<tr>
<td>G</td>
<td>MAB</td>
<td>14.3</td>
<td>9.2 to 19.4</td>
<td>(&lt;.0001)</td>
</tr>
</tbody>
</table>

* \(b\): regression coefficient.

![Fig. 2. Probabilities of staying free of breast cancer for individuals (daughters) from upper and lower halves of distribution of FAB adjusted for parental and daughters’ education, MAB, and BMI and smoking status in daughters as evaluated from the Cox regression model (Model 3) applied to the FHS sample. Respective curves are evaluated at mean values of covariates (parental and daughters’ education, MAB, BMI, and smoking) in corresponding strata; \(p\) denotes \(p\)-value for regression parameter FAB dichot (see Table 3). Numbers of individuals at risk at different ages x in two strata (lower half, \(N_{lower}\)); and upper half, \(N_{upper}\)); \(N_{lower}(25) = 416\), \(N_{upper}(25) = 411\), \(N_{lower}(50) = 377\), \(N_{upper}(50) = 388\), \(N_{lower}(75) = 36\), \(N_{upper}(75) = 66\). Note that the numbers are different from those shown in the legend to Fig. 1 because only women with available data on FAB, MAB and all other covariates were used in Model 3. Note also that the figure shows values of probability (y-axis) from 0.8 to 1 (not from 0 to 1) to better visualize the differences between the curves.](image)(image)
distribution of FAB) were less likely to stay free of breast cancer compared to daughters of younger fathers.

Results of application of Cox regression models (Models 1–4) to the FHSoffs sample are shown in Table 3. The models revealed a statistically significant effect of FABdichot on risks of breast cancer in daughters (p = 0.0015 in Models 1 and 2, p = 0.0012 in Model 3). All other covariates in the models, including MABdichot, did not show a significant effect on the breast cancer risk in daughters. Fig. 2 displays probabilities of staying free of breast cancer for daughters from upper and lower halves of distribution of FAB, adjusted for parental and daughters’ education, MABdichot, BMI and smoking status as evaluated in Model 3.

4. Discussion

The two central findings, derived independently from the NHLBI-Family Heart Study and the FHSoffs, are that FAB strongly affected LTL in the offspring (both sons and daughters) and breast cancer risk in daughters. However, since these associations were observed in independent studies, there is no evidence at present that the FAB effect on daughters’ susceptibility to breast cancer is mediated through telomere length.

The FAB effect on LTL in the offspring of the NHLBI-Family Heart Study not only confirmed observations by us and others (De Meyer et al., 2007; Kimura et al., 2008; Njaiou et al., 2007; Unyon et al., 2005), it also underscored the considerable impact of this effect on LTL in the adult offspring, as LTL was longer by 19.4 bp for daughters and 12.2 bp for sons per each additional year of FAB (Model C, Table 2). Though the FAB effect on the offspring’s LTL is considerable, little is known about whether it is already apparent at birth or is mediated by attenuating age-dependent LTL shortening in the offspring. That said, the FAB effect must emanate from the paternal germ line. And three independent studies observed that in contrast to the progressive shortening of telomeres in replicating somatic cells, telomere length is longer in sperm of older men (Allsopp et al., 1992; Baird et al., 2006; Kimura et al., 2008). One of these studies also found evidence for the emergence of a subset of sperm with longer telomeres in older men (Kimura et al., 2008). How this feature might be translated into a longer LTL in the offspring and transmitted across generation is unknown at present.

Older FAB in the FHSoffs clearly shifted the probability of breast cancer in the daughters towards a younger age (Figs. 1 and 2). For instance, as estimated from the Cox regression model after adjustment for parental and daughters’ education, MAB, smoking and BMI (Model 3, Table 3), by the age of 50 years 1.8% of daughters of older fathers had breast cancer. The age of daughters of younger fathers with the same proportion of breast cancer cases was 62 years. By age 55 years, 4.1% of daughters of older fathers suffered from breast cancer. The age of daughters of younger fathers with the same proportion of breast cancer cases was about 70 years.

The shift in the probability of breast cancer towards a younger age in daughters of older fathers is particularly relevant in light of the effect of FAB on LTL and given that this effect was more pronounced in daughters than in sons. In the NHLBI-Family Heart Study, when dichotomized at the mean FAB (31.4 years), LTL of daughters of older fathers was longer by 162 bps than LTL of daughters of younger fathers (6.988 kb vs. 6.826 kb, respectively, p = 0.0002). This FAB effect amounts to daughters of older fathers being younger by 7.3 years in telomeric year equivalence, based on the average rate of age-dependent LTL shortening of 22.1 bp/year (Table 2, Model C). A similar shift in years towards younger age in the probability of having breast cancer is observed in daughters of older fathers in the FHSoffs (Fig. 2).

We were able to detect the shift towards a younger age in the probability of having breast cancer in the relatively small sample, perhaps due to the longitudinal nature of the Framingham Heart Study, which in the case of the FHSoffs sample used in this study has been going on for about 26 years. However, in light of the small number of breast cancer cases, we cannot exclude the possibility of a false positive finding. That being said and given that epidemiological data cannot infer causality from association, if the increased predilection of daughters of older fathers to breast cancer is linked to telomere biology, our findings are consistent with the concept that a longer LTL, which in large measure reflects telomere length in other cells (Gardner et al., 2007; Kimura et al., 2010a; Okuda et al., 2002), might increase the breast cancer risk.

A long LTL entails diminished susceptibility to atherosclerosis (Benetos et al., 2004; Brouillette et al., 2003, 2007; O’Donnell et al., 2008), the relationships of LTL with atherosclerosis on the one hand and breast cancer on the other hand suggest an “evolutionary” trade off, namely, more susceptibility to some forms of cancer for less susceptibility to atherosclerosis and other cardiovascular diseases (Yashin et al., 2009). It is therefore of considerable import that well-designed, adequately powered studies employing reliable methods of LTL measurements should be undertaken in cohorts with FAB, MAB and breast cancer data with a view to resolve the controversy whether a longer LTL increases the susceptibility to breast cancer in humans.

Finally, growing numbers of not only women but also men choose to postpone parenthood, causing a demographic shift in modern societies in which, on average, both parents conceive their first child at an older age (Hamilton et al., 2005; Office of National Statistics, 2002). Therefore, the FAB effect on the offspring’s LTL and health status is more than just a scientific curiosity but a phenomenon with considerable ramifications.

Disclosure statement

The authors declare no actual or potential conflicts of interest related to this paper.

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