

Does telomere length predict decline in physical functioning in older twin sisters during an 11-year follow-up?

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Abstract Leukocyte telomere length (LTL) is known to be associated with mortality, but its association with age-related decline in physical functioning and the development of disability is less clear. This study examined the associations between LTL and physical functioning, and investigated whether LTL predicts level of physical functioning over an 11-year follow-up. Older mono- (MZ) and dizygotic (DZ) twin sisters ($n = 386$) participated in the study. Relative LTL was measured by qPCR at baseline. Physical functioning was measured by 6-min walking distance and level of physical activity (PA). Walking distance was measured at baseline and at 3-year follow-up. PA was assessed by questionnaire at baseline and at 3- and 11-year follow-ups. The baseline

analysis was performed with path models, adjusted with age and within-pair dependence of twin pairs. The longitudinal analysis was performed with a repeated measures linear model adjusted for age and longitudinal within-pair dependence. A nonrandom missing data analysis was utilized. At baseline, in all individuals, LTL was associated with PA (est. 0.14, SE 0.06, $p = 0.011$), but not with walking distance. Over the follow-up, a borderline significant association was observed between LTL and walking distance (est. 0.14, SE 0.07, $p = 0.060$) and a significant association between LTL and PA (est. 0.19, SE 0.06, $p = 0.001$). The results suggest that LTL is associated with PA and may, therefore, serve as a biomarker predicting the development of disability. Longitudinal associations between LTL and PA were observed only when nonrandom data missingness was taken into account in the analysis.

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Introduction

With increasing age, underlying pathologies, genetic vulnerabilities, physiological and sensory impairments, and environmental barriers increase the risk for decline in physical functioning (Rantakokko et al. 2013). Physical activity prevents functional decline among older people. A low level of physical activity may cause a vicious cycle that leads to disability and increasing

incidence of adverse health outcomes. Walking limitation is often the first sign of further functional decline, increasing the risk for disability, diseases, and mortality (Newman et al. 2006).

To prevent premature adverse aging effects, it is important to identify people who are at risk for accelerated decline in physical functioning and clarify the factors underlying the aging process. Decline in physical functioning accelerates with advancing age, with high interindividual and intraindividual variability in different organ systems. This suggests that chronological age is a rather imprecise estimate of the progression of functional impairments (Newman et al. 2006). To provide more information about individuals' biological aging, several candidate biomarkers have been proposed and tested. Biomarkers of aging should reflect biological aging and potentially identify subjects at risk for accelerated age-related adverse outcomes. Telomere shortening may more accurately predict the development of the biological aging process than chronological age (Blackburn 2005), and therefore, it may serve as a potential biomarker of aging (Mather et al. 2011). Telomere length has several advantages as a potential biomarker of the aging process: it is relatively inexpensive and easy to measure; it has high interindividual variability; it is linked to basic biology; and it correlates well with aging and age-related diseases (von Zglinicki and Martin-Ruiz 2005).

Telomeres are specialized nucleoprotein structures located at the ends of chromosomes that act as a protective caps contributing to genomic integrity and stability. Recent studies have already shown that LTL is inversely associated with mortality (Cawthon et al. 2003) and several age-related diseases such as cardiovascular diseases, COPD, cancer, and type 2 diabetes (Zhao et al. 2013; Haycock et al. 2014; Albrecht et al. 2014). The evidence on the association between LTL and physical functioning is inconclusive. According to a recent meta-analysis, good-quality evidence on the association between telomere length and physical activity remains scarce (Mundstock et al. 2015). In general, the associations between LTL and PA have been weak, moderate, or nonexistent depending on the sample size, methodology, and population examined. Contradictory findings have also been reported on the associations between LTL and walking speed (Harris et al. 2006a; Lee et al. 2013; Gardner et al. 2013). Overall, the evidence of associations between telomeres and indicators of physical aging is equivocal and mostly derived from cross-

sectional and underpowered studies (von Zglinicki and Martin-Ruiz 2005). Very few studies have investigated the association between LTL and indicators of physical functioning in longitudinal designs.

The present study investigated the association between relative LTL and physical functioning in older twin sisters. In addition, we examined whether LTL predicted physical functioning, assessed by 6-min walking distance and level of physical activity, over 3- and 11-year follow-ups. After examining these associations in the twins considered as individuals, potential genetic and environmental influences on the associations between LTL and physical functioning were taken into account by comparing the results between monozygotic and dizygotic twin pairs.

Methods

Study design and participants

The study is part of the Finnish Twin Study on Aging (FITSA), which was set up to investigate genetic and environmental effects on the disablement process in 63- to 76-year-old women (Tiainen et al. 2004; Tiainen et al. 2007). The participants were recruited from the Finnish Twin Cohort, which comprises all the same-sex twin pairs born before 1958 and with both co-twins alive in 1975 (Kaprio et al. 1978; Kaprio and Koskenvuo 2002). Zygosity was determined at the baseline study in 1975 by a validated questionnaire (Sarna et al. 1978) and confirmed in the FITSA study using DNA extracted from a venous blood sample by a battery of ten highly polymorphic gene markers. In August 2000, there were 1260 female twin pairs in the age group 63–76 years who had participated in the Finnish Twin Cohort in 1975. An invitation to participate in the FITSA study was sent to a subsample of 414 twin pairs from this group. Inclusion criteria were willingness of both sisters to participate and the ability to travel to the laboratory. In total, 103 monozygotic and 114 dizygotic twin pairs (434 individuals) participated in the laboratory measurements. LTL analyses were missing or had failed in 48 participants. The total number of participants included in this analysis at baseline was thus 386.

The FITSA study was carried out according to good clinical and scientific practice as laid down by the Declaration of Helsinki. The study protocol has been approved by the Ethics Committee of the Central Hospital

District of Central Finland (K-S shp: Dnro 24/2000). Before the laboratory examinations, the participants were informed about the measurements and gave their written informed consent.

Leukocyte telomere length

LTL was determined from peripheral blood DNA by quantitative real-time polymerase chain reaction (qPCR) as described in Ahola et al. (2012). Briefly, as a single-copy reference gene, β -hemoglobin was used. Each plate included a DNA dilution series (0.5, 1.0, 2.0, 5.0, 10, 20, and 30 ng), which was used to create a standard curve and to perform absolute quantification of each sample. Samples and standard dilutions were analyzed in triplicate. Both the telomere and β -hemoglobin reactions were performed with a CFX 384 Real-Time PCR Detection System (Bio-Rad, Hercules, CA). We used the Bio-Rad CFX Manager v.1.1 software to perform quality control and to calculate the telomere to single-copy gene intensity (T/S) ratios for the samples in order to obtain the relative telomere length. Each qPCR plate included five normal samples and one short telomere length control sample as calibrators to correct for possible interplate batch effects.

Physical functioning

Physical functioning was estimated by 6-min walking distance and level of physical activity (PA). Participants performed a *6-min walking test* in which they attempted to walk as far as possible in the allotted time (Bean et al. 2003) at baseline and at the 3-year follow-up. The 6-min walking test is a performance-based measure of functional exercise capacity, and it can be used as an indicator of exercise tolerance or aerobic capacity. The test was performed on a 50-m indoor track, and participants were instructed to complete as many laps as possible within 6 min. The distance (m) covered in the 6-min walk was recorded and used in the analysis. A standardized protocol and safety guidelines issued by the American Thoracic Society (ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories 2002) were followed.

Physical activity was assessed using a modified version of the seven-point Grimby scale (Grimby 1986) at baseline and at the 3- and 11-year follow-ups. The categories used in questionnaire were as follows: (1) inactive, (2) light activity 1–2 times per week, (3)

light activity several times per week, (4) moderate activity 1–2 times per week, (5) moderate activity several times per week, (6) high activity several times per week, and (7) competitive sports several times per week. For the analysis, categories 5 and 6 were combined, as very few participants (0 to 7) reported category 6 and none category 7.

Participant characteristics

Participants' height and body mass were measured in a laboratory, and body mass index (BMI, kg/m^2) was calculated. Information on smoking behavior, chronic conditions, prescriptive medication, and health status were collected by questionnaires and confirmed by a physician during a clinical examination. In addition, sociodemographic characteristics were collected by questionnaire, depressive symptoms were assessed using the Center for Epidemiologic Studies Depression Scale (Radloff 1977), and cognitive function was assessed by the Mini-Mental State Examination (MMSE) (Folstein et al. 1975).

Statistical analysis

Basic statistical analysis was performed with IBM SPSS statistics 22.0 software. Means and standard deviations (SD) are reported for continuous variables in text and in figures. The equalities of the means and distributions in the descriptive parameters and LTL between the MZ and DZ twins were tested with adjusted Wald and chi-squared tests taking into account the within-pair dependence of twin individuals. Associations between LTL and physical functioning at baseline and during follow-up were analyzed with MPlus statistical software (Muthén and Muthén 1998–2012). LTL, age, and walking distance were used as continuous variables and PA as an ordered five-category variable. The *cross-sectional analysis* was based on a path model with an unstructured within-twin pair outcome correlation matrix and adjustment for age and within-pair dependence of twin pairs (see Appendix S1 for details). The *longitudinal analysis* was performed as a repeated measures linear model, which was adjusted for age and included an unstructured outcome correlation matrix due to additional longitudinal within-person dependence (see Appendix S2 for details). For PA, the model

was estimated for three measurements, but for walking distance, the model was constructed for the two available measurement occasions. The advantage of the present model over the other potential models is the possibility to account for missing data in a flexible modeling framework, which is important in the analysis of a sample of older participants, where the assumption of data missing at random can lead to biased results. Missing data were largely attributable to the poorer health of those unable or unwilling to participate in the follow-ups (see “Results”). Consequently, we estimated the regression coefficient between LTL and the response variables under two missing data settings: assuming (1) data missing at random (MAR) and (2) data missing not at random (MNAR). The latter was based on a selection model (Enders 2010), where factors related to attrition were indicators of missingness regressed on the outcome variables (Enders 2011). All models were constructed first for all participants and then separately for MZ and DZ twins. The differences in the regression coefficients between the twin

groups were tested using a Wald test for equality. A p value less than 0.05 was considered statistically significant.

Results

Baseline statistics

Baseline descriptive statistics for all participants and by zygosity are presented in Table 1. The MZ and DZ twins did not differ in age, basic anthropometrics, or sociodemographic factors. Smoking, prevalence of diseases, and use of prescriptive medication were similar in both twin groups. MMSE test scores were slightly higher in the MZ compared to DZ twins (27.3 vs. 26.8, $p = 0.033$). The CES-D test scores did not differ between the groups.

Mean LTL was 0.88 (0.18) in the MZ twins and 0.91 (0.20) in the DZ twins with no significant difference between the groups ($p = 0.37$).

Table 1 Subject characteristics at baseline

Variable	All ($n = 386$)	MZ ($n = 186$)	DZ ($n = 200$)	Between groups MZ vs. DZ
Age (years)	68.5 (3.4)	68.1 (3.7)	68.6 (3.1)	0.16
Height (m)	158.5 (6.2)	158.0 (6.5)	159.0 (5.8)	0.21
Body mass (kg)	69.9 (12.0)	69.3 (11.7)	70.4 (12.3)	0.44
BMI (kg/m ²)	27.9 (4.8)	27.9 (4.8)	27.9 (4.7)	0.99
Telomere length	0.90 (0.19)	0.88 (0.18)	0.91 (0.20)	0.34
Married or living together ($n, \%$)	205 (53.1)	104 (57.8)	101 (49.0)	0.11
Education (years)	8.6 (3.0)	8.7 (2.9)	8.5 (3.1)	0.62
Current smoking ($n, \%$)	21 (5.4)	8 (4.3)	13 (6.5)	0.42
Number of chronic diseases	2.0 (1.5)	2.0 (1.5)	2.0 (1.4)	0.92
Number of medications	2.0 (2.0)	1.8 (2.0)	2.2 (1.9)	0.065
Astma diagnosis ($n, \%$)	29 (7.5)	12 (6.5)	17 (8.5)	0.51
Diagnosed hypertension ($n, \%$)	142 (36.9)	66 (35.7)	76 (38.0)	0.65
Diagnosed type 2 diabetes ($n, \%$)	22 (5.7)	14 (7.6)	8 (4.0)	0.16
Musculoskeletal disease ($n, \%$)	164 (42.5)	85 (45.9)	79 (39.5)	0.27
MMSE	27.1 (2.2)	27.3 (2.1)	26.8 (2.2)	0.033
CES-D	10.5 (7.4)	10.8 (7.8)	10.2 (6.9)	0.54

Variables are presented in means and standard deviations unless indicated otherwise. Chronic diseases are self-reported; medications are prescriptive medicines

MZ monozygotic, DZ dizygotic, BMI body mass index, MMSE Mini-Mental State Examination, CES-D Center for Epidemiologic Studies Depression Scale

Attendance at the measurements

Of the 386 women with baseline data, 7 (2 %) died before the 3-year and 39 before the 11-year follow-up (10 %); 24 were MZ and 15 DZ individuals. Participants who died during follow-up were older ($p < 0.001$), had more chronic diseases ($p = 0.004$), and prescription medicines ($p = 0.003$) at baseline and scored lower (i.e., poorer cognition) in the MMSE ($p < 0.001$) compared to those who participated in the 11-year follow-up survey. No difference was observed in LTL ($p = 0.069$) or education ($p = 0.084$) at baseline between the deceased and surviving participants who attended the 11-year follow-up.

In total, 85 % of the participants performed the 6-min walking test at baseline. At the 3-year follow-up, 52 % of the total sample participated in the 6-min walking test. Participants who were not able to perform the walking test at the 3-year follow-up had a significantly lower walking test result at baseline compared to those who participated in both measurements ($p < 0.001$). They also had more chronic diseases ($p = 0.008$) and prescriptive medicines ($p = 0.008$).

At baseline, all participants answered the physical activity questionnaire. Of the participants, 97 and 73 % answered the PA questionnaire at the 3- and 11-year follow-ups, respectively.

Associations between LTL and physical functioning at baseline

Mean walking distance was 533 m in the MZ and 528 m in the DZ twins at baseline (between groups, $p = 0.87$,

Table 2). LTL was not associated with baseline walking distance (est. 5.26, SE 4.32, $p = 0.22$, Table 3). A borderline significant difference was observed between the MZ and DZ twins in the association between LTL and walking distance (heterogeneity, $p = 0.053$).

At baseline, 9 % of the participants were inactive and 19 % reported light activities once or twice per week. Light PA several times a week was reported by 33 % and moderate PA 1–2 times per week by 18 %. Moderate or high PA several times a week was reported by 22 %. Mean LTL varied between 0.83 and 0.99 in the different PA categories at baseline (Fig. 1). LTL was associated with baseline PA (est. 0.14, SE 0.06, $p = 0.011$), with no significant difference between the MZ and DZ twins ($p = 0.13$).

Associations between LTL and 6-min walking distance at the 3-year follow-up

At the 3-year follow-up, the MAR analysis for the total sample revealed no association between LTL and walking distance (est. 0.04 m/3.6 s, SE 0.04, $p = 0.300$, Table 4). The longitudinal association between LTL and walking distance did not differ significantly between the MZ and DZ twins ($p = 0.071$).

The MNAR analysis for all participants revealed a borderline significant association between LTL and the walking test result ($p = 0.060$) at the 3-year follow-up; the difference between MZ and DZ twins was not significant ($p = 0.26$).

Table 2 Physical functioning at baseline and after 3- and 11-year follow-up

	Baseline			3-year follow-up			11-year follow-up		
	All	MZ	DZ	All	MZ	DZ	All	MZ	DZ
<i>N</i>	386	186	200	374	181	193	282	135	147
Six-minute walking test (m)	530 (76)	533 (76)	528 (77)	530 (78)	535 (80)	525 (78)			
Activity level, <i>n</i> (%)									
Inactive	33 (9)	17 (9)	16 (8)	36 (10)	15 (8)	21 (11)	57 (20)	30 (22)	27 (18)
Light activity 1–2 a week	72 (19)	32 (17)	40 (20)	81 (22)	40 (22)	41 (21)	63 (22)	26 (19)	37 (25)
Light activity 1–2 times a week	128 (33)	56 (30)	72 (36)	117 (31)	55 (30)	62 (31)	71 (25)	32 (24)	39 (26)
Moderate activity 1–2 times a week	69 (18)	35 (19)	34 (17)	61 (16)	26 (14)	35 (18)	39 (14)	17 (13)	22 (15)
Moderate or high activity several time a week	84 (22)	46 (25)	38 (19)	79 (21)	45 (25)	34 (18)	52 (19)	30 (22)	22 (15)

Values are numbers and percentages of the total group of subjects or means with standard deviations (in 6-min walking test distance)

MZ monozygotic, DZ dizygotic

Table 3 Associations between baseline relative leukocyte telomere length and physical functioning in a path model adjusted for age and within-pair dependency of the twin pairs

Outcome	N	Total			Mz			Dz			Between groups MZ vs. DZ		
		MZ/DZ	Est.	SE	<i>p</i>	Est.	SE	<i>p</i>	Est.	SE	<i>p</i>	Est.	SE
Six-minute walking test (m)	163/173	5.26	4.32	0.223	15.47	6.62	0.019	-1.22	5.33	0.818	16.7	8.63	0.053
Physical activity	186/200	0.14	0.06	0.011	0.26	0.09	0.006	0.08	0.07	0.281	0.18	0.12	0.128

MZ monozygotic, DZ dizygotic, SE standard error

Associations between LTL and PA at the 11-year follow-up

The longitudinal MAR analysis indicated that in the total sample, LTL was not associated with level of PA at the 11-year follow-up (est. 0.03, SE 0.04, $p = 0.410$, Table 4). LTL did not predict PA level in either of the twin groups over the follow-up period.

The MNAR analysis for all participants revealed a statistically significant association between LTL and level of PA (est. 0.19, SE 0.06, $p = 0.001$) over the follow-up period. The MZ and DZ twins did not differ in the association between LTL and PA at the 11-year follow-up ($p = 0.23$).

Discussion

Missing data present a serious challenge in aging research. In longitudinal studies, death and loss to follow-up increase with age. In addition, health problems and functional limitations are more common in older age and interfere with all aspects of data collection. Missing data can bias results, reduce generalizability, and limit

statistical power. In the present study, we used advanced statistical methods to handle missing data that resulted from mortality and decreased physical functioning in a longitudinal design. We found that LTL predicted the level of physical activity at the 11-year follow-up in older twin sisters. A borderline significant association was also observed between baseline LTL and walking distance at the 3-year follow-up. These associations only became evident when we had taken into account the possibility that missing data were generated by a non-random mechanism. In sum, our results suggest that telomere shortening is a weak biomarker in age-related decline in physical functioning.

Cellular aging leads to heterogeneous changes in the composition and function of different tissues and organisms, to decrease regenerative capacity and to increase risk for diseases and mortality. These age-related changes are influenced by numerous genetic, epigenetic, and environmental factors, one of which is telomere erosion. The associations between telomeres, diseases, physiological aging, and decline in physical functioning are ambiguous. Telomere length has shown a modest association with several risk factors (Haycock et al. 2014; Willeit et al. 2014) and various chronic diseases (Zhao et al. 2013; Haycock et al. 2014; Albrecht et al. 2014). Shortened telomeres can result in cell cycle arrest, tumor suppression, and loss of functional tissue via senescence or apoptosis. Telomere length predicts mortality in person aged 60 years and older (Cherkas et al. 2008; Kimura et al. 2008) and current findings also suggest that LTL may predict years of healthy life (Njajou et al. 2009). In addition to genetics, individual differences in LTL can be explained by differences in telomerase expression, white blood cell turnover rate and attrition rate per replication, and amount of oxidative stress. Moreover, recent findings suggest that heritability and early life environment are the main determinants of LTL throughout the human life course (Hjelmborg et al.

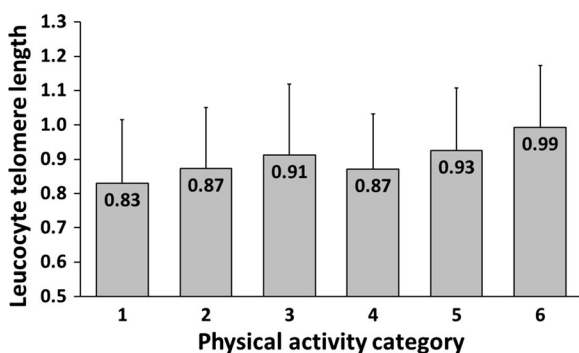


Fig. 1 Relative leukocyte telomere length in physical activity categories at baseline

Table 4 Relative leukocyte telomere length predicting physical functioning at 3- and 11-year follow-ups in a linear model adjusted for age and dependency owing to a longitudinal design nested within twin pairs

Outcome		Total			Mz			Dz			Group comparison MZ vs. DZ		
		Est.	SE	<i>p</i>	Est.	SE	<i>p</i>	Est.	SE	<i>p</i>	Est.	SE	<i>p</i>
Six-minute walking distance	MAR	0.04	0.04	0.300	0.12	0.06	0.040	-0.02	0.05	0.710	-0.14	0.08	0.071
	MNAR	0.14	0.07	0.060	0.24	0.11	0.031	0.11	0.07	0.136	-0.13	0.12	0.257
Physical activity	MAR	0.03	0.04	0.410	0.04	0.07	0.554	0.03	0.05	0.562	-0.01	0.08	0.869
	MNAR	0.19	0.06	0.001	0.25	0.09	0.004	0.13	0.07	0.055	-0.12	0.10	0.227

Six-minute walking distance divided by 100 for analysis; the age-adjusted unit of measurement is meters per 3.6 s

MZ monozygotic, *DZ* dizygotic, *SE* standard error, *MAR* missing at random, *MNAR* missing not at random

2015). Our data support the notion that LTL is a genetically determined biomarker of aging, and more specifically, a biomarker of age-induced change in physical functioning.

The present cross-sectional analysis showed that the participants with longer LTL had a higher level of PA. A similar relationship between LTL and PA has been reported earlier in middle-aged and older subjects (Cherkas et al. 2008; Bendix et al. 2011). Many studies have, however, either failed to confirm this association (Harris et al. 2006b; Cassidy et al. 2010) or reported an inverted U-curve association in which telomeres were longest in participants who were moderately active and shortest among those who were inactive or very active (Ludlow et al. 2008). Very few of the participants in this study were physically very active; thus, conclusions on whether faster telomere erosion is related to a high level of physical activity cannot be drawn. An association between LTL and PA is possible, but it cannot be estimated in all studies due to several confounding factors related to gene-environment interaction, lifespan, stress, and disease, as well as to the measurement techniques and methodology used in the analysis of both telomeres and physical activity.

We did not find an association between telomeres and walking distance in our older participants at baseline. Only a couple of studies have so far investigated associations between telomere length and walking ability in older people. In older Korean women ($n = 117$), LTL was independently associated with the time allotted in 6-m walking distance at their preferred walking speed (Lee et al. 2013), whereas Harris et al. (2006a) found no associations between LTL and 6 m walking in their elderly population, which included both men and women ($n = 190$). Gardner et al. (2013) found weak evidence that LTL is associated with walking speed in 3 to 6 m

walking tests. The previous studies that have estimated the association between LTL and walking have assessed walking speed over shorter (3 to 6 m) distances, whereas we used the total distance covered in the 6-min test at maximal speed. Shorter walking tests are commonly used in clinical practice to estimate individual's functional capacity. In contrast, the 6-min walking test can be used as an estimate of walking endurance and exercise capacity, and it is a useful tool to estimate the impact of multiple comorbidities and the development of disability (Enright 2003).

To date, only a couple of longitudinal papers have been published on the association between LTL and physical functioning (Gardner et al. 2013; Weischer et al. 2014). Our finding that in the total group of participants LTL at baseline was associated with PA at follow-up indicates that LTL is a biomarker of age-related decline in physical functioning. This association between LTL in walking distance was less clear. A lot of methodological difficulties due to selection bias hamper longitudinal studies investigating possible associations between telomeres and other age-related changes. All-cause mortality, changes in health, and decreased physical functioning are associated with telomere length, but shorter telomeres may also reduce the likelihood of the participants being (re)examined. The statistical methods generally used often assume that dropout is allocated randomly, although it is clear that participants lost in longitudinal aging studies have worse outcomes, even after adjustment for baseline characteristics (Hardy et al. 2009). Several advanced statistical methods have been used to analyze data missing due to death, but very few analytical techniques can handle both mortality and other nonrandom missingness. New statistical approaches and software (MPlus 7) allow more complex mixed-model types of analyses and missing data to be

handled in a flexible modeling framework. In this study, we analyzed the associations between LTL and physical functioning by assuming data both missing at random and MNAR. The advantages of MNAR modeling is that it allows us to take account of nonignorable missingness and also allows all the available data to be used in the analysis, thereby increasing statistical power. The method used to cope with missing data in the statistical analysis had a marked influence on the findings of this study. The MNAR analysis revealed that LTL predicted the level of PA at the 11-year follow-up. In addition, the association between LTL and walking distance at the 3-year follow-up was close to significant. These findings were not confirmed in the models where data were assumed to be missing at random. The dropout analysis confirmed that the missing data resulted from mortality, diseases, and low levels of physical functioning at baseline, which did not enable travel or participation in the either the follow-up measurements or specific functional tasks. Thus, it was clear that the participants with and without follow-up data were different, and therefore, an analysis based on the assumption of data missing at random would have led to biased results.

Genetic factors may regulate the association between LTL and age-related changes in physical functioning. In the present study, LTL was associated with baseline physical functioning and level of physical functioning at follow-up. Telomere length is highly dependent on genetic factors (Bischoff et al. 2005; Broer et al. 2013). A recent meta-analysis reported that the heritability rate of LTL is about 0.70 (Broer et al. 2013), but lower and higher estimates have also been presented, depending on, for example, the age of the population studied (Vasa-Nicotera et al. 2005; Andrew et al. 2006). Genetic factors can influence telomere length through several mechanisms: through initial telomere length and through the rate of telomere erosion and its effect on the degree of telomere attrition. In contrast, a smaller proportion of the variation observed in walking ability can be accounted for by genetic factors (Pajala et al. 2005). Genetic influences on walking distance, however, have been shown to increase with aging (Ortega-Alonso et al. 2006). The present findings suggest that the associations found between telomere length and physical functioning may be related to genetic factors, which explains why significant associations were evident in these pairs; however, there was only minor evidence for heterogeneity between MZ and DZ pairs. Further study is thus warranted to clarify the precise

contributions of genetic and familial and environmental influences on the association between telomere length and physical functioning among older people.

Our study provides novel information on the associations between LTL and physical functioning over 3- and 11-year follow-up periods. In this study, we estimated physical functioning by both standardized performance measures, also usable in clinical practice (6-min walking test), and validated self-reported questionnaire data (PA). Telomere length was determined from blood leukocytes, and hence may not correlate well with telomeres assayed from other cells such as myocytes which may be more involved in disability development. Our study was limited to older women within a rather narrow age range and without severe physical functioning limitation at baseline. Therefore, the results cannot be generalized to men or to persons with more severe functional limitations. In addition, we did not have information on the changes in LTL. It should be pointed out that, to address the question of whether LTL can be used as a biomarker of age-related physical functioning decline, knowledge is needed on the changes in LTL, taking confounding factors into account, that take place across the life course (Zhang et al. 2014).

In summary, our results extend the previous findings that longer LTL is associated with higher levels of PA. Our findings at the 3- and 11-year follow-ups indicate that LTL may be a biomarker of age-related changes in physical functioning. The longitudinal associations became evident when missing data, which resulted mainly from mortality and decreased physical functioning, was modeled nonrandomly. The possible associations of LTL with indicators of physical functioning and its decline are more likely related to genetic mechanisms.

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Compliance with ethical standards The study protocol has been approved by the Ethics Committee of the Central Hospital District of Central Finland (K-S shp: Dnro 24/2000)

References

- Ahola K, Siren I, Kivimaki M, Ripatti S, Aromaa A, Lonnqvist J, Hovatta I (2012) Work-related exhaustion and telomere length: a population-based study. *PLoS One* 7:e40186. doi:10.1371/journal.pone.0040186; 10.1371/journal.pone.0040186.
- Albrecht E, Sillanpaa E, Karrasch S, Alves AC, Codd V, Hovatta I, Buxton JL, Nelson CP, Broer L, Hagg S, et al. (2014) Telomere length in circulating leukocytes is associated with lung function and disease. *Eur Respir J* 43:983–992. doi:10.1183/09031936.00046213
- Andrew T, Aviv A, Falchi M, Surdulescu GL, Gardner JP, Lu X, Kimura M, Kato BS, Valdes AM, Spector TD (2006) Mapping genetic loci that determine leukocyte telomere length in a large sample of unselected female sibling pairs. *Am J Hum Genet* 78:480–486
- ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories (2002) ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med* 166:111–117. doi:10.1164/ajrccm.166.1.at1102
- Bean JF, Leveille SG, Kiely DK, Bandinelli S, Guralnik JM, Ferrucci L (2003) A comparison of leg power and leg strength within the InCHIANTI study: which influences mobility more? *J Gerontol A Biol Sci Med Sci* 58:728–733
- Bendix L, Gade MM, Staun PW, Kimura M, Jeune B, Hjelmborg JV, Aviv A, Christensen K (2011) Leukocyte telomere length and physical ability among Danish twins age 70+. *Mech Ageing Dev* 132:568–572. doi:10.1016/j.mad.2011.10.003; 10.1016/j.mad.2011.10.003.
- Bischoff C, Graakjaer J, Petersen HC, Hjelmborg J, Vaupel JW, Bohr V, Koelvraa S, Christensen K (2005) The heritability of telomere length among the elderly and oldest-old. *Twin Res Hum Genet* 8:433–439. doi:10.1375/183242705774310141
- Blackburn EH (2005) Telomerase and Cancer: Kirk A. Landon–AACR prize for basic cancer research lecture. *Mol Cancer Res* 3:477–482
- Broer L, Codd V, Nyholt DR, Deelen J, Mangino M, Willemsen G, Albrecht E, Amin N, Beekman M, de Geus EJ, et al. (2013) Meta-analysis of telomere length in 19,713 subjects reveals high heritability, stronger maternal inheritance and a paternal age effect. *Eur J Hum Genet* 21:1163–1168. doi:10.1038/ejhg.2012.303
- Cassidy A, De Vivo I, Liu Y, Han J, Prescott J, Hunter DJ, Rimm EB (2010) Associations between diet, lifestyle factors, and telomere length in women. *Am J Clin Nutr* 91:1273–1280. doi:10.3945/ajcn.2009.28947
- Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA (2003) Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* 361:393–395. doi:10.1016/S0140-6736(03)12384-7
- Cherkas LF, Hunkin JL, Kato BS, Richards JB, Gardner JP, Surdulescu GL, Kimura M, Lu X, Spector TD, Aviv A (2008) The association between physical activity in leisure time and leukocyte telomere length. *Arch Intern Med* 168:154–158. doi:10.1001/archinternmed.2007.39
- Enders CK (2010) Applied missing data analysis. Guilford Press, New York
- Enders C (2011) Missing not at random models for latent growth curve analyses. *Psychol Methods* 16:1–16. doi:10.1037/a0022640
- Enright PL (2003) The six-minute walk test. *Respir Care* 48:783–785
- Folstein MF, Folstein SE, McHugh PR (1975) “Mini-mental state”: a practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 12:189–198. doi:10.1016/0022-3956(75)90026-6
- Gardner MP, Martin-Ruiz C, Cooper R, Hardy R, Sayer AA, Cooper C, Deary IJ, Gallacher J, Harris SE, Shiels PG, et al. (2013) Telomere length and physical performance at older ages: an individual participant meta-analysis. *PLoS One* 8:e69526. doi:10.1371/journal.pone.0069526
- Grimby G (1986) Physical activity and muscle training in the elderly. *Acta Med Scand Suppl* 711:233–237
- Hardy SE, Allore H, Studenski SA (2009) Missing data: a special challenge in aging research. *J Am Geriatr Soc* 57:722–729. doi:10.1111/j.1532-5415.2008.02168.x
- Harris SE, Deary IJ, MacIntyre A, Lamb KJ, Radhakrishnan K, Starr JM, Whalley LJ, Shiels PG (2006a) The association between telomere length, physical health, cognitive ageing, and mortality in non-demented older people. *Neurosci Lett* 406:260–264
- Harris SE, Deary IJ, MacIntyre A, Lamb KJ, Radhakrishnan K, Starr JM, Whalley LJ, Shiels PG (2006b) The association between telomere length, physical health, cognitive ageing, and mortality in non-demented older people. *Neurosci Lett* 406:260–264
- Haycock PC, Heydon EE, Kaptoge S, Butterworth AS, Thompson A, Willeit P (2014) Leucocyte telomere length and risk of cardiovascular disease: systematic review and meta-analysis. *BMJ* 349:g4227. doi:10.1136/bmj.g4227
- Hjelmborg JB, Dalgard C, Moller S, Steenstrup T, Kimura M, Christensen K, Kyvik KO, Aviv A (2015) The heritability of leucocyte telomere length dynamics. *J Med Genet* 52:297–302. doi:10.1136/jmedgenet-2014-102736
- Kaprio J, Koskenvuo M (2002) Genetic and environmental factors in complex diseases: the older Finnish Twin Cohort. *Twin Res* 5:358–365. doi:10.1375/136905202320906093
- Kaprio J, Sarna S, Koskenvuo M, Rantasalo I (1978) The Finnish Twin Registry: formation and compilation, questionnaire study, zygosity determination procedures, and research program. *Prog Clin Biol Res* 24 Pt B:179–184.
- Kimura M, Hjelmborg JV, Gardner JP, Bathum L, Brimacombe M, Lu X, Christiansen L, Vaupel JW, Aviv A, Christensen K (2008) Telomere length and mortality: a study of leukocytes in elderly Danish twins. *Am J Epidemiol* 167:799–806. doi:10.1093/aje/kwm380; 10.1093/aje/kwm380.
- Lee JY, Bang HW, Ko JH, Kim JH, Lee DC (2013) Leukocyte telomere length is independently associated with gait speed in elderly women. *Maturitas* 75:165–169. doi:10.1016/j.maturitas.2013.03.008
- Ludlow AT, Zimmerman JB, Witkowski S, Hearn JW, Hatfield BD, Roth SM (2008) Relationship between physical activity level, telomere length, and telomerase activity. *Med Sci Sports Exerc* 40:1764–1771. doi:10.1249/MSS.0b013e31817c92aa
- Mather KA, Jorm AF, Parslow RA, Christensen H (2011) Is telomere length a biomarker of aging? A review. *J Gerontol A Biol Sci Med Sci* 66:202–213. doi:10.1093/gerona/gql180

- Mundstock E, Zatti H, Louzada FM, Oliveira SG, Guma FT, Parisi MM, Rueda AB, Machado DG, Stein RT, Jones MH, et al. (2015) Effects of physical activity in telomere length: systematic review and meta-analysis. *Ageing Res Rev* 22:72–80
- Muthén LK, Muthén BO (1998–2012) *Mplus User's Guide*. Los Angeles, CA, Muthén & Muthén
- Newman AB, Simonsick EM, Naydeck BL, Boudreau RM, Kritchevsky SB, Nevitt MC, Pahor M, Satterfield S, Brach JS, Studenski SA, et al. (2006) Association of long-distance corridor walk performance with mortality, cardiovascular disease, mobility limitation, and disability. *JAMA* 295: 2018–2026
- Njajou OT, Hsueh WC, Blackburn EH, Newman AB, Wu SH, Li R, Simonsick EM, Harris TM, Cummings SR, Cawthon RM, et al. (2009) Association between telomere length, specific causes of death, and years of healthy life in health, aging, and body composition, a population-based cohort study. *J Gerontol A Biol Sci Med Sci* 64:860–864. doi:10.1093/gerona/ glp061
- Ortega-Alonso A, Pedersen NL, Kujala UM, Sipilä S, Törmäkangas T, Kaprio J, Koskenvuo M, Rantanen T (2006) A twin study on the heritability of walking ability among older women. *J Gerontol Ser A Biol Med Sci* 61: 1082–1085
- Pajala S, Era P, Koskenvuo M, Kaprio J, Alen M, Tolvanen A, Tiainen K, Rantanen T (2005) Contribution of genetic and environmental factors to individual differences in maximal walking speed with and without second task in older women. *J Gerontol A Biol Sci Med Sci* 60:1299–1303
- Radloff LS (1977) The CES-D Scale: a self-report depression scale for research in the general population. *Appl Psychol Meas* 1: 385–401. doi:10.1177/014662167700100306
- Rantakokko M, Manty M, Rantanen T (2013) Mobility decline in old age. *Exerc Sport Sci Rev* 41:19–25. doi:10.1097/JES. 0b013e3182556fle
- Sarna S, Kaprio J, Sistonen P, Koskenvuo M (1978) Diagnosis of twin zygosity by mailed questionnaire. *Hum Hered* 28:241–254
- Tiainen K, Pajala S, Sipilä S, Kaprio J, Koskenvuo M, Alen M, Heikkinen E, Tolvanen A, Rantanen T (2007) Genetic effects in common on maximal walking speed and muscle performance in older women. *Scand J Med Sci Sports* 17:274–280. doi:10.1111/j.1600-0838.2006.00553.x
- Tiainen K, Sipilä S, Alen M, Heikkinen E, Kaprio J, Koskenvuo M, Tolvanen A, Pajala S, Rantanen T (2004) Heritability of maximal isometric muscle strength in older female twins. *J Appl Physiol* 96:173–180. doi:10.1152/jappphysiol.00200. 2003
- Vasa-Nicotera M, Brouillette S, Mangino M, Thompson JR, Braund P, Clemitson JR, Mason A, Bodycote CL, Raleigh SM, Louis E, et al. (2005) Mapping of a major locus that determines telomere length in humans. *Am J Hum Genet* 76: 147–151
- von Zglinicki T, Martin-Ruiz CM (2005) Telomeres as biomarkers for ageing and age-related diseases. *Curr Mol Med* 5:197–203
- Weischer M, Bojesen SE, Nordestgaard BG (2014) Telomere shortening unrelated to smoking, body weight, physical activity, and alcohol intake: 4,576 general population individuals with repeat measurements 10 years apart. *PLoS Genet* 10:e1004191. doi:10.1371/journal.pgen.1004191
- Willeit P, Raschenberger J, Heydon EE, Tsimikas S, Haun M, Mayr A, Weger S, Witztum JL, Butterworth AS, Willeit J, et al. (2014) Leucocyte telomere length and risk of type 2 diabetes mellitus: new prospective cohort study and literature-based meta-analysis. *PLoS One* 9:e112483. doi: 10.1371/journal.pone.0112483
- Zhang WG, Zhu SY, Bai XJ, Zhao DL, Jian SM, Li J, Li ZX, Fu B, Cai GY, Sun XF, et al. (2014) Select aging biomarkers based on telomere length and chronological age to build a biological age equation. *Age (Dordr)* 36:9639. doi:10.1007/s11357- 014-9639-y
- Zhao J, Miao K, Wang H, Ding H, Wang DW (2013) Association between telomere length and type 2 diabetes mellitus: a meta-analysis. *PLoS One* 8:e79993. doi:10.1371/journal.pone. 0079993