Telomere Length and Frailty: The Helsinki Birth Cohort Study

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Abstract

Objectives: Telomere length is associated with aging-related pathologies. Although the association between telomere length and frailty has been studied previously, only a few studies assessing longitudinal changes in telomere length and frailty exist. Design: Longitudinal cohort study. Setting and participants: A subpopulation of the Helsinki Birth Cohort Study consisting of 1078 older adults aged 67 to 79 years born in Helsinki, Finland, between 1934 and 1944. Measures: Relative leukocyte telomere length (LTL) was measured using quantitative real-time polymerase chain reaction at the average ages of 61 and 71 years, and at the latter the participants were assessed for frailty according to Fried criteria. Results: The mean ± SD relative LTLs were 1.40 ± 0.29 (average age 61 years) and 0.86 ± 0.30 (average age 71 years) for the cohort. A trend of shorter mean relative LTL across frailty groups was observed at 61 years (P = .016) and at 71 years (P = .057). Relative LTL at age 61 years was significantly associated with frailty: per 1-unit increase in relative LTL, the corresponding relative risk ratio (RRR) of frailty was 0.28 (95% confidence interval [CI] 0.08−0.97), adjusting for several confounders. Also, LTL at age 71 years was associated with frailty (RRR 0.18, 95% CI 0.04−0.81) after adjustment for sex, age, and adult socioeconomic status, but further adjustment attenuated the association. No associations between telomere shortening and frailty were observed during the 10-year follow-up. Conclusions: Shorter relative LTL was associated with frailty in cross-sectional and longitudinal analyses, but telomere shortening was not, suggesting that short LTL may be a biomarker of frailty.

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Keywords:
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frailty
telomere
explained by chronological age alone. \(^3\) To account for this variation, knowledge of the aging process has been applied to identify markers of biological aging.\(^3\)

Telomeres consist of tandem DNA repeats located at the ends of eukaryotic chromosomes and function to maintain chromosomal integrity.\(^3\) Progressive shortening of telomeres occurs at each somatic cell division, unless their length is maintained by the enzyme telomerase.\(^3\) Because the number of cell divisions is expected to increase with age, shorter leukocyte telomere length (LTL) has been associated with aging-related markers of inflammation\(^1\) and oxidative stress,\(^1\) as well as pathologies including cardiovascular disease,\(^1\) type 2 diabetes,\(^1\) and dementia,\(^1\) as critically short telomeres may compromise chromosomal stability and predispose the cell to senescence and apoptosis.\(^1\) Furthermore, in some but not all studies, LTL has been found to predict all-cause mortality.\(^1,12\) As a result, telomere length has been proposed to act as a marker for biological aging.\(^1\)

Seven previous studies have failed at demonstrating associations between telomere length and frailty.\(^14-20\) However, with the exception of a longitudinal study\(^1\) on telomere length measured at one time point in relation to changes in frailty status in 2006 older Chinese adults, these previous studies have been cross-sectional in design. Our aim was to explore cross-sectional and longitudinal associations between LTL measured at 2 time points over a 10-year interval and frailty according to the criteria of Fried et al.\(^1\) in a cohort of 1078 older individuals born in Helsinki, Finland, between 1934 and 1944.

Methods

Study Design

The present study is a substudy of the Helsinki Birth Cohort Study that includes a subpopulation of 8760 individuals who were all born in Helsinki between 1934 and 1944, had visited child welfare clinics at that time, and lived in Finland in 1971 when a unique personal identification number was assigned to all Finnish residents.\(^3\) Random-number tables were used to select a subset of people who attended a clinical examination between 2001 and 2004. Of the 2902 invited subjects, 2003 were examined clinically. From this clinical study cohort, 1094 of the invited 1404 cohort members participated in a clinical follow-up between 2011 and 2013. Of these, 1078 individuals had information on frailty.\(^22\) LTL was measured at the clinical examination (n = 1042) in 2001–2004 and at follow-up (n = 1061) in 2011–2013. A total of 1037 participants had both LTL measurements available, and for these participants telomere shortening was calculated.\(^23\) The clinical study protocol was approved by the Coordinating Ethics Committee of The Hospital District of Helsinki and Uusimaa. Written informed consent was obtained from each participant before initiating any study procedures.

DNA Extraction and Telomere Length

Relative LTL was measured twice: at the baseline clinical examination between 2001 and 2004 and at the 10-year follow-up between 2011 and 2014. In brief, DNA was extracted from peripheral whole blood using a commercially available kit and then assessed for purity and integrity using detailed methodology described previously.\(^23\) Using slightly different methods, a real-time quantitative polymerase chain reaction (PCR) approach was applied at both time points to measure relative LTL. First in 2001–2004, the ratio of telomere DNA to hemoglobin beta single-copy gene signal intensities was used to determine relative LTL, as previously described.\(^24-26\) Later in 2011–2014, relative LTL was measured using a multiplex quantitative real-time PCR method, described previously by Cawthon\(^27\) and Guzzardi et al.\(^28\) Four genomic DNA control samples were included in all plates to calibrate the plate effect and for monitoring the coefficient of variation (CV), which was 21.0% and 6.2% at the first and second time points, respectively. Telomere measurements are expressed as T/S ratios, which equals the ratio of telomere repeat copy number to single gene copies in experimental samples compared with a reference sample. Significant correlation was observed between the 2 relative LTL measurements (r = 0.254, P < .001). Telomere shortening during the 10-year period was calculated adjusting for the baseline relative LTL measurement (relative change in LTL = [(LTL at T1) − (LTL at T2)]/[LTL at T1] × 100) to avoid error due to different methodology used.

Frailty

Frailty was defined as the sum of 5 criteria, including weight loss, exhaustion, low physical activity, slowness, and weakness, at the clinical examination in 2011–2013.\(^1\) A question from the Beck Depression Inventory\(^29\) was used to assess recent weight loss. Those who reported losing at least 5 kg met the criterion. Exhaustion was assessed using the following question: “How many times during the last week have you felt unusually tired or weak?” The criterion was met if the response was “On 3 days or more.” The validated Kuopio Ischemic Heart Disease Risk Study (KIHD) questionnaire was used to evaluate leisure time physical activity (LTPA).\(^29\) Those whose total LTPA time (including walking, resistance training, and gardening) was 1 hour or less per week met the criterion of low physical activity. In case of missing KIHD LTPA data (n = 37), physical activity was assessed using the question: “In total, how many hours a week do you do the following sports (walking, jogging, cycling, swimming, gymnastics, group exercise)?” The criterion was met if the total duration of physical activity was 1 hour or less per week. Slowness was assessed based on maximal walking speed over a 4.57-m distance. For walking speed, sex-specific cutoffs for medium height (for men <175.9 cm cutoff was 1.65 m/s and >175.9 cm 1.83 m/s, and for women <162.2 cm cutoff was 1.47 m/s and >162.2 cm 1.55 m/s) were used to identify the slowest 20% who met the criterion. Weakness was assessed by measuring isometric grip strength of the dominant hand with an adjustable dynamometer chair (Good Strength; Metitour Ltd, Jyväskylä, Finland). For grip strength, sex-specific quartiles of body mass index were used to identify the weakest 20% who met the criterion. Cohort members were classified as frail if they met 3 or more, prefrail if they met 1 or 2, and nonfrail if none of the criteria were met.

Covariates

Body composition was assessed in 2001–2004 using bioelectrical impedance by the InBody 3.0 8-polar tactile electrode system (Bio-space Co. Ltd, Seoul, Korea). Segmental multifrequency analyses (5, 50, 250, and 500 kHz) were performed separately for each limb and trunk to estimate body fat percentage.\(^30,31\) Smoking status (smoker, former smoker, never smoked) and self-reported diabetes and cardiovascular disease were assessed using questionnaires at the clinical examination Data on adult socioeconomic status (SES), which was obtained from Statistics Finland, was coded based on occupational status attained at 5-year intervals between 1970 and 2000 as follows: upper and lower middle class, self-employed, and manual workers.

Statistics

Results for continuous variables are expressed as means and SDs and as proportions for dichotomous or categorical values. Significance between groups was evaluated using 1-way analysis of variance and cross tabulation, respectively for continuous and categorical values. Multinomial logistic regression analysis was used to study the association between telomere length and frailty. The analyses were first adjusted for age and sex and then additionally for adult SES, adult body fat percentage, smoking, and the prevalence of cardiovascular disease and diabetes. Because no significant interactions were
observed between sex and relative telomere measurements on frailty (all P > 0.05), we report results pooled by sex. The analyses were 2-tailed, the level of significance was set at P < 0.05, and analyses were carried out with SPSS (IBM SPSS Statistics for Windows, version 23.0 released 2015; IBM Corp, Armonk, NY).

Results

Characteristics of the 1078 men and women included in the study are presented in Table 1. In addition to having longer telomeres at baseline and at follow-up, women experienced a slower rate of telomere shortening than the men in the cohort over a 10-year period (all P < 0.05). The prevalence of frailty was 2.7% and 4.3% at the mean age of 70.9 years, respectively, for men and women. No significant sex differences in frailty were observed.

In Table 2, those who were classified as frail at the follow-up had the lowest mean T/S ratios at baseline and follow-up. This graded decrease in the T/S ratio was significant at baseline (P = .016) and borderline significant at follow-up (P = .057).

Relative LTL was associated with frailty in cross-sectional and longitudinal analyses, shown in Table 3. At the average age of 71 years, the age- and sex-adjusted relative risk ratio (RRR) of frailty was 0.16 (95% confidence interval [CI] 0.04–0.73) per 1-unit increase in the T/S ratio compared with the nonfrail. The association persisted after adjusting for adult SES, but was attenuated when adjusted further for adulthood body fat percentage, smoking, prevalence of cardiovascular disease, and diabetes and relative LTL at the mean age of 61 years. Longitudinally, relative LTL at a mean age of 61 years was associated with frailty after a 10-year follow-up; per 1-unit increase in the T/S ratio, the age and sex-adjusted RRR for frailty was 0.24 (95% CI 0.07–0.83) compared with the nonfrail. The association changed little after additional adjustments (RRR 0.28, 95% CI 0.08–0.97). No significant associations were observed between telomere shortening and frailty.

Discussion

Short LTL, which is a potential marker of biological age, is associated with aging-related chronic diseases8–10; however, no associations between LTL and frailty have been reported previously14–20 to the best of our knowledge, this study is the first to provide evidence of an inverse association between LTL and frailty, and furthermore, to study longitudinal associations between LTL measured at 2 time points and frailty.

The absence of previous cross-sectional and longitudinal evidence14–20 may be the result of 3 things. First, the sample sizes of these studies may have been lacking in statistical power to detect significant associations. Second, although sample sizes may have been adequate, participants might present a rather large age spectrum, which could be a problem because telomere length may vary significantly at different ages, leading to a more general sample than could be expected with participants of similar age. Third, the use of only a single LTL measurement may not be sufficient in measuring a multi-dimensional syndrome such as frailty because LTL has been suggested to be dynamic in nature so that neither its length nor shortening rate is fixed at a given point in time.32

Despite previously found associations between telomere shortening and grip strength,33 a subcomponent of frailty, no associations between telomere shortening over a 10-year period and frailty were observed. Although considerable shortening of telomere length may occur during a given period, relative changes in LTL may not result in

### Table 1

<table>
<thead>
<tr>
<th>Characteristics of the Study Population</th>
<th>Whole Cohort</th>
<th>Men</th>
<th>Women</th>
<th>P*</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean (SD)</td>
<td>n</td>
<td>Mean (SD)</td>
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<tr>
<td>Adult Characteristics at 61 y</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Body fat, %</td>
<td>1039</td>
<td>28.7 (8.0)</td>
<td>454</td>
<td>22.9 (5.5)</td>
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<tr>
<td>Current smoker, %</td>
<td>1071</td>
<td>19.1</td>
<td>471</td>
<td>20.8</td>
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<tr>
<td>Cardiovascular disease, %</td>
<td>1075</td>
<td>5.6</td>
<td>473</td>
<td>4.7</td>
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<tr>
<td>Diabetes, %</td>
<td>1075</td>
<td>5.2</td>
<td>473</td>
<td>6.8</td>
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<tr>
<td>Adult socioeconomic status</td>
<td>1078</td>
<td>28.7</td>
<td>475</td>
<td>23.6</td>
</tr>
<tr>
<td>Upper middle class, %</td>
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<td>16.8</td>
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<td>23.6</td>
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<tr>
<td>Lower middle class, %</td>
<td></td>
<td>46.0</td>
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<td>28.0</td>
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<td>Self-employed, %</td>
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<td>8.5</td>
<td></td>
<td>9.3</td>
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<td>Manual workers, %</td>
<td></td>
<td>28.7</td>
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<td>39.2</td>
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<tr>
<td>Telomere measurements</td>
<td></td>
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<tr>
<td>LTL at 61 y, T/S ratio</td>
<td>1042</td>
<td>1.40 (0.29)</td>
<td>458</td>
<td>1.37 (0.29)</td>
</tr>
<tr>
<td>LTL at 71 y, T/S ratio</td>
<td>1061</td>
<td>0.86 (0.30)</td>
<td>465</td>
<td>0.81 (0.27)</td>
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<tr>
<td>Telomere shortening rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Between 61 and 71 y, change T/S ratio</td>
<td>1037</td>
<td>–0.54 (0.36)</td>
<td>455</td>
<td>–0.57 (0.35)</td>
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<tr>
<td>Frailty classification at 71 y</td>
<td>1078</td>
<td>–37.1 (24.5)</td>
<td>475</td>
<td>–39.5 (21.2)</td>
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<td>Nonfrail, %</td>
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<td>56.4</td>
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<td>Prefrail, %</td>
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<td>Frail, %</td>
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<td>3.6</td>
<td></td>
<td>2.7</td>
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</table>

LTL, leukocyte telomere length; T/S ratio, ratio of telomere repeat copy number to single gene copies in experimental samples compared with a reference sample.

*Difference between men and women.

### Table 2

<table>
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<tr>
<th>Measurements According to Frailty Classification in the Whole Cohort</th>
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<tr>
<td>T/S shortening rate at 61 y/T/S ratio</td>
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<tr>
<td>Mean (SD)</td>
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<tr>
<td>Mean LTL at 71 y/T/S ratio</td>
</tr>
<tr>
<td>T/S shortening rate (change T/S ratio)</td>
</tr>
<tr>
<td>T/S shortening rate (change T/S ratio percent)</td>
</tr>
</tbody>
</table>

LTL, leukocyte telomere length; T/S ratio, ratio of telomere repeat copy number to single gene copies in experimental samples compared with a reference sample.

*Trend across frailty classes.
critically short telomeres, and again a clinically significant reduction in function and reserve that characterize frailty. In fact, telomere shortening has been observed to be greater in individuals with longer LTL at baseline; however, the results of the present study remained essentially unchanged after additional adjustment for baseline LTL.

Because telomere length has been suggested to be a marker of biological age, individuals with shorter telomeres can be expected to be at increased risk of aging-related pathologies such as frailty, which has been proposed as a clinical representation of biological age. The absence of telomerase, which is an enzyme that promotes telomere elongation and the function of which is often impaired in individuals with critically short telomeres, may promote the loss of telomere integrity. As a result, activation of the p53-pathway may lead to impaired mitochondrial function and promote cell dysfunction. Senescent cells are more likely to secrete aging-related markers of inflammation and oxidative stress, which may underlie the decline of muscle mass characteristic of sarcopenia, a major risk factor for frailty. In fact, increased levels of inflammatory markers and oxidative stress also have been observed among frail older adults.

The notion of little variance in telomere length at birth stresses the importance of genetic and environmental factors in determining later telomere length and shortening. Associations between shorter telomeres and, for example, a less active lifestyle, proinflammatory nutritional agents, and a less healthy cardio-metabolic profile may give insight to the reported associations between LTL and several chronic diseases. Greater simultaneous presence of these diseases may predispose an individual to disturbances in homeostasis, and consequently frailty, as illustrated by the concept of comorbidity.

A key strength of the present study is its longitudinal design; we were able to study cross-sectional and longitudinal associations between LTL and frailty in a well-characterized population in excess of 1000 individuals through a period of 10 years. Relative LTL was measured twice, which enabled us to study associations between telomere shortening and frailty according to the criteria put forward by Fried et al.

The study has some limitations. Frailty was measured only at follow-up, which limits our capability of understanding whether short LTL is actually a cause or a consequence of frailty. Although the 2 LTL measurements correlated significantly (P < .001), relatively high interassay variability (CV 21.0%) was observed at baseline. Although several confounding factors were accounted for, no information on the participants’ nutritional status was available, resulting in limited ability to control for the effects of dietary factors on LTL. Selective survival at the clinical follow-up may have led to relatively longer telomeres and an underrepresentation of frailty among the study participants. As we studied only white individuals aged 67–79 years born in Helsinki in 1934 to 1944 and who attended child welfare clinics at the time, the results may not be generalizable to other populations or age groups.

Conclusions

In conclusion, in this longitudinal study of older Finnish persons, relative LTL was observed to be inversely associated with frailty, after adjusting for potential confounders. The findings support the role of short LTL as a predictor of frailty in that it can detect processes that will eventually lead to frailty, and therefore act as a possible biomarker of frailty. Future longitudinal studies with several telomere and frailty measurements are needed to address these associations in more detail.

References


