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A prospective study of leukocyte telomere length and risk of phobic anxiety among women

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Abstract

We prospectively examined the relation of relative telomere lengths (RTLs), a marker of biological aging, to phobic anxiety in later-life. RTLs in peripheral blood leukocytes were measured among 3,194 women in the Nurses' Health Study who provided blood samples in 1989/90. The Crown-Crisp Phobic Index (CCI, range=0-16) was assessed in 1988 and 2004. Only participants with CCI≤3 (consistent with no meaningful anxiety symptoms) in 1988 were included. We related baseline RTLs to odds ratios (ORs) of incident high phobic anxiety symptoms (CCI≥6). To enhance clinical relevance, we used finite mixture modeling (FMM) to relate baseline RTLs to latent classes of CCI in 2004. Overall, RTLs were not significantly associated with high phobic anxiety symptoms after 16 years of follow-up. However, FMM identified 3 groups of phobic symptoms in later-life: severe, minimal/intermediate, non-anxious. The severe group had non-significantly shorter multivariable-adjusted mean RTLs than the

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CONFLICTS OF INTEREST

None.

minimal/intermediate and non-anxious groups. Women with shorter telomeres vs. longest telomeres had non-significantly higher likelihood of being in the severe vs. non-anxious group. Overall, there was no significant linear association between RTLs and incident phobic anxiety symptoms. Further work is required to explore potential connections of telomere length and emergence of severe phobic anxiety symptoms during later-life.

Keywords

Epidemiology; Anxiety disorders; Phobic anxiety; Panic disorder; Agoraphobia; Social phobia; Specific Phobia; Crown-Crisp Index score; Telomere length; Finite mixture modeling

1. INTRODUCTION

Anxiety disorders, the most common of psychiatric disorders in the U.S. population (Kessler et al., 2005), are associated with significant functional impairment and increased risk of morbidity (de Beurse et al., 1999; Lenze et al., 2001; Albert et al., 2005; Sareen et al., 2006; Roy-Byrne et al., 2008). According to the National Comorbidity Survey Replication, the U.S. lifetime prevalence of anxiety disorders is 28.8%, and the 12-month prevalence is 18.1% (Kessler and Wang, 2008). New onset of anxiety disorders primarily occurs among younger persons, and the median onset of anxiety disorders is in childhood/adolescence. Yet, anxiety disorders can still emerge in later-life (Le Roux et al., 2005; Chou, 2009) and late-life onset of anxiety may be under-recognized (Jeste et al., 2005). Given that the etiology of new onset late-life anxiety remains largely unknown, further work is needed to elucidate potential biological mechanisms and to determine whether processes intrinsic to biological aging may increase the risk of newly emergent anxiety in later-life. In recent years, a limited number of cross-sectional epidemiologic studies (Kananen et al., 2010; Okereke et al., 2012) observed that anxiety disorders and high anxiety symptoms, both likely indicators of chronic psychological distress, are associated with accelerated biological aging, as evidenced by shorter telomeres; however, not all studies have found an association between anxiety and telomere length (Surtees et al., 2011; Needham et al., 2015).

Existing epidemiologic studies (Kananen et al., 2010; Surtees et al., 2011; Okereke et al., 2012; Hoen et al., 2013; Shalev et al., 2014; Needham et al., 2015) have typically framed research questions from the standpoint of the potential impact of psychiatric disorders and symptoms, including anxiety, on telomere length. However, emerging basic science evidence demonstrates that mechanisms relevant to accelerated biological aging, such as higher oxidative stress, may increase the risk of anxiety disorders (Hovatta et al., 2005; Masood et al., 2008; Lee et al., 2010; Salim, 2014). Telomeres, repetitive TTAGGG sequences at the distal ends of linear chromosomes (Blasco, 2005), progressively shorten with each cell division in somatic cells, and eventually enter replicative senescence or undergo apoptosis (Harley et al., 1992; Blackburn, 2005). Oxidative stress and inflammation may accelerate telomere attrition (Harley et al., 1992; von Zglinicki, 2002; Epel et al., 2004; Blackburn, 2005; Cattan et al., 2008); thus, progressive telomere shortening represents a “molecular clock” that may serve as an indicator of biological aging. Recently, rodent models have demonstrated that increased oxidative stress may be involved in the

pathogenesis of psychiatric symptoms, including anxiety (Hovatta et al., 2005; Masood et al., 2008; Ng et al., 2008; Rammal et al., 2008; Lee et al., 2010;); however, whether there may be similar effects in humans is unknown. Despite the compelling basic evidence relating oxidative stress to development of anxiety, the established connection of oxidative stress with abnormal telomere length and function, and preliminary cross-sectional associations of shorter telomeres and higher anxiety in humans (Kananen et al., 2010; Okereke et al., 2012), no epidemiological studies have yet addressed prospectively whether telomere length, an index of the process of accelerated biological aging, is independently associated with later-life emergence of anxiety.

Thus, in this study we examined the relation of relative telomere length (RTLs), measured among mid-life and older ages, to risk of incident phobic anxiety at later-life among 3,194 participants of the Nurses' Health Study.

2. METHODS

2.1. The Nurses' Health Study (NHS)

The NHS began in 1976 when 121,700 female nurses, aged 30 to 55 years and living in the United States, completed an initial questionnaire. Subsequent questionnaires have been mailed every two years to collect information on a wide variety of lifestyle and health factors. Total follow-up in the NHS is ≥90%. Further details on the NHS cohort and validation of various risk factor and outcome measures have been described previously (Colditz et al., 1986). Blood samples, along with information from a supplementary questionnaire, were collected from 32,826 women in 1989-1990. Methods for blood collection have been described elsewhere (Hankinson et al., 1995). For the current analysis, we used RTLs measured among controls from prior nested case-control studies of: endometrial, ovarian, breast, pancreatic, and colon cancer; colon polyps; basal and squamous cell carcinoma; melanoma; stroke; myocardial infarction; and rheumatoid arthritis (Han et al., 2008; De Vivo et al., 2009; Prescott et al., 2010; Nan et al., 2011). RTLs that were assayed from a random sample of healthy participants aged ≥ 70 years and with no history of stroke as part of the NHS cognitive function sub-study were also included (Devore et al., 2011). The current study was approved by the Human Research Committee at Brigham and Women's Hospital, Boston, MA, USA.

2.2. Assessment of relative telomere length

Using the blood samples collected in 1989-1990, genomic DNA was extracted from peripheral blood leukocytes with the QiAmp (Qiagen Inc., Valencia, CA) 96-spin protocol. RTL was measured using quantitative polymerase chain reaction (qPCR) (Cawthon, 2002; Wang et al., 2008). Each sample was assayed in triplicate by laboratory technicians who were masked to participant characteristics; variability was assessed with inclusion of quality control samples on each plate. RTL was calculated as the exponentiated ratio of telomere repeat copy number to single gene (36B4) copy number (T/S) (Livak and Schmittgen, 2001; Cawthon, 2002; Du et al., 2013). In our hands, coefficients of variation (CVs) for the telomere and single gene assay were <4%, and CVs for the exponentiated T/S ratio were ≤18%. Although this assay measures relative telomere length, in work by Cawthon

(Cawthon, 2002), the T/S ratio correlates well with absolute telomere length provided by Southern blot (r=0.82, p<0.0001).

2.3. Assessment of phobic anxiety

Symptoms of phobic anxiety were assessed with the Crown-Crisp Index (CCI) in 1988 and 2004. The CCI contains 8 questions that assess fear and desire for avoidance (Crown and Crisp, 1966). Each item has 2 to 3 levels of possible responses, yielding a sum score of responses on all items that ranges from 0 to 16 points; higher scores indicate greater phobic anxiety. If women were missing 1-2 items on the CCI, we computed the sum score based on non-missing responses (i.e., we did not generate scores by imputing missing responses to the mean), as this is a more conservative approach used in our prior work (Okereke et al., 2012). We did not calculate the sum score for women who were missing >2 items from CCI assessed in 1988 or 2004 (Kroenke et al., 2010; Okereke et al., 2012). The CCI has been validated in psychiatric outpatient clinics and has been shown to have good discrimination (Crown and Crisp, 1966; Burgess et al., 1987). Previous analyses (Okereke et al., 2012) in the NHS have found that the CCI is reliable within this cohort and has moderate internal consistency (Cronbach coefficient alpha for CCI in 1988=0.62) comparable to that originally reported by Crown and Crisp (Crown and Crisp, 1966) among a clinical sample (Cronbach coefficient alpha=0.69).

2.4. Assessment of covariates

Information on covariates was obtained using the supplementary questionnaire completed at the time of blood collection or the most proximal cohort questionnaire (i.e., the 1988-1990 questionnaire cycle). We ascertained information on a variety of *a priori* potential confounding factors that have been related to anxiety and/or telomeres in previous literature: age (in years) (Kananen et al., 2010), educational attainment (Regier et al., 1990; Steptoe et al., 2011), race (Breslau et al., 2006; Needham et al., 2013), physical activity (in MET-hours/week) (Goodwin, 2003; Du et al., 2012), cigarette smoking (pack-years) (Cuijpers et al., 2007; Du et al., 2012), body mass index (BMI) (kg/m^2) (Petry et al., 2008; Du et al., 2012), and paternal age-at-participant's birth (Prescott et al., 2012). Anti-depressant use at blood draw was collected as a proxy for history of clinical depression. Furthermore, we considered demographic factors, including spouse/partner's education, and employment status; lifestyle factors, such as alcohol intake (grams/day); medical comorbidities (diabetes, hypertension, high cholesterol, and chronic respiratory disease); and medication use (e.g., regular use of hormones and multivitamins) to help further address potential sources of confounding by socio-economic/socio-demographic, lifestyle, and/or other health factors.

2.5. Determination of sample for analysis

There were 5,808 women who provided blood samples as controls in the previous nested case-control studies or healthy participants in the cognitive function sub-study. We performed a series of exclusions to determine the analytic sample. First, we excluded women with insufficient data on the outcome (i.e., missing >2 items on the CCI) in 1988 (n=163) or in 2004 (n=856). We further excluded women with a total CCI score >3 points in 1988 (n=1,452) to remove women who might be prevalent cases of phobic anxiety. This

cutoff was supported by preliminary data, from separate ongoing NHS clinical validation work, which indicates that cases of phobic or other anxiety disorders are very rare among those scoring 0-3 points. Lastly, we excluded women who reported prior regular use of diazepam (information collected on the 1980 and 1982 questionnaires), as benzodiazepine use could indicate past cases of phobic anxiety (*n*=143). Thus, the final population for analysis included 3,194 women. In general, characteristics of the analytic sample were similar to those of all women who provided blood samples in 1989/90; however, women in the analytic sample were slightly older with lower BMI and higher levels of physical activity compared to women in the entire blood cohort (mean age 58.3 vs. 56.5 years, mean BMI 25.1 vs. 25.5 kg/m^2, mean physical activity 16.7 vs. 15.2 MET-h/week).

2.6. Statistical analysis

To improve normality, RTLs were natural log-transformed, and Rosner's extreme studentized deviate (ESD) test for outliers was used to identify and remove any outliers (Rosner, 1983). To account for batch-to-batch variability among the nested case-control studies and the cognitive function study, we computed z-scores for RTLs, within each nested case control study, using the natural log-transformed values. To preserve relative rankings, categories of RTL (e.g., quintiles, quartiles) were based on the entire distribution before analytic exclusions among controls with RTL measurements (*n*=5,808). Rather than allowing "floating" sample sizes for different models, we used indicators to account for missing covariate data. However, only 6 women were missing data for BMI and values for this covariate were imputed to the median to improve model convergence.

Our primary approach was to use logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI) to assess baseline RTLs and incident high phobic anxiety symptoms. Women with high phobic anxiety symptoms were defined as CCI score ≥6 points. Preliminary data from an ongoing validation in the NHS suggests a high overall performance (i.e., area-under-curve [AUC]=0.9; unpublished data) of this cut point for Diagnostic and Statistical Manual (DSM)-IV (American Psychiatric Association, 1994) phobic disorders and anxiety disorders more broadly. The fifth quintile of RTLs (longest) was used as the reference group. To test for a linear trend, we assigned the median value to each RTL category and modeled this variable as a continuous term.

As an alternative approach to enhance clinical relevance, we used finite mixture modeling (FMM, a latent variable modeling method; see Supplementary online methods) (Titterington et al., 1985; McLachlan and Peel, 2000) to empirically derive latent classes reflecting phobic symptoms at later-life, in 2004. Thus, with FMM, we were able to characterize incident phobic anxiety symptoms more dimensionally – without relying on binary categorization of participants according to a pre-specified CCI cut point consistent with presence/absence of DSM-IV anxiety disorders. We evaluated CCI scores in 2004 for up to five possible latent classes and used the Bayesian Information Criterion (BIC), as well as interpretability of latent classes of phobic symptom scores (i.e., phobic symptom groups) in 2004, to determine the optimal number of classes. We used generalized linear models to estimate least-squares mean RTLs by phobic symptom groups in 2004. We further used polytomous regression models to assess the relation of RTLs to the likelihood of membership in phobic symptom

groups in 2004. Due to small numbers in these polytomous regression models, which could lead to unstable estimates due to low cell sizes, we used RTL quartiles to create a binary variable (i.e., quartiles 1-3 vs. quartile 4) to assess extreme groups (shorter telomere lengths vs. longest telomere lengths). These models were adjusted for the same covariates as logistic regression models, plus CCI in 1988 to account for significant baseline differences across phobic symptom groups.

We conducted several secondary analyses. First, to account for the fact that some participants could not be assessed in 2004 due to earlier attrition (Robins, 1994; Breslow et al., 2009), we conducted a sensitivity analysis that incorporated inverse probability of attrition weighting in the logistic regression models. We calculated the survival probability for each telomere category using the data from the entire study population in 1988 (n=5,808) and used the reciprocal as the weights in calculating the risk of incident high phobic anxiety symptoms. Second, we assessed potential effect modification by baseline age (<60 years/≥ 60 years), using multiplicative interaction terms, as we had hypothesized that RTLs may have particular biologic relevance to emergence of late-life anxiety among the oldest women (Kananen et al., 2010). As there were low case numbers in stratified analyses, we created RTL quartiles and collapsed into two groups (i.e., quartiles 1-3 vs. quartile 4), similar to the above-mentioned polytomous regression analyses.

For all analyses, we report multivariable-adjusted results, which were similar to age-adjusted results. All p-values were two-sided and p>0.05 was considered statistically significant. We used SAS Version 9.3 (SAS Institute, Cary, NC) for all analyses.

3. RESULTS

Table 1 shows age and age-adjusted characteristics of the study population by RTL quintiles. Characteristics were generally similar across RTL quintiles. However, women in the first (shortest) quintile of RTL were slightly older and had higher pack-years of smoking, alcohol intake, and lower levels of physical activity compared to women in the fifth (longest) quintile of RTL.

3.1. Results from primary models (logistic regression)

Table 2 presents age- and multivariable-adjusted ORs and 95% CIs for the association between RTLs among mid-life and older ages and emergent phobic anxiety symptoms in later life (n=3,194). We identified 153 women with incident high phobic anxiety symptoms (defined as CCI≥6) in 2004. Overall, there were no associations between baseline RTLs and risk of high incident phobic anxiety symptoms among women in these models (e.g., multivariable OR [95% CI]=0.98 [0.56-1.72] comparing shortest vs. longest RTL quintile, p-for-linear trend=0.69). Results were similar when we adjusted for comorbidity and disease-related factors (history of hypertension, diabetes, cardiovascular disease, dyslipidemia, cancer, and respiratory disease) in extended multivariable models (OR [95% CI]=0.99 [0.56-1.74] comparing shortest vs. longest RTL quintile; p-for-linear trend=0.73).

3.2. Results from alternative models (FMM)

The FMM analyses identified three classes of incident phobic anxiety symptoms scores in 2004. Three classes were selected as optimal based on BIC as well as three other fit indices (Supplementary Table S1). Classes were interpreted as a severe anxiety group (*n*=80) (median CCI=8 [range:7-13]), a minimal/intermediate anxiety group (*n*=2,436) (median CCI=2 [range:1-6]), and a non-anxious group (*n*=678) (median CCI=0 [range:0-0]) (see Supplementary Table S2 for age and age-adjusted characteristics across phobic symptom groups in 2004). Table 3 shows that women in the severe group had shorter least-squares mean RTLs (multivariable-adjusted mean RTLs=−0.12 standard units) compared to women in the minimal/intermediate (multivariable-adjusted mean RTLs=0.03 standard units) and non-anxious group (multivariable-adjusted mean RTLs=−0.03 standard units); however mean RTLs were not significantly different across the groups (*F*-test *p*-value=0.17).

In multivariable-adjusted polytomous regression models (Table 4), there was a non-significant suggestion of a relation between baseline RTL and likelihood of being in the group with emergence of severe later-life phobic anxiety. Specifically, women with shorter RTLs (quartiles 1-3) vs. longest RTLs (quartile 4) had non-statistically significant higher odds of being in the severe vs. non-anxious group (OR [95% CI]=1.78 [0.91-3.51]; *p*-value=0.09). Results were null for the contrast between minimal/intermediate vs. non-anxious group (e.g., OR [95% CI]= 0.95 [0.76-1.17; *p*-value=0.60]).

3.3. Results from secondary analyses

3.3.1. Sensitivity analysis—Logistic regression models that incorporated inverse probability weighting of attrition produced estimates that did not differ from those in the primary analysis (data not shown).

3.3.2. Effect modification—In stratified analyses to address the possibility of a stronger relation between shorter RTLs and incident high phobic anxiety risk among women ≥60 years at blood draw, we did not find evidence of effect modification (*p*-interaction=0.26) (data now shown in tables).

4. DISCUSSION

In this study of 3,194 mid-life and older women, baseline RTLs were not significantly associated with risk of developing incident high phobic anxiety symptoms (defined as CCI≥6 points) 16 years later. Analyses using latent modeling approaches to empirically identify patterns of incident phobic symptoms in later life suggested the possibility that having longer telomeres may be protective against severe phobic anxiety emergent in later-life among women; however results were not statistically significant.

Previous epidemiologic studies of anxiety disorders and telomere length have been somewhat limited by reliance on cross-sectional designs, which cannot determine temporality of the association between anxiety and telomere length, and results have been inconsistent (Kananen et al., 2010; Surtees et al., 2011; Okereke et al., 2012; Needham et al., 2015). These inconsistencies may be due in part to differences in study populations (e.g., sample size, age, and gender), telomere measurements, ascertainment of anxiety disorders,

or type of anxiety disorders. A prior cross-sectional analysis (Okereke et al., 2012) in the NHS cohort (*n*=5,243 women aged 42-69 years) observed that women with CCI≥6 had significantly shorter RTLs compared to women below that symptom threshold. Furthermore, stronger associations were observed among women with high phobic anxiety symptoms who were overweight, non-smokers, or born to fathers who were ≥40 years old. However, a larger sample was available for the cross-sectional analysis. Thus, future studies, with larger sample sizes and adequate statistical power to assess effect modification, might address more comprehensively the range of potential moderators of prospective telomere-anxiety associations. Three additional cross-sectional studies (Kananen et al., 2010; Surtees et al., 2011; Needham et al., 2015) (*n*=974 individuals aged 30-87 years, *n*=1,290 individuals aged 20-39 years, and 4,441 women aged 41-80 years, respectively) found no overall association with telomere length and anxiety disorders among community samples. However, one of these studies (Kananen et al., 2010) reported that older participants (aged 48-87 years) with anxiety had significantly shorter telomeres compared to controls.

To date, limited studies have prospectively addressed whether psychiatric disorders and symptoms predict telomere length and results remain inconsistent (Hoen et al., 2013; Shalev et al., 2014). Future research is needed to evaluate the temporality and/or directionality of telomere and anxiety associations (e.g., whether high phobic anxiety symptoms significantly predict faster shortening in telomeres, or whether *change* in telomere length is associated with subsequent risk of high phobic anxiety). Furthermore, due to the complexities involved in examining telomere length change, considerable methodologic care will be required in performing such work (Chen et al., 2011).

Adding to this literature, our study is the first to address whether telomere length is prospectively related to risk of late-life anxiety in a large sample of community-dwelling female participants. Given that our study suggests no overall linear relation of shorter telomere length to incident high phobic anxiety, it is possible that previously observed cross-sectional associations may be driven by potential impacts of anxiety disorders on telomere shortening. Additionally, telomere length may have different relations to short vs. long-term risk of high phobic anxiety symptoms, such that telomere length may be relevant to predicting risk of anxiety within a few years, but not up to 16 years of follow-up. To place our results into further context, prospective studies that have assessed the relationship between telomere length and risk of disease have been inconsistent; yet overall there is a suggestion that shorter telomere length is associated with an increased risk of disease, including cardiovascular disease and cancer (Wentzensen et al., 2011; Haycock et al., 2014). Variation in results on telomere length and risk of diseases may also be due to study design, RTL measurements and sample size (Schürks et al., 2013).

Notably, using FMM analyses, we did observe a non-significant suggestion that long telomeres may be protective against emergence of severe anxiety in later life, although this suggestion was seen among a small group of women with such symptoms (*n*=80) and results may have been underpowered. Nonetheless, future research should explore this suggestion among larger samples with better characterization of anxiety symptoms on the severe end of the spectrum.

Furthermore, it is plausible, but currently unsubstantiated, that late-life onset of anxiety may have different underlying etiology compared to earlier-onset anxiety; however further work is needed to understand the etiology of onset among older adults. Late-life onset of anxiety often involves comorbid disorders, such as cardiac and metabolic problems (Kessler et al., 2005; Kessler and Wang, 2008; Wolitzky-Taylor et al., 2010), which are commonly seen in older age and additionally may be associated with shorter telomeres (Demissie et al., 2006; Fitzpatrick et al., 2007; Salpea et al., 2010). Thus, abnormal telomere dynamics and risk of subsequent emergence of anxiety in late life may be particularly relevant among older persons. Although, we did not observe significant effect modification by age (<60/≥60 years), we acknowledge that this analysis was likely underpowered. Additional prospective studies, with larger numbers of participants across the older age groups at blood collection, are needed to examine the role of telomere length on late-life emergence of severe anxiety, particularly among the older-old.

Although research is still at an early stage, other mechanisms in aging besides oxidative stress, such as inflammation, may play a role in the relationship between anxiety and shorter telomeres. Specifically, elevated levels of inflammatory biomarkers have been associated with risk of some anxiety and stress disorders, such as posttraumatic stress disorder (PTSD) (Pitsavos et al., 2006; Brennan et al., 2009; Eraly et al., 2014). Similarly, inflammation may be a particularly important process affecting risk of late-life depression – a question examined in the Leiden 85-plus cohort, where study authors found that higher levels of C-reactive protein and pro-inflammatory cytokines were associated with increased depressive symptoms over 5 years of follow-up (van den Biggelaar et al., 2007). Future work is needed to clarify the potential role of biological aging via both oxidative stress and inflammation on risk of late-life-onset psychiatric disorders.

The strengths of our study include the use of a validated phobic anxiety scale, a large cohort with 16 years of follow-up between initial and follow-up anxiety measures, as well as high-quality information on a variety of health, lifestyle, and socio-demographic factors. Furthermore, the prospective design provides the foundation for establishing a temporal relation.

Our study also has several potential limitations. First, shorter telomere length may introduce potential estimate bias by earlier attrition from the cohort. However, in sensitivity analyses, we conducted inverse-probability of attrition weighting to account for this potential bias and results did not differ from our main analysis. Second, we used a convenience sample of controls from prior nested case-control studies and healthy women from a cognitive function sub-study in our cohort, rather than using a random sample of participants. Third, given the considerable variability in RTL measurements, it is possible that there may be random measurement error in RTLs, which would generally tend to impair the ability to detect possible true associations between telomere length and late-life-emergent anxiety. Fourth, potential misclassification of incident high phobic anxiety symptoms is a concern. Although, preliminary data from an ongoing NHS validation suggest strong concordance between the CCI≥6 cut point and diagnoses of DSM-IV criteria phobic disorders from structured interviews and also support the very low likelihood of true cases of phobic anxiety among those with CCI=0-3 points, outcome misclassification is still possible. Fifth, given the

observational nature of the study, residual confounding is possible. However, multivariable adjustment – considering extensive social, health and lifestyle variables – did not alter findings from age-adjusted estimates. Although women with a total CCI score >3 points in 1988 were excluded from the analysis, this restriction was necessary to mitigate risk of bias away from the null. We also lacked data on clinical details and could not address whether telomeres relate to development of late-life anxiety symptoms with greater persistence or greater tendency toward treatment-resistance. Finally, our results may lack generalizability. As the CCI is focused primarily on phobic symptoms (e.g., phobic anxiety), our results may be less informative regarding how telomere length relates prospectively to the broader spectrum of anxiety (including generalized anxiety or subclinical anxiety) in later life. Furthermore, the NHS cohort is predominately composed of women of European ancestry and results from our analysis may lack generalizability for men or women of other ethnicities. However, the homogeneity of the NHS cohort improves the internal validity of the study, as it reduces the influence of potential unmeasured social, health, or lifestyle factors.

In conclusion, there was no significant linear association between baseline telomere length and incident high phobic anxiety symptoms. However, further work is needed to explore whether long telomere length may be protective against emergence of severe patterns of phobic anxiety symptoms during later-life. Future prospective studies should target these investigations among larger samples of individuals, among older ages, and with better characterization of anxiety symptoms on the severe end of the spectrum.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- We examined telomere length in 1989/90 with incident phobic anxiety in 2004.
- Logistic regression models suggest no significant association between telomere length and phobic anxiety.
- Finite Mixture Modelling (FMM) identified 3 groups of phobic anxiety symptoms in 2004: 1) severe, 2) minimal/intermediate, and 3) non-anxious.
- Analyses using FMM groups suggest that shorter telomeres may be associated with emergence of severe phobic anxiety symptoms; however, results were not significant.

Table 1

Baseline age and age-standardized characteristics by quintiles of relative telomere length (*N*=3,194)[a,b]

	Relative telomere length (z-scores)				
	Q1 (shortest)	**Q2**	**Q3**	**Q4**	**Q5 (longest)**
N	618	618	657	662	639
Relative telomere length (z-scores)[c]	−1.3(−2.0 to −0.9)	−0.5(−0.8 to −0.3)	0.0(−0.2 to 0.2)	0.5(0.3 to 0.8)	1.2(0.9 to 2.0)
Age (years)[d]	59.2(6.4)	58.7(6.4)	58.7(6.5)	57.8(6.6)	57.2(6.8)
Age (5-year groups)[d]					
<50	10.5	11.8	11.1	14.2	18.2
50-<55	15.9	16.5	18.3	21.0	19.3
55-<60	22.7	23.1	22.2	24.8	23.5
60-<65	29.3	29.8	28.2	24.0	23.3
≥65	21.7	18.8	20.2	16.0	15.8
Non-Hispanic whites, %	99.1	98.3	98.4	98.6	98.1
Nurses' education level, %					
RN	65.5	68.4	66.2	65.9	63.3
Bachelor	22.8	18.9	23.6	22.1	23.5
Graduate	11.7	12.7	10.3	12.0	13.2
Paternal age at participant's birth (years)	30.9(6.6)	31.2(7.1)	31.7(7.1)	31.9(7.4)	31.6(6.8)
Body mass index (kg/m^2)	25.2(4.4)	25.2(4.3)	25.1(4.2)	24.8(4.4)	25.2(4.1)
Physical activity (MET-hours/week)	16.5(17.7)	16.9(18.4)	16.0(17.7)	16.7(17.8)	17.5(18.1)
Pack-years of smoking	11.9(18.4)	11.5(17.0)	11.6(18.1)	12.4(18.3)	10.7(16.9)
Alcohol intake (g/day)	5.6(8.9)	5.4(9.6)	6.2(11.3)	4.7(8.1)	4.7(7.6)
Anti-depressant use, %	3.9	2.4	3.6	3.5	2.5
Current hormone use, %	36.2	35.9	34.4	36.9	34.1
History of hypertension, %	25.5	25.9	23.1	28.3	27.1
History of diabetes, %	4.3	2.7	2.5	3.5	3.1
History of heart disease, %	4.1	6.0	3.9	7.0	3.9
History of elevated cholesterol, %	38.1	37.9	38.5	44.4	39.7
History of cancer, %	2.8	2.2	2.6	2.2	2.0
Respiratory disease, %	4.8	5.0	4.8	5.3	4.7

Values are means (SD) or percentages and are standardized to the age distribution of the study population unless otherwise noted.

[a] All women had no meaningful anxiety symptoms at baseline (Crown-Crisp Index=0-3).

[b] Due to rounding, percentages may not sum to 100.0.

[c] Values are medians (10^{th} to 90^{th} percentile).

[d] Value is not age adjusted.

Table 2

Odds ratio (95% Confidence Interval) of high phobic anxiety (CCI≥6) in later life by quintiles of relative telomere length (*N*=3,194)[a]

	Relative telomere length (z-scores)					P_{trend} [e]
	Q1 (shortest)	**Q2**	**Q3**	**Q4**	**Q5 (longest)**	
Cases/non-cases	27/591	29/589	38/619	34/628	25/614	
Model I[b]	1.00 (0.57-1.75)	1.12 (0.64-1.93)	1.38 (0.82-2.33)	1.29 (0.76-2.20)	1.00 (ref)	0.78
Model II[c]	0.98 (0.56-1.72)	1.07 (0.62-1.86)	1.37 (0.81-2.31)	1.28 (0.75-2.19)	1.00 (ref)	0.69
Model III[d]	0.99 (0.56-1.74)	1.08 (0.62-1.88)	1.36 (0.81-2.30)	1.28 (0.75-2.18)	1.00 (ref)	0.73

Abbreviations: CCI=Crown-Crisp Index.

[a] All women had no meaningful anxiety symptoms at baseline (CCI=0-3).

[b] Adjusted for age (continuous, in years).

[c] Adjusted for model I covariates plus body mass index (BMI) (continuous, in kg/m^2), physical activity (continuous, MET-hours/week), pack years of smoking (continuous), paternal age at participant's birth (15 to <25 years, 25 to <30 years, 30 to <35 years, 35 to <40 years, ≥40 years, missing), antidepressant use (no, yes, missing), race (non-Hispanic white, other), and education (RN, bachelor, graduate degree).

[d] Adjusted for model II covariates plus hypertension (yes, no), dyslipidemia (yes, no), diabetes (yes, no), cardiovascular disease (yes, no), respiratory disease (yes, no), and cancer (yes, no).

[e] P_{trend} calculated with median values of each category of relative telomere length as a continuous variable.

Table 3

Least-squares mean relative telomere length z-scores (standard errors) by latent classes of phobic symptom scores in 2004 (*N*=3,194)[a,b]

	CCI score in 2004			
	Severe	**Minimal/ intermediate**	**Non-anxious**	***F*; P_{value} [f]**
N	80	2436	678	
Median CCI in 1988 (range)	2 (0-3)	2 (0-3)	1 (0-3)	
Median CCI in 2004 (range)	8 (7-13)	2 (1-6)	0 (0-0)	
Model I[c]	−0.15 (0.11)	0.03 (0.02)	−0.02 (0.04)	1.88; 0.15*
Model II[d]	−0.12(0.11)	0.03 (0.02)	−0.03 (0.04)	1.80; 0.17**
Model III[e]	−0.12 (0.11)	0.03 (0.02)	−0.03 (0.04)	1.77; 0.17***

Abbreviations: CCI=Crown-Crisp Index. Degrees of freedom=df.

[a] Generalized linear model analysis conducted across three mutually exclusive classes of CCI scores in 2004: 1) women with severe CCI in 2004 (*N*=80), 2) women with minimal/intermediate CCI in 2004 (*N*=2,436), and 3) women who were non-anxious in 2004 (*N*=678).

[b] All women had no meaningful anxiety symptoms at baseline (CCI=0-3).

[c] Adjusted for age (continuous, in years).

[d] Adjusted for model I covariates plus CCI in 1988 (continuous), body mass index (BMI) (continuous, in kg/m^2), physical activity (continuous, MET-hours/week), pack years of smoking (continuous), paternal age at participant's birth (15 to <25 years, 25 to <30 years, 30 to <35 years, 35 to <40 years, ≥40 years, missing), antidepressant use (no, yes, missing), race (non-Hispanic white, other), and education (RN, bachelor, graduate degree).

[e] Adjusted for model II variables plus hypertension (yes, no), dyslipidemia (yes, no), diabetes (yes, no), cardiovascular disease (yes, no), respiratory disease (yes, no) and cancer (yes, no).

[f] *F*-test statistic and P_{value} are from the global test for differences in least-squares means.

* $F_{\text{numerator df, denominator df}} = F_{2, 3190}$

** $F_{\text{numerator df, denominator df}} = F_{2, 3174}$

*** $F_{\text{numerator df, denominator df}} = F_{2, 3168}$

Table 4

Relation of relative telomere length to the likelihood of membership in latent classes of phobic symptom scores in 2004 (*N*=3,194)[a,b]

	Relative Telomere Length (z-scores)		
	Quartile 1-Quartile 3 OR (95% CI)	**Quartile 4 (longest) OR (95% CI)**	***P* value**
Model I[c]			
Minimal/intermediate	0.91 (0.74-1.11)	1.00 (ref)	0.34
Severe	1.78 (0.92-3.44)	1.00 (ref)	0.09
Model II[d]			
Minimal/intermediate	0.95 (0.76-1.17)	1.00 (ref)	0.60
Severe	1.78 (0.91-3.51)	1.00 (ref)	0.09
Model III[e]			
Minimal/intermediate	0.95 (0.77-1.18)	1.00 (ref)	0.65
Severe	1.76 (0.90-3.47)	1.00 (ref)	0.10

Abbreviations: OR=odds ratio. CI=confidence interval.

[a] Polytomous regression analysis conducted across three mutually exclusive classes of Crown-Crisp Index (CCI) scores in 2004: 1) women with severe CCI in 2004 (*N*=80), 2) women with minimal/intermediate CCI in 2004 (*N*=2,436), and 3) women who were non-anxious in 2004 (*N*=678) (reference).

[b] All women had no meaningful anxiety symptoms at baseline (CCI=0-3).

[c] Adjusted for age (continuous, in years).

[d] Adjusted for model I covariates plus CCI in 1988 (continuous), body mass index (BMI) (continuous, in kg/m^2), physical activity (continuous, MET-hours/week), pack years of smoking (continuous), paternal age at participant's birth (15 to <25 years, 25 to <30 years, 30 to <35 years, 35 to <40 years, ≥40 years, missing), antidepressant use (no, yes, missing), race (non-Hispanic white, other), and education (RN, bachelor, graduate degree).

[e] Adjusted for model II covariates plus hypertension (yes, no), dyslipidemia (yes, no), diabetes (yes, no), cardiovascular disease (yes, no), respiratory disease (yes, no) and cancer (yes, no).