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Leukocyte Telomere Length and Physical Ability among Danish Twins age 70+

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Abstract

Leukocyte telomere length (LTL) shortens with age and is potentially a biomarker of human aging.

We examined the relation of LTL with physical ability and cognitive function in 548 same-sex twins from the Longitudinal Study of Aging Danish Twins. LTL was measured by Southern blots of the terminal restriction fragments (TRF). Physical ability was evaluated using a self reported scale of 11 questions, while cognitive function was scored by MMSE and a cognitive composite score sensitive to age-related decline.

A random intercept model revealed a positive, significant association between LTL and physical ability. For every unit increase in physical ability score, LTL increased by 0.066 kb ($p=0.01$), equal to approximately three years of age-dependent LTL shortening. A matched case-co-twin design showed that the group consisting of the twins from each pair with the longer LTL also displayed better physical ability ($p<0.01$). Moreover, the intra-pair difference in LTL was associated with intra-pair difference in physical ability ($p<0.01$), confirming the association. However, we found no association between cognitive function and LTL.

The LTL-physical ability association in the elderly provides further support to the premise that LTL is an index of somatic fitness in the narrow context of human physical health.

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1 Introduction

Telomeres are specialized nucleoprotein structures at the end of chromosomes. They provide an important buffer for gene-coding regions, since cell replication entails the loss of nucleotides from both ends of the DNA strands due to the end replication problem (Olovnikov 1973). Leukocyte telomere length (LTL) undergoes age-dependent shortening, reflecting replication of hematopoietic stem cells (HSCs). As epidemiological studies have often found that LTL is associated with indices of aging (Benetos et al. 2001; Martin et al. 2004; Okuda et al. 2000; Panossian et al. 2003; Zhai et al. 2006; Zhang et al. 2003), it seems that LTL might serve as a biomarker of human aging.

Both physical ability and cognitive function decline with age (Andersen-Ranberg et al. 1999; Starr et al. 1997) and are closely associated with morbidity and mortality (Andersen et al. 2002; Cesari et al. 2008; Harris et al. 1989; Lee et al. 2006). The inter-individual variation in the absolute decline as well as rate of decline of physical function and cognitive performance with age, is due to both genetic and environmental factors (Johnson et al. 2009; McGue and Christensen 2002). Reduced cognitive function, physical function, and some aging-related diseases are thought to relate in part to the cumulative burden of oxidative stress stemming from chronic, low-grade inflammation. Because oxidative stress augments telomere shortening with each replication (Von Zglinicki et al. 1995) and chronic inflammation increases the demand for more replication of HSCs, it is plausible that impaired cognitive function as well as physical ability should be associated with LTL shortening. As such, LTL might serve as a biomarker of the general decline in somatic fitness seen with normal aging.

Few studies have explored the association of LTL with indicators of physical function. In these studies (including 190 79-years old (Harris et al. 2006), 646 individuals of 44–70 years of age (Mather et al. 2010b) and 1048 70-years old (Harris et al. 2010))) no significant association was observed between LTL and physical function (evaluated by dynamometer grip strength, time taken to walk six meters and FEV₁). The same groups also observed no association of LTL with cognitive level or with changes in cognitive performance during life (Harris et al. 2006; Harris et al. 2010; Mather et al. 2010a). However, a large study of community dwelling elders (N=2734) (Yaffe et al. 2009) as well as a study of 382 women (Valdes et al. 2010) concluded that cognitive performance correlated significantly with LTL. These studies cover a broad age range and cognitive function has been assessed in various ways. This possibly explains the contradictory findings, justifying more studies that address potential links of LTL with indices of physical function and cognition.

Inconsistent results have also previously been reported on the association between LTL and mortality (Bischoff et al. 2006; Cawthon et al. 2003; Fitzpatrick et al. 2011; Kimura et al. 2008; Martin-Ruiz et al. 2005; Njajou et al. 2009). This can in part be explained by the vast variety of mortality causes, not all necessarily being an outcome of a result of the general bodily decline, but could also be due to methodological constraints related to telomere length measurements. We have previously found association of LTL with mortality (Kimura et al. 2008) and with perceived age (Christensen et al. 2009) using refined telomere length measurements by Southern blots and capitalizing on intra-pair twin comparisons, which control for genetic factors and rearing environment. The present study employs the same powerful approach to test whether shorter LTL is associated with diminished physical ability and cognitive performance.

2 Material and Methods

2.1 Description of twin sample

The Longitudinal Study of Aging Danish Twins (LSADT) began in 1995 with an assessment of members of same-sex twin pairs born in Denmark before 1920 (McGue and Christensen 2007). In 1997 a total of 2174 twins from the LSADT participated in a comprehensive face-to-face interview. The interview included detailed questions about physical ability, alcohol consumption, smoking, height, and weight as well as assessment of the participants' cognitive performance (Andersen et al. 2002; Frederiksen et al. 2002). Blood samples were collected from 283 twin pairs and of these, 274 pairs (548 individuals) had their LTL measured (Kimura et al. 2008). For further information on the participants, see Table 1 (for the 548 individuals who had LTL measured using the HphI/MnII restriction digest, see below) and Supplement Table S1 (for the 476 individuals who had LTL measured using the HinFI/RsaI restriction digest).

The study has been approved by the scientific-ethical committee for Vejle and Funen counties and all participants provided written informed consent.

2.2 LTL measurement

Leukocyte DNA was extracted using standard techniques (Bischoff et al. 2005). DNA quality was assessed by electrophoresis. Telomere length was measured using Southern blots of terminal restriction fragments after digestion with HphI and MnII as well as with HinFI and RsaI restriction enzymes as previously described (Kimura et al. 2010). The two LTL measures correlate strongly ($r=0.88$, $p=0.000$), the HphI/MnII products resulting in lengths that are on average 1.1kb shorter than the HinFI/RsaI products.

Each sample was measured in duplicate (performed on different gels) and the mean of the two samples was used (in those cases where one sample was discarded due to technical matters, the mean was based on only one sample. This was the case in 134 out of 548 for the HphI/MnII digest, and in 144 out of 476 for the HinFI/RsaI digest.). The inter-assay coefficient of variation was 3.4% for the HphI/MnII digest and 2.5% for the HinFI/RsaI digest. The samples from twin pairs were run side by side on the same gel.

2.3 Physical ability score

Physical ability was evaluated using a self reported strength scale of 11 questions to calculate a physical ability score. Each question was rated on a scale from 1 to 4 points. 1 = can do the activity without fatigue, 2 = can do the activity with fatigue or minor difficulties, 3 = can do the activity with aid or major difficulties, 4 = cannot do the activity. The result of the scale is five subtracted the average score of the 11 individual answers. Examples of the 11 questions are: Can you: "walk up two flights of stairs", "walk around the house" "run 100 meters" and "carry 5 kg". A high strength score indicates a better physical ability. The physical ability scale has high consistency and reliability (Christensen et al. 2000).

2.4 Physical activity

A five point scale of physical activity was deducted from self reported level and frequency of physical activity. The answers were scored as: 0 - "no physical activity", 1 - "seldom low intensity physical activity", 2 - "frequent low intensity physical activity", 3 - "seldom high and frequent low intensity physical activity" and 4 - "frequent high intensity physical activity".

2.5 Cognitive performance scores

MMSE: This is an international standard 30-point questionnaire that is used to screen for cognitive impairment. The functions that are tested are orientation, memory, learning, attention and arithmetic. A score from 24–30 points reflects a normal cognitive function; a score of 18–23 is considered a mild cognitive impairment, while 0–17 points is considered severe cognitive impairment (Tombaugh and McIntyre 1992).

Cognitive composite: Because the MMSE is used primarily to identify cognitive impairment, cognitive function was also assessed using standardized scores on five individual cognitive measures. These were selected to represent tasks that are sensitive to normative age changes and that can be briefly and reliably assessed by lay interviewers. The specific tasks were 1) a verbal fluency test (number of animals named in one min); 2) forward and 3) backward digit span; and 4) immediate and 5) delayed recall of a 12-item list. All tests were inter correlated; the average correlation was .34. The standard scores for each task were summed to give an overall score. A higher cognitive composite score indicates a better cognitive function (McGue and Christensen 2001; McGue and Christensen 2002).

2.6 Statistical methods

To test if mental and physical factors were associated with LTL we applied a multiple regression model with y as the outcome and a, b, c,... as independent covariates. The random intercept regression model is considered to account for non-independence within pairs (Christensen et al. 2001; Wu 2010). Effects of interactions among covariates are checked and the model with the best parsimonious fit to data is selected. The assumption of normality in distribution of residuals was tested by standard methods and was not found violated. In case of doubt the appropriate stabilizing transformation of outcome was tested and found to show similar results. No outliers were detected from diagnostic plots. Possible association was further studied using an intra-pair twin design in which difference in LTL was regressed on difference in explanatory variables. Stata 11 was used for the analysis (Stata Corp, College Station, Texas, USA)

3 Results

Of the 548 twins (90 pairs of men, 184 pairs of women), 153 pairs were dizygotic (DZ) and 121 pairs were monozygotic (MZ). Descriptive statistics of participants are presented in Table 1 (and S1). The mean ages of the participants were 78.3 years for men (range 73 – 88) and 79.0 years for women (range 73 – 94). Women were found to have longer LTL than men (age adjusted: 4.63kb and 4.45kb, respectively). (We present data from the HphI/MnII digest, however, comparable results were obtained with HinfI/RsaI digest. These results can be found in the Supplementary tables.)

Men achieved higher physical ability scores compared to women (age adjusted: 3.21 and 3.15, respectively) although this was not significant. In total 23/548 (4.2%) individuals had a MMSE score <18 indicating cognitive impairment and 55/548 (10.0%) individuals had a MMSE of 18–23 indicative of mild cognitive impairment.

3.1 The best fitting random intercept model

The best fitting random intercept model revealed a positive association between LTL and physical ability when adjusting for effects of sex and age and taking into account that the co-twins are not independent. For every unit increase in physical ability score, LTL increases by 0.066 kb (See table 2 and S2). When stratifying for sex, the association was attenuated for men, by a factor 2 to 0.033kb (p=0.48). The association between LTL and physical

ability was not attenuated when stratifying by zygosity although it no longer reaches significance (See Table 2 and S2).

No association between LTL and cognitive function was found (See Table 2 and S2). There was also no association when excluding individuals with cognitive impairment (MMSE score < 24) (data not shown).

LTL associated negatively with age (an attrition of 0.025 kb/year). We further found that LTL was negatively associated to smoking status for the MnlI/HphI digest (Table 2). The association was smaller and did not reach significance for the HinfI/RsaI digest (Table S2). Smoking status was not associated to physical ability and neither influenced, nor explained, the association between LTL and physical ability. Neither did stratifying by smoking status alter the association (data not shown). There was no association to BMI or alcohol consumption in this population (data not shown). These covariates were evaluated since the literature has suggested that they could be associated with LTL. In the final model only sex and age were included as confounders, due to the lack of association of the other covariates.

We found a strong correlation between physical ability and physical activity level ($r=0.78$, $p=0.000$). We also found a positive association between physical activity level and LTL (Coefficient: 0.028 kb/point, $p=0.034$). The association between physical ability and LTL was slightly reduced and no longer significant when taking physical activity level into account (Coefficient was diminished from 0.066 kb/point to 0.055 kb/point, $p=0.142$), suggesting that physical activity level explains a smaller fraction of the association.

3.2 Matched case-co-twin design

The same-sex twin design of this study can be exploited to minimize the impact of age and sex and laboratory measurement errors such as batch variation. We have done this by grouping the twins by LTL so that group 1 consists of the co-twins who had the longer LTL and group 2 consists of the co-twins who had the shorter LTL. A comparison of the co-twins with the longer LTL with their co-twins with the shorter LTL showed a positive association between LTL and physical ability ($P<0.01$) (Table 3 and 3S). The same trend was found separately in men ($p=0.066$, $N=90$) and women ($p=0.0341$, $N=184$); the association was also maintained when analyzing MZ and DZ twins separately.

We also examined whether the magnitude of the intra-pair difference in LTL was associated with that of the intra-pair difference in the physical ability score. For every one unit increase in physical ability score within a pair, LTL increased by 0.086 kb ($p=0.005$); however, including sex as covariate diminished this association (Coefficient = 0.028 kb, $p=0.131$). Using the same approach, we again found no association between LTL and cognitive function.

4 Discussion

The aim of this study was to test whether LTL in elderly Danish Twins is associated with physical ability and cognitive performance. We hypothesized that twins with shorter LTL had poorer physical ability and lower cognitive performance. The best fitting random intercept model indicated that when physical ability increased by one unit, LTL increased by 0.066 kb, equivalent to approximately three years of age-dependent LTL shortening. The intra twin-pair analysis of the difference in LTL vs. difference in physical ability further confirmed the finding. We undertook the same approach in a previous study exploring the relation between LTL and mortality in this cohort (Kimura et al. 2008). However, we found no association between LTL and cognitive performance in this cohort.

Harris et al. (2006 and 2010) and Mather et al (2010b) found no correlation between LTL and physical health. The reason for the discrepancy between our study and previous studies may be due to different approaches of evaluating physical ability. We have used a self-reported scale of 11 questions to evaluate the physical ability of the participating twins, a scale that has shown to have high consistency and reliability (Christensen et al. 2000). Further, we used the Southern blot method to measure LTL, which, in this cohort, had an inter-assay CV of 3.4% (Kimura et al. 2008) while other groups have used qPCR based methods, reporting a CV as high as 8.8% (Mather et al. 2010b). Since the reported differences are small, results could be missed with a higher CV.

Valdes et al (2010) and Yaffe et al (2009) found that cognitive function was significantly correlated with LTL, concluding that LTL might be a potential biomarker to detect individuals at risk of cognitive decline. We, however, found no significant association between LTL and cognitive function, consistent with Harris et al. (2006 and 2010) and Mather et al (2010a). This was still true when using the co-twin design, as well as when excluding individuals with cognitive impairment defined as MMSE <24. The discrepancies between these studies might relate to the different ages of participants in the studies. Our study has focused on an aged population with a different demography than the other studies. Further the use of different test-instruments could also contribute to the discrepancies found.

Selection bias might also be a problem; an elderly person with cognitive impairment might not be interested in participating in a comprehensive face to face interview, let alone agree to donate a blood sample. This could result in a study population that is too homogenous and a possible type 2 error. Still, 14.2% of our subjects were cognitively impaired. Finally, the blood samples were drawn approximately half a year after the interview likely causing an underestimation of an association.

A dizygotic intra-pair comparison controls for rearing environment and on average 50% of the genetic material, whereas a monozygotic intra-pair comparison control for 100% genetic factors and rearing environment. Since none of the associations we observed were attenuated by zygosity, the association was unlikely to be driven by genetic factors or early common environment (Osler et al. 2007) Being aware that the statistical power of such an assumption is limited in this study due to the relative small sample size, we turned to examining a potential influence of late environmental factors, namely smoking and physical activity.

Oxidative stress is thought to be a major contributor to cellular ageing according to the free radical theory (Harman 1992). Further evidence suggests that oxidative damage can accelerate shortening of telomeres (Starr et al. 2008; Von Zglinicki 2000). One major contributor of extrinsic oxidative stress is smoking (Church and Pryor 1985). We found that smoking was negatively correlated to LTL, but not to physical ability and did thus not explain the association.

Our physical ability score is a composite mostly of measures of “able to do” and only briefly considers if a person is doing exercise. It thus does not take the frequency level into account. Since we had information on frequency and intensity of physical activity we generated a scale of the physical activity level, enabling us to evaluate if our findings could be explained by physical activity affecting both LTL and physical ability. Physical activity interacts with physical ability (Chin et al. 2008) and has been shown to correlate with LTL in a hormesis like fashion, with moderate physical active individuals showing the longest LTL (Cherkas et al. 2008; Ludlow et al. 2008). It has been suggested that this association can be due to the up-regulation of telomerase activity or due to an influence on the immune system (Werner et al. 2009). Our study confirmed that physical activity correlated with both physical ability and LTL. The association between physical ability and LTL was slightly diminished when

taking physical activity into account, suggesting that the level of physical activity partly explains this association.

In conclusion, our findings are in line with the LTL-longevity nexus in humans. Since multiple genetic and environmental factors impact LTL, individually, each of these factors appears to have only a small to modest effect on LTL. Clearly, the links between LTL and human longevity are mediated via a complex network of interacting factors, which is unlikely to be as complicated as aging itself, given that aging is the most complex of all complex traits. From this standpoint, deciphering the factors that fashion LTL will take us a long way towards understanding the biology of human aging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Leukocyte telomere length (LTL) is associated to physical ability in elderly Danish Twins
- LTL is not associated to cognitive function in our study
- LTL is associated to physical activity

Table 1

General characteristics of studied population. Data are given as mean (SE) unless stated.

Characteristics	Total (N=548)	Female (N=368)	Male (N=180)	p-value (sex diff)
LTL MnlI/HphI (kb)	4.57 (0.02)	4.62 (0.03)	4.46 (0.04)	<0.001
<i>Age adjusted</i>		4.63 (0.04)	4.45 (0.05)	
Age (years)	78.8 (0.18)	79.0 (0.23)	78.3 (0.27)	
Zygosity				
Monozygotic (N)	242	164	78	
Dizygotic (N)	306	204	102	
Smoking				
Current	152 (28%)	91 (25%)	61 (34%)	
Past	198 (36%)	110 (30%)	88 (49%)	
Never	197 (36%)	167 (45%)	30 (17%)	
Physical activity level	2.22 (0.06)	2.19 (0.08)	2.28 (0.11)	0.511
<i>Age adjusted</i>		2.21 (0.07)	2.23 (0.11)	
Score 0 (N)	120 (22%)	83 (23%)	37 (21%)	
Score 1 (N)	22 (4%)	15 (4%)	7 (4%)	
Score 2 (N)	186 (34%)	124 (33%)	62 (34%)	
Score 3 (N)	57 (10%)	41 (11%)	16 (9%)	
Score 4 (N)	163 (30%)	105 (29%)	58 (32%)	
Physical ability Score	3.17 (0.03)	3.13 (0.04)	3.25 (0.05)	0.098
<i>Age adjusted</i>		3.15 (0.04)	3.21 (0.06)	
Cognitive function				
MMSE Score	26.01 (0.15)	25.89 (0.19)	26.26 (0.26)	0.21
<i>Age adjusted</i>		25.88 (0.19)	26.12 (0.29)	
Cognitive composite	1.14 (0.14)	1.27 (0.17)	0.87 (0.25)	0.24
<i>Age adjusted</i>		1.26 (0.19)	0.75 (0.30)	

Table 2

Univariate regression coefficients for LTL and physical ability and cognitive function, as well as for smoking status and physical activity level. Coefficients are adjusted for sex and age as well as twin-pair dependency.

LTL <i>MnII/HphI</i>	Coef	SE	P
Sex			
Male	Ref		
Female	.178	.063	.005
Age	-.025 ^a	.007	.001
Physical ability			
Total	.066	.026	.011
Women	.079	.031	.012
Men	.033	.047	.48
DZ	.065	.035	.063
MZ	.061	.037	.10
Cognitive function			
MMSE	.006	.005	.246
Cognition	.007	.006	.278
Smoking			
Current	Ref		
Former	.039	.047	.409
Never	.106	.050	.035
Physical activity	.028	.013	.034

The coefficients are expressed in kb per point on the respective scales. E.g

^a the coefficient is equal to an attrition in LTL of 0.025 kb per year.

Table 3

Association between LTL and physical ability within twin pairs. Twins of each pair divided in two groups by LTL.

	All (N=274)	Men (N=90)	Women (N=184)	DZ (N=153)	MZ (N=121)
Group 1: Longest LTL	3.25	3.32	3.21	3.16	3.35
Group 2: Shortest LTL	3.10	3.18	3.06	3.03	3.19
Difference (SE)	0.148 (.053)	0.148 (.079)	0.148 (.069)	0.133 (.075)	0.167 (.075)
p-value (Group 1 vs. 2)	0.0059	0.066	0.034	0.079	0.027