Shorter telomere length is linked to brain atrophy and white matter hyperintensities

MIKAEL WIKGREN1, THOMAS KARLSSON2, HEDVIG SÖDERLUND3, ANNELIE NORDIN1, GÖRAN ROOS4, LARS-GÖRAN NILSSON5, ROLF ADOLFSSON1, KARL-FREDRIK NORRBACK1

1Department of Clinical Sciences, Division of Psychiatry, Umeå University, Umeå 90187, Sweden
2Department of Behavioral Sciences and Learning, Disability Research Division, Linköping University, Linköping, Sweden
3Department of Psychology, Uppsala University, Uppsala, Sweden
4Department of Medical Biosciences, Division of Pathology, Umeå University, Umeå, Sweden
5Department of Psychology, Stockholm University, Stockholm, Sweden

Address correspondence to: Mikael Wikgren. Tel: +46703038942; Fax: +4690135324. Email: mikael.wikgren@psychiat.umu.se

Abstract

Background: leukocyte telomere length (TL) is considered a marker of biological aging. Several studies have investigated the link between leukocyte TL and aging-associated functional attributes of the brain, but no prior study has investigated whether TL can be linked to brain atrophy and white matter hyperintensities (WMHs); two prominent structural manifestations of brain aging.

Methods: we investigated whether leukocyte TL was related to brain atrophy and WMHs in a sample of 102 non-demented individuals aged 64–75 years.

Results: shorter TL was related to greater degree of subcortical atrophy ($\beta = -0.217$, $P = 0.034$), but not to cortical atrophy. Furthermore, TL was 371 bp shorter ($P = 0.041$) in participants exhibiting subcortical WMHs, and 552 bp shorter ($P = 0.009$) in older participants exhibiting periventricular WMHs.

Conclusion: this study provides the first evidence of leukocyte TL being associated with cerebral subcortical atrophy and WMHs, lending further support to the concept of TL as a marker of biological aging, and in particular that of the aging brain.

Keywords: brain atrophy, older people, telomere length, white matter hyperintensities

Introduction

With aging, the brain undergoes several macrostructural changes. Atrophy of the brain begins at a slow rate already in early adulthood and accelerates with increasing age. Cerebral atrophy is considered part of the normal aging process of the brain, but greater extent and higher rates of atrophy are associated with cognitive decline and dementia. Atrophy is global, but can progress at different rates in different regions of the brain [1]. Another macrostructural change of the brain associated with aging is white matter hyperintensities (WMHs). WMHs appear as bright hyperintense foci on T2-weighted magnetic resonance images (MRI), and as hypodense areas on T1-weighted MRI and computer tomography scans. WMHs represent several different changes in the white matter; for instance, these changes comprise partial loss of myelin, oligodendroglia, and axons, fibrohyalinosis, activation of macrophages and dilated perivascular spaces [2]. This spectrum of alterations is often seen as manifestations of incomplete infarcts. Even though WMHs are considered part of normal aging (occurring in over 90% of older individuals), they are associated with increased risks of dementia and stroke. Vascular risk factors, such as high blood pressure, are believed to be important factors underlying atrophy and development of WMHs [3].

A growing body of literature brings forward leukocyte telomere length (TL) as a candidate biomarker of aging. Telomeres are the specialised structures forming the end parts of eukaryotic chromosomes, consisting of tandemly repeated short stretches of DNA (TTAGGG in humans) and associated nucleoproteins. Due to the so-called end replication problem, telomeres are inescapably shortened with each chromosomal replication event, subsequently leading to telomere shortening in actively dividing tissues [4]. Only a small fraction of the observed telomere loss can be attributed to the end replication problem, instead cellular environmental
Factors such as inflammation and oxidative stress are important factors contributing to telomere loss. Critically short telomeres will trigger cellular senescence or apoptosis. The insights that TL to a degree reflects past replicative history and determine future potential, and reflects the cell's cumulative exposure to inflammation and oxidative stress, led to the idea that TL is an index of biological aging [5]. Measures of TL across different tissues have shown that there is synchronicity across tissues, meaning that relatively long TL in one tissue is accompanied by relatively long TL in another tissue in the same individual [6]. For this reason, leukocyte TL may not only be an index of cumulative systemic oxidative stress and inflammation but also a surrogate measure of relative TL in other tissues. Studies in leukocytes have linked TL, independent of age, with several aging-related disorders and mortality; for example, cardiovascular disease [7], osteoporosis [8] and cancer [9]. However, the findings are not always consistent; some studies fail to find a link between TL and mortality or aging-associated parameters such as cognitive deficits [10, 11].

Regarding the aging of the brain, leukocyte TL has been associated with dementia and cognition [12, 13], but little is known concerning TL’s relationship with the structural aspects of the brain. Currently, no studies have investigated whether leukocyte TL is linked to brain atrophy or WMHs.

We measured leukocyte TL and used MRI to assess brain atrophy and WMHs in 102 non-demented community-dwelling subjects. Our aim was to investigate whether brain atrophy and WMHs were associated with leukocyte TL.

Methods

Study participants

The study sample consisted of the Swedish subsample of the CASCADE study. The CASCADE (Cardiovascular Determinants of Dementia) study is a European multicenter study aimed at studying cardiovascular risk factors for WMHs and atrophy, and their impact on cognition [14]. The Swedish sample was recruited from the Betula Study, a prospective cohort study investigating various aspects of memory, health and aging [15]. Recruitment of participants from the Betula Study was performed by random selection from the population registry of Umeå, Sweden. The study population has been shown to be representative of the general population of northern Sweden. Exclusion criteria for enrolment into the Betula Study are dementia, mental retardation, serious visual or auditory handicaps, not having Swedish as a mother tongue and any other feature that would compromise the ability to comply with the study protocol. For details regarding the Betula Study recruitment process, see Nilsson et al. [15].

After exclusion of 21 participants from the original sample [3, 16] due to lack of blood samples for TL measurements, the final sample consisted of 102 individuals (51 males, 51 females) with a mean age of 69 years (ranging from 64 to 75 years). All participants had ≥24 in Mini-Mental State Examination score (MMSE) [17].

Laboratory procedures

Leukocyte DNA was extracted from whole blood using standard procedures. Leukocyte TL was measured using a quantitative real-time PCR as described earlier [18, 19]. In short, each DNA sample was amplified on two parallel 96-well PCR plates, one amplifying telomere repeats and the other amplifying a single copy gene (β2-globin). The ratio of the mean telomere repeat copy number to the mean single copy gene copy number (T/S ratio) as related to that of a reference sample (DNA from the cell line CCRF-CEM) reflects relative TLs. Relative TLs (measured in relative T/S ratios) were converted to telomere restriction fragment lengths (measured in base pairs) based on correlation data derived from a sample (healthy subjects from the same geographical region as the participants in the current study) in which TL was measured using both quantitative real-time PCR and the conventional Southern blot method (n = 44, r = 0.93).

Magnetic resonance imaging

Supplementary data are available in Age and Ageing online, Appendix 1.

Statistics

Partial correlations and ANCOVAs were used to assess relationships (adjusted for age and gender) between TL and BMI, education, blood pressure, MMSE score, smoking, diabetes and cardiovascular disease. Hierarchical multiple linear regression was applied to evaluate if TL was significantly associated with measures of cerebral atrophy while controlling for potential confounders, and to evaluate if TL added a significant degree of variance explanation above that offered by a standard set of explanatory variables (age, gender and systolic and diastolic blood pressure). WMHs rating scores were dichotomised according to having (>0 rating) or not having (0 in rating) WMHs, followed by dividing the scores of those with WMHs into tertiles. ANCOVAs were then used to analyse for associations between TL and WMHs, while adjusting for age, gender, and systolic and diastolic blood pressure. TL was compared between subjects with and without WMHs, and across all four groups as a test for trend.

P-values of <0.05 were considered significant and were not corrected for multiple testing. IBM SPSS Statistics 19 was used for all statistical analyses.

Results

The 102 participants had a mean age of 69.3 years (SD = 3.8; median age = 69.6), with 51 (50%) of them being male (Table 1). The mean TL of the sample was 5543 bp (SD = 586), and TL was 255 bp longer in women compared with men (P = 0.028). TL was not significantly associated with age (P = 0.913).
TL was not associated with BMI ($P = 0.216$), education ($P = 0.640$), systolic blood pressure ($P = 0.139$), MMSE score ($P = 0.842$), smoking ($P = 0.366$), diabetes ($P = 0.919$) or cardiovascular disease ($P = 0.409$). Shorter TL was, however, associated with lower diastolic blood pressure ($r = 0.275$, $P = 0.006$).

A regression model including age, gender and blood pressure (diastolic and systolic) significantly predicted parietal cortical atrophy ($R^2 = 0.178$); insular cortical atrophy ($R^2 = 0.109$); temporal cortex atrophy ($R^2 = 0.119$); and total cortical atrophy ($R^2 = 0.141$; Table 2). Frontal cortex atrophy and occipital cortex atrophy were not significantly predicted by this model. Adding TL to the model did not result in any significant increase in predicted variance of any model regarding cortical atrophy.

Total as well as occipital subcortical atrophy were significantly predicted by a regression model including age, gender and blood pressure ($R^2 = 0.102$ and 0.119, respectively; Table 2). These two atrophy measures were however significantly better predicted when TL was added to the models. TL explained an additional 5.5% of the variance in occipital subcortical atrophy to a total of 17.4%. Regarding the variance of total brain atrophy, adding TL to the model explained a further 4.1% of the variance to a total of 14.4%. In both these models, age and TL were the only two predictors which were significantly associated with the atrophy measures. Thus, both age and TL independently and significantly predicted occipital and total subcortical atrophy. Both variables were approximately equally powerful in predicting atrophy (e.g. $\beta_{\text{age}} = 0.253$, $\beta_{\text{TL}} = -0.217$ for total subcortical atrophy). Dividing the sample at the median age (69.6 years) revealed that the association with TL was stronger among the older participants; TL was only significantly associated with subcortical occipital atrophy among participants aged above the median age.

Across the entire sample, TL showed no significant association with periventricular WMHs (Table 3). However, participants above the median age (69.6 years) with periventricular WMHs had significantly shorter TL than those without (difference of 552 bp; $P = 0.009$). Regarding subcortical WMHs, TL was 371 bp shorter ($P = 0.041$) among participants with subcortical WMHs, compared with those not exhibiting subcortical WMHs. This difference grew to 624 bp when looking only at the participants above the sample median age ($P = 0.006$). Analyses were adjusted for age, gender and blood pressure.

### Discussion

This study provides the first evidence that two prominent manifestations of human aging—cerebral subcortical atrophy and WMHs—are linked to short leukocyte TL. For subcortical atrophy, the age-independent predictive power of TL was comparable to that of age, supporting the value of TL as a marker of biological aging in general (independent of chronological age), and of subcortical structural change in particular.

Our contribution suggests that TL is related to measures of subcortical age-related alterations, but not cortical...
manifestations of aging. The occurrence of subcortical and periventricular WMHs was associated with shorter TL, although the association was more prominent with respect to periventricular WMHs as TL was significantly associated with periventricular WMHs only in the older half of the sample. Shorter TL was also linked with greater degree of subcortical atrophy. Considering that demyelination and microinfarction within the white matter, appearing as WMHs on the MRI scans, contribute to subcortical atrophy [20], it is plausible that the common denominator of our findings was an association of TL with white matter changes.

There are several possible reasons as to why this pattern emerges. Small vessel disease and inflammation-related demyelination have been related to subcortical atrophy, and due to the anatomy of the cerebral vascularisation white matter is more vulnerable to ischemia than cortical grey matter [2, 21, 22]. Inflammation might be the mediating factor in the relationship between TL and subcortical structural changes, as it is implicated in both vascular disease and leukocyte telomere shortening. Vulnerability to inflammatory processes varies across brain regions. Enzymes synthesising pro-inflammatory leukotrienes are, for example, more highly expressed in the subcortical limbic structures than in the primary sensory cortices, making the subcortical limbic structures more sensitive to inflammatory processes [23]. Inflammation, in turn, involves increased productions of reactive oxygen species which is directly involved in TL shortening through not entirely clear mechanisms, although likely by damaging DNA bases in the telomere sequence which in turn interferes with the replication fork [24].

The difference in TL was greater when comparing occurrence with non-occurrence of subcortical WMHs than in the corresponding comparison of periventricular WMHs. A frequent correlate of periventricular WMHs is disruption of the ependymal lining, while it is common to find that the walls of the arterioles in these regions are uncompromised. In contrast, vascular changes are considered a major determinant of subcortical WMHs [2]. Thus, considering this difference in pathophysiology between subcortical and periventricular WMHs, it seems that a link between TL and vascular changes is likely to explain the stronger association of TL with subcortical WMHs.

The notion that associations between brain phenotypes and shorter leukocyte TL can be linked with cerebral vascular factors is supported by previous studies. In a study by von Zglinicki et al. [25], leukocyte TL was shorter in patients with vascular dementia, a form of dementia characterised by cerebrovascular lesions often related to stroke or atherosclerosis. Interestingly, the same study did not find shorter leukocyte TL in patients with Alzheimer’s disease. Another study, by Martin-Ruiz et al. [26], showed that shorter leukocyte TL predicted risk of death and of developing dementia following stroke.

Age-associated volume decline has been described as greater in white matter relative to that of grey matter. Decline in grey matter seems to begin earlier and progress more gradually compared with white matter atrophy which becomes prominent later in life (70 years of age), but at that point progresses more steeply than that of grey matter [2]. This age-related pattern might explain why TL was associated with subcortical structural change, particularly among participants aged above the median sample age (69.6 years).

TL did not correlate with age in this sample. Reasons for this might be the relatively narrow age range of the sample.
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Medical Faculty, Umeå University and the County Councils of Västerbotten and Norrbotten, Sweden. The Betula Study is supported by grants from the Swedish Research Council [grant numbers 345-2003-3883, 315-2004-6977] and the Bank of Sweden Tercentenary Foundation, the Swedish Council for Planning and Coordination of Research, the Swedish Council for Research in the Humanities and Social Sciences and the Swedish Council for Social Research. CASCADE was mainly funded by the European Union Directorate General XII.

Supplementary data

Supplementary data mentioned in the text are available to subscribers in Age and Ageing online.

References


Key points

- Shorter leukocyte TL was associated with greater degree of subcortical brain atrophy.
- Shorter leukocyte TL was associated with subcortical WMHs.
- Shorter leukocyte TL was associated with periventricular WMHs in older participants.

Acknowledgement

Research nurse Eva Lundberg is thankfully acknowledged for her help and expertise.

Conflicts of interest

None declared.

Funding

This work was supported by the Swedish Research Council [grant numbers 2006-4472, 2009-5269, 2009-3413] the


Received 21 November 2012; accepted in revised form 3 September 2013