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Telomeres and the ageing process

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Summary

Telomere length is a reflection of cumulative oxidative and inflammatory stress throughout the life course, as well as oestrogen exposure. There is evidence that telomere length is associated with lifespan; however, it does not appear to be a better indicator of biological age than chronological age, even though it can be considered a biomarker of ageing. There are studies showing association between leukocyte telomere length and many of the common diseases of ageing, lifestyle factors and socioeconomic status. However, the associations are less marked among older people. Research in the area of manipulation of genetic control of telomerase activity controlling telomere length may have clinical applications in future.

Keywords: telomeres, lifespan, ageing, frailty, chronic disease.

Introduction

Telomeres are the DNA–protein complex located at the ends of chromosomes, comprising several telomere-binding proteins and DNA tandem repeat of TTAGGG.[1,2] The total number of nucleotides in the repeating sequence represents telomere length (units: base pairs or kilobase). There is a 3' overhanging DNA strand in each telomere, which folds back on itself to form the t-loop. This structure, together with specific and non-specific proteins, forms the telomere cap at the end of the chromosome.[3] In the absence of the telomere cap, broken chromosomes are earmarked by cells for degradation. Telomeres also maintain genomic integrity during DNA replication, without which normal regions for DNA transcriptions may be erased by the end replication problem.[3,4] Thus telomeres act as the buffering zone for the loss of DNA during DNA replications.

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The number of times that a somatic cell is able to divide is finite.[5] With each cell division, telomere shortening occurs until no further replication takes place (replicative senescence). This process is of evolutionary significance in the balance between preventing the formation of cancer cells (with unlimited proliferation capacity) and substantial telomere attrition which may result in failure of normal cell functions, the trade-off being suppression of cancer cell formation at the expense of limitation in lifespan. Telomere length is maintained by a protein–RNA complex, telomerase, which includes a RNA template (TERC) and a reverse transcriptase catalytic subunit (TERT).[6] TERT is encoded by the *hTERT* gene located at chromosome 5 with 1132 amino acids, while the RNA template TERC is encoded by the *hTERC* gene located at chromosome 3 with 451 nucleotides.

It has been shown that telomere erosion is accelerated under hyperoxic conditions.[7] Cellular oxidative stress may adversely affect repair in the telomeric region of DNA.[8] Telomere shortening may also be accelerated by inflammatory processes, where cell turnover rate is increased. It has been observed that shorter telomere length is associated with surrogate markers of inflammation such as IL-6, hs-CRP and fetuin-A, adjusted for age and gender.[9]

Telomere attrition is most rapid during early life, when about 1 kb of the telomere is lost annually,[10] probably caused by a high rate of proliferation in the most immature subsets of haematopoietic progenitors. Partially differentiated progenitors with already shortened telomere would be required to sustain haematopoiesis throughout the life by substantial cell divisions. Later, environmental influences resulting in oxidative or inflammatory stress contribute to varying telomere attrition rate. Thus telomere length is shortened with increasing age. There is also a gender difference in telomere length, in that length in women is often longer than that of men.[11–13] Since there is no significant gender difference in telomere

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length at birth,[14] the observed longer telomere length in women may be related to the oestrogen-associated increase in telomerase activity via the hTERT gene expression,[15] as well as oestrogen-augmented anti-oxidative and anti-inflammatory capacity.[16–19] Gender difference in telomere length may partly account for the longer life expectancy in women compared with men.

Studies of telomere length in humans is of interest in various aspects of the ageing process. Telomere dynamics allow the balance between cell senescence and immortalization to be examined: cell senescence results in limited lifespan, while the latter is of relevance to the development of cancer. Studies of how various environmental factors affect telomere length would be a cellular confirmation of epidemiological studies of association between lifestyle and environmental factors, and lifespan and health outcomes throughout the life course.[20] Telomere length may be considered as a marker of biological ageing[21] where, intriguingly, number of years of lifespan may be equated with kb of telomere length.[22] It may be a reflection of cumulative exposure to oxidative and inflammatory stress throughout the life course and therefore associated with the occurrence of chronic diseases, or the phenotype of frailty, whether frailty is regarded as progressive loss of function in various systems[23] or accumulation of multiple deficits.[24] The objectives of this review are:

1 to summarize the evidence relating to telomere length and lifespan;
2 to review the use of telomere length as a biological marker of ageing;
3 to examine the associations between telomere length and chronic diseases commonly encountered with ageing, and the frailty phenotype;
4 to examine the associations between telomere length and risk factors predisposing to chronic diseases;
5 to examine clinical implications and need for further studies.

Telomere length and lifespan

Since cell mortality and immortality is inextricably linked to ageing and the cancer process, telomere shortening can be regarded as a determinant of longevity of cell lineage.[25] In turn, replicative cell senescence would affect functioning of tissues and organs and contribute to the ageing process. Demonstration of telomere shortening with age ideally should be carried out within the same individual. Unfortunately such studies are rare, but do show loss of telomere length with age.[26] Cross-sectional studies in general show a negative association between age and telomere length, but there is wide variation.[20] Among people aged 60 years and older, survival is shorter among those with shorter telomere length.[27] A negative correlation between age and telomere length has also been observed in men aged 65 years and older but not women.[28] However, no association was found in other studies of older people aged 85 years and older.[29,30] It is possible that, at extreme old age, there are many confounding factors relating to selective survival accounting for the lack of association. The gender difference in life expectancy of some 7–8 years and the observation of longer telomeres in women compared with men provide strong evidence of the role of telomere length in lifespan.[31]

Telomere length as a biological marker of ageing

Since telomere length contributes to lifespan and is a reflection of oxidative and inflammatory stress, it may be a better indicator of biological ageing, and predictor of future functional outcomes, than chronological age. A biomarker of ageing should ideally predict the rate of ageing, monitor a basic process that is part of the ageing process and not the effects of disease, be able to be tested repeatedly without harming the individual, and be simple and inexpensive. Telomere attrition has been included as one of these markers, together with inflammatory cytokine levels, allostatic load, cognitive functioning and socioeconomic status, mitochondrial DNA deletions, and heat shock proteins.[21] However, thus far, no computational model of biological age has performed better than chronological age.[32]

Telomere length and common diseases of ageing, and the frailty phenotype

Studies on telomere dynamics are largely carried out in leukocytes, as they mirror the replicative history of haematopoietic stem cells.

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Atherosclerosis

Within individuals, telomere length has been shown to track cardiovascular health and ageing, and telomere attrition may underlie the fetal origins of cardiovascular disease.[33] Associations between telomere length and heart diseases have been reported extensively.[34–40] In a pilot study of 10 coronary artery disease patients and 20 controls, the average telomere length in patients was shorter than that of the controls by 303 bp ($P = 0.002$).[38] Benetos *et al.* found that hypertensive men with carotid artery plaques generally have shorter telomere length than those without plaques ($P < 0.01$).[34] In a randomly selected cohort of 419 participants, Fitzpatrick *et al.* found that shorter telomere length of 1000 bp is associated with 3-fold increases in the risk of myocardial infarction (95% CI: 1.22–7.73) and stroke (95% CI: 1.29–8.02).[37] Brouilette *et al.* found an association between shorter telomere length and higher risk of coronary heart disease ($P < 0.01$). They suggested that mean leukocyte telomere length may be useful in identifying individuals who may benefit most from treatment with statins.[35]

Inflammation and oxidative stress are associated with coronary heart disease.[41–43] These two factors have been shown to accelerate telomere erosion rate.[37]

Vascular smooth muscle cells from atherosclerotic plaques from patients undergoing bypass surgery showed 1 kb telomere loss compared with cells from normal blood vessels, and telomere length in cells from plaques is inversely related to plaque severity. Plaque senescence could be reversed by telomerase, while atorvastatin reduced the rate of telomere shortening during chronic oxidative stress *in vitro*.[44] A decline in the ankle-brachial index in 2000 women aged 65 years and over during a follow-up period of 4 years has been shown to be associated with shorter telomere length.[45]

Cancer

Mechanisms that avoid cell senescence may, at the same time, promote unlimited proliferation, and telomere shortening in the absence of other alterations has been considered a potent tumour suppressor mechanism.[46] Therefore, telomere dysfunction may contribute to carcinogenesis as well as ageing.[47] With shortening of telomeres, genomic instability occurs with increased chance of mutation of proto-oncogenes and tumour suppressor genes. These changes may explain the increased incidence of cancer with age.[48] Leukocyte telomere length has been shown to be associated with breast, lung, bladder and skin cancers in various clinical studies.[49–52]

Infections and chronic inflammation

During infective processes, immune cells proliferate more rapidly resulting in a faster telomere attrition rate. Moreover, the process of phagocytosis produces oxygen radicals that increase oxidative stress, and thus may lead to further telomere erosion. Previous studies showed that patients with chronic viral infections had shorter telomere length.[53,54] This phenomenon was also observed in the hepatocytes of patients with chronic inflammatory liver diseases.[55–57] Ilmonen *et al.* showed that lymphocyte telomeres were significantly shorter in male mice infected by *Salmonella* ($P = 0.01$). Lower bacterial load was also found in mice with initially longer telomeres ($P = 0.0006$).[58]

Bone mineral density

The association between bone mineral density (BMD) and telomere length has been shown in both elderly men ($n = 84$) and women ($n = 2150$) within a broad age range in previous studies.[59,60] Bekaert *et al.* found significant associations between age-adjusted telomere length and BMD changes of mid-region forearm, ultradistal forearm and total forearm ($P = 0.03$, $P = 0.10$ and $P = 0.04$, respectively).[59] Individuals with bone loss at the mid-region forearm had a significantly shorter telomere length of 423 bp when compared with those without bone loss ($P = 0.001$). With a much larger sample size, Valdes *et al.* confirmed the association of telomere length and forearm BMD ($P < 0.013$) in an adjusted model.[60] In addition, spine BMD and the risk of clinical osteoporosis were also associated with telomere length ($P < 0.005$ and $P < 0.003$, respectively). The association may be explained by increased bone turnover as a result of increased levels of inflammatory cytokines, which affect telomere dynamics.

However, in a study of 1867 community-living elderly Chinese aged 65 years and over, leukocyte telomere length measured using Q-PCR was not

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associated with baseline bone mineral density or bone loss over 4 years, after adjusting for confounding factors, and accounted for less than 1.6% of the baseline bone mineral density.[61]

Neurodegenerative diseases

In a preliminary study of 62 participants, shorter telomere length was found to be associated with the development of mild cognitive impairment (MCI, a preclinical state of dementia).[62] The odds of MCI is 12-fold higher (95% CI: 1.24–116.5) in individuals with shorter telomere length compared with those who had longer telomere length. Moreover, smaller hippocampal volume is significantly associated with shorter telomere length ($P = 0.038$). These results suggest a possible role of telomere length as a biomarker to identify the risk of dementia. In another prospective study, Martin-Ruiz *et al.* used the mini mental status exam (MMSE) to examine if cognitive function was associated with telomere length.[63] For 1 kb decreases of telomere length, the score of MMSE is lower by 0.77 points ($P = 0.04$). A similar association is also found in Alzheimer's disease (AD) patients.[64] Telomeres are found to be shorter in AD patients compared with controls ($P = 0.04$). Telomere length of T-cells was correlated with AD disease status as measured by MMSE scores ($P = 0.025$). In a study of 257 older Hispanic, Caucasian and African-American individuals, Honig *et al.* found that AD patients have shorter telomere length compared with that of controls ($P < 0.03$).[65]

The association between telomere length and Parkinson's disease (PD) is not consistent.[66,67] Although Guan *et al.* found a significant association between telomere length and the risk of PD, the sample size was small ($n = 24$) and confined to a narrow age group.[66] Since oxidative stress and inflammation in the substantia nigra is implicated in the pathogenesis of PD,[68,69] the association with telomere erosion is not unexpected. A significant telomere difference (5 kb) is found in PD patients in their 50s and 60s ($P < 0.01$). Wang *et al.* did not observe any association in a study of 96 PD patients and 172 age-matched controls.[67] In contrast to Guan, Wang found that shorter telomere length is observed in PD controls than PD patients, although this correlation is not significant ($P = 0.09$). Wang suggested that there may be other unknown lifestyles and environmental factors affecting their results.

Frailty

Frailty represents an excess of deficits over assets in a dynamic state of balance, covering physical, functional, psychological, nutritional and social domains as reported by previous reports.[70–72] To quantify frailty, one measure is the frailty index (FI). In an aged cohort ($n = 2914$), FI increased with age in a log-linear relationship ($r = 0.91$; $P < 0.001$).[73] In the same study, people with higher FI had greater mortality.

Lack of standardized definitions, disease and disability confounders, and complex multifactorial aetiology has made establishing of the underlying biological mechanisms of frailty difficult.[74] Since telomere length has been shown to be associated with age-related diseases, such as CHD and cancers[34,35,75] that are components of the functional deficits included in the FI, and frailty represents the accumulation of multiple deficits that might have been caused by telomere shortening, it is reasonable to hypothesize associations between FI and telomere length.

Although oxidative stress may increase the rate of telomere attrition, and high oxidative stress markers have been shown to be correlated with frailty in elderly Chinese subjects,[76] correlation between telomere length and frailty (assessed using a composite index of multiple deficits) has not been demonstrated. In a study of 976 men and 1030 women aged 65 years and over living in the community, women were more frail than men but had longer telomere length. In men, while there was a negative correlation between telomere length and age, and a positive correlation between frailty index and mortality, there was no correlation between telomere length and frailty index in either sex.[77] It appears that, while telomere length may be a marker of cellular senescence, this relationship may not be extrapolated to the functional level represented by the frailty phenotype.

Lifestyle

Obesity

In 1122 Caucasian women aged 18 to 76 years, Valdes *et al.* showed that the mean telomere length of the obese women was significantly shorter than that of the lean women ($P = 0.026$).[78] The age-adjusted telomere length was found to be significantly associated with the body mass index

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(BMI) and serum leptin (a marker and regulator of body fat) concentration ($P = 0.031$ and $P = 0.019$, respectively). The underlying mechanism is uncertain, but may be related to the increased secretion of inflammatory cytokines by adipocytes.

Smoking

In the same study of obesity mentioned above, Valdes *et al.* found a significant dose-dependent association between smoking and telomere length,[78] the underlying mechanism being oxidative stress caused by the chemicals in cigarettes. In this study, each pack-year smoked was equivalent to 18% annual loss of age-adjusted telomere length when compared with the overall population in their cohort ($P = 0.045$). Nevertheless, this association could not be confirmed in another study of 812 elderly and the oldest old.[30] Differences in their cohort structure and age group studied may explain the discrepancy.

Associations between dietary patterns and food groups have been reported. In a study of a US multi-ethnic population of 840, after adjustment for age, other demographics, lifestyle factors, and intakes of other foods or beverages, processed meat intake was inversely associated with telomere length, but not with foods with anti-oxidant or anti-inflammatory properties.[79] In contrast, in a study of 2006 elderly Chinese aged 65 years and over, in men, Chinese tea consumption was significantly associated with telomere length after adjustment for confounders.[80] The mean difference in telomere length for those in the highest quartile of consumption (>3 cups/day or >750 ml/day) and those in the lowest quartile (< = 0.28 cups/day or < = 70 ml/day) was 0.46 kb, corresponding to approximately 5 years of life.

Physical activity

Cherkas *et al.* examined associations between physical activity and telomere length in 2152 women and 249 men.[81] They used a 4-point scale (inactive, light activity, moderate activity and heavy activity) to represent the physical activity level in the leisure time of their participants. After adjusting for age, sex and year of extraction, telomere length was significantly associated with the level of physical activity. Participants with a higher level of leisure time activity generally have longer telomere length ($P < 0.001$), even after adjustment for BMI, smoking and socioeconomic status ($P = 0.002$).

Similarly, Ludlow *et al.* also found a significant association between physical activity level and telomere length in 34 men and 35 women. However, the findings differed in that individuals with moderate activity had longer telomere length compared with those having low or high levels of activity ($P = 0.001$ and $P = 0.04$, respectively). Furthermore, the stratification of physical activity level used was different in the two studies. The 4-point scale of physical activity used by Cherkas was based on self-reported activity hours per week of the participants, while Ludlow used both time and intensity of the physical activities in a week to determine the exercise energy expenditure (EEE) of each participant.

The benefits of physical activity on telomere length in middle-aged adults were not observed in elderly Chinese, suggesting that the benefits may be much attenuated by age 70 years.[28] However, methodological differences and differences between different social settings may account for these contrasting observations.

Psychological stress

Depression has been found to be associated with higher oxidative DNA damage.[82] Since oxidative stress has a profound effect on telomere erosion,[8,56] an association between psychological stress and telomere dynamics might be expected. Epel *et al.* demonstrated a relationship between psychological stress and reduced telomere length in 39 caregivers and 19 controls.[83] Both chronicity of caregiving and the overall perceived stress are negatively correlated with telomere length ($P < 0.01$ and $P < 0.05$, respectively). Individuals having higher psychological stress have also been shown to have lower telomerase activities ($P < 0.05$). However, the exact mechanism for this phenomenon is unclear.

Socioeconomic factors

Socioeconomic status (SES) has been associated with human health and mortality in some previous studies.[84,85] However, whether SES affects the ageing process was uncertain until Cherkas *et al.* found, in a study of 1552 Caucasian woman twins,

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a difference in telomere length between people in higher compared with lower SES groups.[86] A general decreasing trend of telomere length was found in the lower SES group ($P < 0.024$); the mean difference of telomere length was 163.2 bp between the 'non-manual' and 'manual' workers ($P < 0.01$). Other than the influence of income and education level, it has been suggested that psychological stress may be a factor.

However, in a homogeneous birth cohort of 318 samples, peripheral blood mononucleocyte (PBMC) telomere length was not associated with life-course SES after adjusting for lifestyle variables, gender and paternal age at birth. The authors suggested the large inter-individual differences in blood cell telomere length[14] may mask any correlations, particularly if sample size were small.

In contrast to previous studies showing that lower socioeconomic status is associated with shorter telomere length, a study in elderly Chinese showed that older men with higher self-rated socioeconomic status have shorter telomeres,[87] suggesting that there may be cultural and age-related differences in the social determinants of health. Since psychological stress has been associated with shorter telomeres,[83] it is possible that stress related to retirement among men in higher ranks in this society may be a contributory factor.

Living environment

The natural environment may affect health directly, independently of socioeconomic factors, the relationship being mediated by either psychological factors or physical activity.[88] The demonstration of geographic variation in telomere length in elderly Chinese after adjusting for confounding lifestyle factors (including physical activity), age and socioeconomic status suggested that the presence of green spaces could be directly health promoting.[89] Residents in areas with more open/green spaces have longer telomeres, the association probably to have been mediated by psychological factors independent of lifestyle, age, or socioeconomic status.

Methodological issues

In epidemiological studies of the association between telomere length and various population or clinical characteristics, there are many methodological issues that need to be considered in interpretation and comparison between studies. Firstly, the laboratory methods used may be different. These include terminal restriction fragment analysis by Southern blot technique (TRF), quantitative PCR, quantitative fluorescence *in situ* hybridization (Q-FISH), Q-FISH coupled with flow cytometry (Flow-FISH), Q-FISH in histological sections and single telomere length analysis (STELA).[90,91] Depending on the research question, Q-PCR methods may be more suitable for large population studies due to ease of use and high throughput. The value of coefficient of variation is important as it has been pointed out that, given that telomere attrition is 20–30 bp per year, a 2% deviation from the true value may be equivalent to 5 years in biological age.[31] Furthermore, due to the large inter-individual variations, appropriate sample size and consideration of age range, as well as other confounders, are important in epidemiological studies.

Clinical implications and future studies

In view of the evidence implicating abnormalities in telomere dynamics in the ageing process, onset of chronic diseases and cancer, and the role of telomerase in the regulation of telomere length, it is theoretically possible that manipulation of genetic control of telomerase activity may have clinical applications in future.[92] Alterations that affect telomeres at the level of chromatin structure might also offer potential therapeutic strategies.[93] Studies in mitigating telomere-directed DNA damage responses in neuronal plasticity and survival may be promising as future therapeutic strategies for neurodegenerative diseases.[94]

Measurement of telomere length as a predictor of lifespan or disease progression is unlikely to have any utility in the clinical setting as, currently, measurement methods have not developed to the stage of meeting the usual criteria for laboratory tests. It therefore remains a research tool for examining the pathological basis for ageing and diseases at the cellular level.

Conflicts of interest

The authors declared no conflicts of interest.

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