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Review Article

Is Telomere Length a Biomarker of Aging? A Review

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Telomeres, the DNA–protein structures located at the ends of chromosomes, have been proposed to act as a biomarker of aging. In this review, the human evidence that telomere length is a biomarker of aging is evaluated. Although telomere length is implicated in cellular aging, the evidence suggesting telomere length is a biomarker of aging in humans is equivocal. More studies examining the relationships between telomere length and mortality and with measures that decline with "normal" aging in community samples are required. These studies would benefit from longitudinal measures of both telomere length and aging-related parameters.

Key Words: Telomere length—Biomarker of Aging.

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INCREASED inter-individual and intra-individual variability is observed within sensory, motor, and cognitive and health domains with increasing chronological age (1–6). This inter-individual and intra-individual variability is found among individuals of the same age, suggesting that chronological age is a relatively imprecise measure of an individual's functional or health status. The concept of biomarkers of aging was developed to provide more information about an individual's biological health or functional status than chronological age. Biomarkers of aging are quantifiable parameters that reflect biological aging, which potentially can identify those at risk of aging-related conditions, disease, and mortality. Biomarkers could also be used to monitor and evaluate interventions designed to delay the onset or retard the progression of aging-related conditions and disease. Even though there has been little agreement on the validation criteria for candidate biomarkers (7–10), the promise of the utility of biomarkers of aging continues to be a driving force for research (11,12).

Telomere length has been proposed as a candidate biomarker of aging (7,10,13–17). Telomeres are nucleoprotein structures located at the ends of eukaryotic chromosomes. The observation that telomeres shorten with increasing age and are implicated in cellular aging has led to the proposal that telomere length is a biomarker of aging. Support for this hypothesis is provided by human studies that have found a significant inverse relationship between telomere length and several age-sensitive measures, aging-related conditions, disease, and mortality. However, the evidence is equivocal.

In a 2005 review of the in vitro and in vivo evidence, von Zglinicki and Martin-Ruiz (16) found that telomere length satisfied several criteria for a biomarker of aging, as it changes with age, has high inter-individual variability, is linked to basic biology, and correlates with aging and aging-related disease. The authors acknowledged that the majority of the evidence was cross-sectional and that many studies were underpowered. Since this review, a wide range of human studies have been published examining the relationship of telomere length with aging-related measures and mortality, prompting the need to reexamine the evidence using a set of well-defined biomarker of aging criteria developed by the American Federation of Aging Research (10,11):

1. It must predict the rate of aging. In other words, it would tell exactly where a person is in their total life span. It must be a better predictor of lifespan than chronological age.
2. It must monitor a basic process that underlies the aging process, not the effects of disease.
3. It must be able to be tested repeatedly without harming the person. For example, a blood test or an imaging test.
4. It must be something that works in humans and in laboratory animals, such as mice. This is so that it can be tested in lab animals before being validated in humans.

Methods

Published human studies in English were searched using the following databases: PubMed, Web of Science, and Scopus.

Various combinations of the following keywords were used as search criteria: telomere*, biomarker (aging or aging), function, cogn*, mortality, lifespan, survival. Studies were included if they used a representative population sample and had a sample size of 100 or more. Conference proceedings were excluded. Other published articles were identified by cross-checking cited references. All relevant studies were examined and compiled into tables detailing the age of sample, size of sample, sex ratio, adjustments, type of telomere length assay, the result, and, in addition for longitudinal studies, the length of follow-up (Tables 1 and 2). Over 3,830 studies were examined with only 19 meeting the criteria for this review (Tables 1 and 2).

Results

Criterion 1. It must predict the rate of aging. In other words, it would tell exactly where a person is in their total life span. It must be a better predictor of lifespan than chronological age.

This criterion states that telomere length should act as a predictor of the rate of aging and life span, with the expectation that shorter telomere length will be associated with an increase in the rate of aging and shortened life span. It must also be a better predictor than chronological age.

Telomeres and Life Span/Mortality

The first study to report that shortened telomeres were predictive of mortality was published by Cawthon and colleagues in 2003. In a study of 143 individuals aged 60 years or older, individuals with the shortest telomeres for their chronological age had a significantly higher mortality rate (18). This was explained by a threefold higher mortality rate from heart disease and more than eightfold higher mortality rate from infectious diseases. However, when the analysis was stratified by age group, the result was no longer significant for those aged 74 years and older, although the sample size was reduced when undertaking these analyses. Since the publication of this work, there have been several other studies examining telomere length and mortality that are summarized in Table 1.

To date, 5 of 10 studies did not find a significant relationship between shortened telomeres and increased risk of mortality. Telomere length did not predict mortality in a prospective population study of the oldest old (≥85 years) (19) nor in three cross-sectional studies examining individuals in their 70s (21,22,26). In the fifth study, Bischoff and colleagues (20) found no correlation between telomere length and survival in a cohort of Danish twins and singletons (mean age = 81 years), with chronological age being a better predictor of survival.

In addition to the Cawthon and colleagues study (18), four other studies have found a significant relationship between mortality and shorter telomere length. In an alternate analytical strategy, Kimura and colleagues (24), using a sample drawn from one of the cohorts originally used by Bischoff and colleagues (20), found an association between mortality and shorter telomeres in the lower quartile/half of the telomere distribution but not for mean telomere length. Bakaysa and colleagues (23) in a prospective study of Swedish twins (mean age = 79 years) found that the twin with the shorter telomeres had a threefold higher mortality risk than the cotwin over an average follow-up period of 7 years, regardless of zygosity. In a recent study, shorter telomeres length predicted cardiovascular mortality, but not overall mortality, for women only in a small U.S. sample (25). In a larger Italian sample, Ehrlenbach and colleagues (27) observed that shorter baseline telomere length was associated with all-cause mortality.

Survivor Effects

Many of the aforementioned studies have used samples from the latter stages of the life span, and there may be survivor effects when using older cohorts (e.g., 19,20). If as proposed, individuals with shorter telomere length are more susceptible to aging-related diseases and at a higher risk of mortality, then these individuals will die earlier. Therefore, cross-sectional studies of older adults comprised survivors with relatively long telomeres, and variability may be reduced (26,36). In fact, reduced telomere length variability in older samples has been observed (37).

Longitudinal Telomere Length Change and Mortality

Two studies to date have examined the relationships between longitudinal measures of telomere length and mortality. In 2005, Martin-Ruiz and colleagues (19) estimated telomere length in the oldest old at baseline and at follow-up (3.7–12.9 years) in 81 participants. They observed that the change in telomere length at follow-up was highly variable with some participants showing an increase in telomere length. They did not observe an association between mortality and rates of change nor any difference in mortality between individuals who showed a decrease versus an increase in telomere length. Due to the instability of their telomere length measurements, these authors concluded that telomere length may not be a useful biomarker in the oldest old (aged 85 years and older). This phenomenon may explain why several other studies have failed to find significant relationships when examining telomere length in the oldest old (37), although a small centenarian study (n = 38) found shorter telomere length was associated with poorer general health (38). In a slightly younger sample, Epel and colleagues (25) found that telomere shortening over 2.5 years was associated with mortality (follow-up of 12 years) from cardiovascular disease (n = 53), but not overall mortality (n = 102), for men only. Similar to Martin-Ruiz and colleagues (19), they observed telomere lengthening in a proportion

Table 1. Telomere Length and Mortality/Lifespan Studies

Study	*p* Value	Details of Result	Sample	*n*	Age (y)	% ♂	Follow-up (y)	No. of Deaths	Adjusted For?	TL Assay
Cawthon 2003 (18)	<.05*	Individuals with shorter TL ~2× higher mortality rate: 3× higher mortality rate from heart disease (p = .008**) and 8× higher mortality rate from infectious disease (p = .015*)	USA	143	Range 60–97	50	~20	101	Age	Q-PCR
Martin-Ruiz 2005 (19)	>.40	Not associated with all-cause mortality	Leiden 85+ Study, The Netherlands	598	Range 85–101; *M* age = 89.9	27	~20	598	Age, sex	Q-PCR
Bischoff 2006 (20)	n.s.	N/A	Danish prospective study—singletons and twins	812	73–101	32	7–8	412	Age (♀ and ♂ analyzed separately)	TRF
Harris 2006 (21)	>.05	N/A	Lothian Birth Cohort 1921, Scotland, prospective	190	79	43	5	36	Sex, TL assay	Q-PCR
Fitzpatrick 2007 (22)	.19	N/A	Cardiovascular Health Study subsample, USA	419	Range 65–92; *M* = 74.2	41	~7	176	Age, race, sex	TRF
Bakaysa 2007 (23)	.03*	~3× higher mortality rate for the cotwin with shorter *M* telomere length	Swedish twins (same sex)	350	Range 63–95; *M* = 79	31	~7	156	Assay	TRF
Kimura 2008 (24)	≤.014*	Increased mortality rate for the cotwin with shorter telomeres in the lower 25%–50% of the telomere length distribution	Longitudinal Study of Aging Danish Twins (same sex)	548	Range 73–94; *M* ~79	33	4.4	289	Age	TRF
Epel 2009 (25)	<.05* for ♀	Mortality from CVD associated with baseline TL for ♀ (OR = 2.3) but not for ♂. No associations with overall mortality	MacArthur Health Aging Study, USA	235	Range 70–79; *M* = 73.7	~49	12	102 overall; *n* = 53 deaths from CVD	Age	Q-PCR
Njajou 2009 (26)	>.05	Not associated with survival or specific causes of death	Health ABC Study, USA	2,721 (~58% white)	Range 70–79; *M* ~73.6 yrs	~43 to 52	10	975	Age, sex, race, recruitment site, assay	Q-PCR
Ehrlenbach 2009 (27)	<.001***	Shorter telomeres at baseline for those who subsequently died	Bruneck Study, longitudinal, Italy	669	Baseline: 53–71; *M* = 62.0	46%	10	159	Unclear	Q-PCR
Longitudinal telomere change										
Martin-Ruiz 2005 (19)	>.05	Change in TL over 3.7–12.9 y; no assn	Leiden 85+ Study, The Netherlands	81	Range 85–101; *M* age = 89.9	27	~20	81	Age, sex	Q-PCR
Epel 2009 (25)	<.04*	Change in TL over 2.5 y associated with CVD mortality for ♂ only (OR = 3.0); no associations with overall mortality	MacArthur Health Aging Study, USA	235	Range 70–79; *M* = 73.7	~49%	12	102 overall; *n* = 53 CVD deaths	Age	Q-PCR

Notes: References are listed by first author only. Studies used peripheral blood samples for TL estimation.
CVD = cardiovascular disease; n.s. = not significant; Q-PCR = quantitative real-time polymerase chain reaction; TL = telomere length; TRF = terminal/telomere restriction fragment analyses.
*p < .05; **p < .01; ***p < .001.

Table 2. Telomere Length and Age-Sensitive Functional Measures

Trait	Study	*p* Value	Details of Result	Sample	*n*	Age (y)	% ♂	Adjusted For?	TL Assay
Physical function									
Lung function	Harris 2006 (21)	>.05	N/A	Lothian Birth Cohort 1921	182	79	43	Sex, TL assay	Q-PCR
	Mather 2010 (28)	>.05	N/A	PATH Study, Australia: two narrow-age range cohorts	~646	Cohort 1 *M* = 47; Cohort 2 *M* = 67	(1) 44; (2) 54	Age, sex, physical activity, alcohol, height, smoking	Q-PCR
Grip strength	Bekaert 2005 (29)	>.05	N/A	Belgian men	110 ♂	Range 71–86; Median = 75	100	Sex, TL batch number	TRF
	Harris 2006 (21)	>.05	N/A	Lothian Birth Cohort 1921, Scotland	182	79	43	Sex, TL assay	Q-PCR
	Mather 2010 (28)	>.05	N/A	PATH Study, Australia: two narrow-age range cohorts	~646	Cohort 1 *M* = 47; Cohort 2 *M* = 67	Cohort 1: 44; Cohort 2: 54	Age, sex, phys. activity, alcohol, height	Q-PCR
Blood pressure	Bekaert 2007 (30)	>.05	N/A	Belgian (Asklepios Study)	~2,509	Range ~35 to 55; *M* ~46	49	Age, sex. Hypertensives excluded	TRF
	Brouilette 2007 (31)	≥.24	N/A	Scottish men	1,058	*M* = 56.7	100	Age	Q-PCR
	Fitzpatrick 2007 (22)	.06 for diastolic and .15 for systolic	N/A	Cardiovascular Health Study, USA	293	Range 65–92; *M* = 74	41.1	Age, race, sex	TRF
	Epel 2009 (25)	>.05 for entire sample and when split by sex	N/A	MacArthur Health Aging Study, USA	235	Range 70–79; *M* = 73.7	~49	Unclear	Q-PCR
	Mather 2010 (28)	Cohort 1: .017* for ♀, n.s. for ♂; Cohort 2: >.05	For younger cohort, systolic +vely correlated with TL for younger cohort ♀ (partial *r* = .181)	PATH Study, Australia: two narrow-age range cohorts	~646	Cohort 1 *M* = 47; Cohort 2 *M* = 67	Cohort 1: 44; Cohort 2: 54	Age, sex, physical activity, alcohol, BMI, antihypertensive medication	Q-PCR
Pulse pressure	Benetos 2001 (32)	≤.0005*** for ♂ only; n.s. for ♀	For men, shorter TL associated with higher pulse pressure	French	193	*M* = 56	62	Age, mean arterial pressure	TRF
	Lee 2005 (60)	0.45	N/A	Korean ♀	130	Range 55–69	0	Unclear	Q-PCR
Cognitive function									
Cognitive Test Battery	Harris 2006 (21)	>.05 for 3/4 tests and .022* for verbal fluency	No correlation for 3/4 tests. Exception: verbal fluency & TL (partial *r* = –.17)	Lothian Birth Cohort 1921, Scotland	185	79	43	Sex, childhood IQ, TL assay	Q-PCR
	Valdes 2010 (33)	≤.032*for 4/6 tests and >.05 for remaining tests	Better performance correlated with longer TL on 4/6 measures (partial *r* = .12–.16)	UK Twin Cohort (♀)	382 ♀	Range 19–78; *M* = 50.65	0	Age, NART scores	TRF
DSST	Yaffe 2009 (34)	.02*	At baseline, better DSST scores associated with longer TL	Health ABC Study, USA	2,734	*M* = 74 at baseline; range 70–79	~48	Age, gender, race, education, assay	Q-PCR
MMSE	Martin-Ruiz 2005 (19)	.90	N/A	Leiden 85+ Study, The Netherlands	598	Range 85–101; *M* = 89.8	27	Age, sex	Q-PCR
MMSE	Harris 2006 (21)	>.05	N/A	Lothian Birth Cohort 1921, Scotland	185	79	43	Sex, childhood IQ, TL assay	Q-PCR

Table 2. (*Continued*)

Trait	Study	*p* Value	Details of Result	Sample	*n*	Age (y)	% ♂	Adjusted For?	TL Assay
MMSE	Canela 2007 (35)	For entire sample >.05; for 60–69 y age group .003**	Inverse assn with TL for age group 60–69 y only (n = 33)	ZincAge, Italian	198	Range 60–99	45	Age	Q-FISH
3MS	Yaffe 2009 (34)	.28	N/A	Health ABC Study, USA	2,734	M = 74; range 70–79	~48	Age, gender, race, education, assay	Q-PCR
Longitudinal cognitive change									
MMSE change over 3 y	Martin-Ruiz 2005 (19)	.48	N/A	Leiden 85+ Study, The Netherlands	598	Range 85–101; M = 89.8	27	Age, sex	Q-PCR
Lifetime cognitive change from age 11 to 79 y	Harris 2006 (21)	>.05	N/A	Lothian Birth Cohort 1921, Scotland	185	79	43	Sex, childhood IQ, TL assay	Q-PCR
DSST: change over 7 y	Yaffe 2009 (34)	.99	N/A	Health ABC Study, USA	2,734	M = 74 at baseline; range 70–79	~48	Age, gender, race, education, assay	Q-PCR
Change in 3MS scores over 7 y	Yaffe 2009 (34)	0.01*	Less decrease in 3MS scores for those with longer telomeres	Health ABC Study, USA	2,734	M = 74 at baseline; range 70–79	~48	Age, gender, race, education, assay	Q-PCR

Notes: References are listed by first author only. Studies used peripheral blood samples for TL estimation.
BMI = body mass index; 3MS = Modified Mini-Mental State Examination; DSST = Digit Symbol Substitution Test; MMSE = Mini-Mental State Examination; NART = National Adult Reading Scores; n.s. = not significant; Q-PCR = Quantitative real-time polymerase chain reaction; TL = telomere length; TRF = terminal/telomere restriction fragment analyses.
*p < .05; **p < .01; ***p < .001.

of participants in addition to telomere length maintenance and shortening.

As survival bias may be a potential factor in studies of older adults, stronger findings may emerge from studies that have measured baseline telomere length earlier in life, such as middle age. Limitations of many mortality studies also include the length of the follow-up period, with some studies reporting only a small number of deaths due to a short follow-up period (e.g., <40 deaths, 20).

Given that there is wide inter-individual variability in telomere length of individuals of the same age, intra-individual rates of telomere decline may be a more valid and useful measure than cross-sectional measures. Apart from the Martin-Ruiz and colleagues study (19), several prospective studies have only recently been established to measure telomere length longitudinally, such as the Bogalusa Heart Study (39) and the Bruneck Study (27), which will provide useful data in the future.

Whether shortened telomere length predicts mortality awaits the results of further research. Prospective longitudinal studies of telomere length would provide the most valuable insights into the relationship between telomere length and mortality by examining the importance of rates of change in addition to cross-sectional analyses.

Criterion 2: It must monitor a basic process that underlies the aging process, not the effects of disease.

There is clear evidence that telomeres are involved in cellular aging and human diseases of premature aging. Telomeres in somatic dividing cells shorten at each cell division due to the inability of the cell to completely replicate the extreme ends of each chromosome (40) and from stochastic telomere loss events, such as damage from high levels of oxidative stress, recombination, and unequal sister chromatid exchange (41–44). Critically, shortened telomeres are one of the triggers of cellular replicative senescence (45–51), which may contribute to organismal aging (47,52). Shortened telomeres are also associated with several inherited diseases of premature aging (e.g., dyskeratosis congenita, Werner's syndrome, ataxia telangiectasia) (16,53). Furthermore, mutations in a number of genes that are responsible for telomere maintenance have been identified as one of the causes of the disease, dyskeratosis congenita (54).

However, whether telomere length is correlated with measures of "normal" aging is still unclear. If telomere length is a biomarker of aging, then it should reflect aging throughout the life span, as chronological aging is accompanied by physical, sensory, cognitive, and health changes. Studies examining the relationships between measures of "normal" aging-related functional decline and telomere length are summarized in Table 2. Many of these measures have also been identified as potential biomarkers of aging themselves (8,55). For example, lung function declines with age (56) and predicts cognitive performance (57) and mortality

(58). Analyses examining telomere length and measures of aging-related decline also provide evidence on whether telomere length is a general indicator of biological aging or specific for certain biological systems (e.g., cardiovascular) throughout the life span.

Physical Aging and Telomere Length

A significant inverse relationship between pulse pressure and telomere length was observed in a French study, but for men only (32), whereas no relationship was found in a Korean sample of postmenopausal women (59). Blood pressure was not significantly associated with telomere length in several studies (22,25,30,31). In the most recent study examining telomere length and measures of physical function in two narrow age range Australian cohorts, a positive correlation was observed between systolic blood pressure and telomere length in middle-aged women but not in older adults. The direction of this relationship was contrary to expectations. There were no significant correlations observed between diastolic blood pressure and telomere length (28). No relationships were observed between another marker of physical health, grip strength, and telomere length in a study of older Belgian men (29), in a Scottish sample all aged 79 years (21), and in middle-aged and older Australians (28). Similarly, lung function was not correlated with telomere length in the Scottish and Australian samples (21,28).

Cognitive Aging and Telomere Length

The findings for telomere length and cognitive performance in cross-sectional studies have been mixed and may be partly due to the differences in the cognitive tests used, the telomere length estimation methods utilized, and the samples employed (e.g., age range). Harris and colleagues (21) found a significant relationship with only one of the four cognitive tests in a modest sized Scottish sample all aged 79 years (adjusted for sex, childhood IQ, and assay), whereas Valdes and colleagues (33) found significant correlations with four of the six cognitive tests in a larger cohort of female twins adjusted for age and National Adult Reading Test scores. However, for the Valdes and colleagues study (33), only two of the correlations remained significant after adjusting for multiple covariates, and the sizes of the reported correlation coefficients were small (see Table 2). There were differences between the two studies as the Valdes and colleagues (33) sample was larger, consisted of females only, and had a wide age range (19–78 years), with a mean age that was almost 30 years younger than that of the Harris and colleagues sample (21). In the largest study to date (n = 2,734, mean age = 74 years), Yaffe and colleagues (34) found higher Digit Symbol Substitution Test (60) scores, a measure of attention, psychomotor speed, and executive function, were associated with longer telomeres, after adjustments for covariates. The Harris and colleagues (21) and the Valdes and colleagues (33) studies did not assess a similar cognitive test to the Digit Symbol Substitution Test.

The relationships between telomere length and performance on the dementia-screening tests, the Mini-Mental State Examination (61), and the modified Mini-Mental State Examination (62) have also been examined. Several studies did not find any significant results (19,21,34), whereas Canela and colleagues (35) found significant results for a small subset of their sample aged 60–69 years only. However, the Mini-Mental State Examination has ceiling effects (63), especially for younger age groups, and is generally used for older cohorts only (see Table 2).

As shown in Table 2, very few studies have examined telomere length and longitudinal cognitive change. Of the three prospective studies, only one study found significant results. Yaffe and colleagues (34) using a large American sample found that longer telomere length was associated with less decline in modified Mini-Mental State Examination scores over 7 years but did not observe such a relationship with the Digit Symbol Substitution Test.

Overall, these results suggest that there are few relationships between telomere length and age-sensitive measures of function. The strongest support was observed for a relationship between cognitive performance and telomere length, although the results are not unequivocal. This evidence may suggest that telomere length is not a "universal" biomarker of aging and hence does not reflect general underlying aging processes. However, it is acknowledged in the literature that markers of biological aging may change over the life span and that a single biomarker may not be sufficient to reflect aging across a variety of biological systems. Therefore, different panels of biomarkers may be necessary at different ages to assess biological age (64). This raises the possibility that telomere length may be related to different aging-related measures at different ages. Little is known about telomere length dynamics over the life span but telomere change rates may be dependent upon genetics, environmental, and stochastic factors that can vary over the lifetime (36,65).

General Health and Telomere Length

An alternate strategy is to examine the relationships between general health and telomere length rather than specific functional domains. Njajou and colleagues (26) examined the relationship between telomere length and (a) self-rated general health and (b) a cumulative measure of years of self-rated general health based on longitudinal data. In a population sample of more than 2,500 individuals aged 70–79 years, they found a significant association between longer telomere length (cross-sectional data) and better self-rated health (both cross-sectional and longitudinal data). In a small study (n = 38), longer telomeres were observed in a cohort of healthy centenarians compared

with a cohort of sex and age-matched centenarians in poor health (38).

More studies are required to adequately assess these relationships. Ideally, longitudinal assessment of both telomere length and measures of interest are the optimal method for examining these relationships. Given the wide inter-individual variability in telomere length, rates of change may prove to be more informative than absolute estimates.

Criterion 3: It must be able to be tested repeatedly without harming the person. For example, a blood test or an imaging test.

In humans, the most commonly collected sample for telomere length estimation is peripheral blood, which can be sampled repeatedly without harming the participant. Most researchers assume that telomere length estimated in peripheral blood samples is a surrogate marker of telomere length in other tissues, where aging-related changes may be occurring. Several studies suggest that telomere length may act as a proxy for telomere length in other somatic cell types (17,66). Conversely, there is also evidence that this may not be true for all tissue types (67). The relationships between leukocyte telomere length and tissues, where there is little cell turnover, such as neurons, are also not clear. Assessing telomere length in tissue types relevant to the domain under investigation, such as lung tissue for respiratory phenotypes, may be more appropriate than using telomere length estimated in leukocytes. However, if samples are required from tissues that are not easily accessible, it is unlikely that this criterion would be met due to the difficulties of sampling without harming the individual.

Criterion 4: It must be something that works in humans and in laboratory animals, such as mice. This is so that it can be tested in lab animals before being validated in humans.

Animal models have been employed to investigate the role of telomeres in aging. Mouse models are commonly used, but there are fundamental differences in telomere biology between mice and humans. Inbred mice strains have extremely long telomeres (20–150 kilobases) compared with humans (up to 15 kilobases) (13). Additionally, the enzyme responsible for telomere maintenance, telomerase, is readily detectable in most mouse somatic dividing cells, whereas telomerase levels are generally low in the equivalent human cells (68,69). Although inbred mice have very long telomeres, they have a short life span, suggesting that there may be different factors involved in the aging of the mouse. However, mouse telomeres shorten in mice with increasing chronological age in a number of tissues in at least two species, the wild mouse *Mus spretus* (70) and in the laboratory mouse *Mus musculus* [unpublished data in (71)]. Furthermore, telomeres of telomerase knockout mice display progressive shortening over generations, with later generations showing impaired function, such as hypertension (72), cardiac dysfunction (73), and reduced tissue regeneration, premature aging, and a shortened life span (74). There is also some evidence that mice overexpressing telomerase have an extended life span (75). This suggests that telomerase and/or telomeres play a role in aging, aging-related conditions and disease, and life span in the mouse. Recently, nonhuman primates have been proposed as an alternate animal model as they have similar levels of telomerase activity and are genetically more closely related to humans than to other mammals, such as rodents (76). Future animal studies may contribute further insights into telomere biology and biological aging. However, due to the differences in biology between disparate species, human studies provide important evidence regarding the roles that telomeres play in human aging.

Discussion

This review suggests that the evidence that telomere length is a biomarker of aging is inconclusive. Currently, telomere length does not fully meet American Federation of Aging Research criteria that telomere length is (a) a better predictor of life span than chronological age (Criterion 1) and that (b) it monitors a basic process underlying normal aging at the population level (Criterion 2). Mortality studies are few, and outcomes may be biased in older cohorts because of survivor effects. Fewer studies have assessed the relationship between telomere length and age-sensitive measures of function that decline with normal aging in human population studies.

On the other hand, the evidence does suggest that telomere length fulfills the biomarker criterion that the samples generally used for telomere length estimation (peripheral blood) can be taken repeatedly with minimal harm (Criterion 3). It should be noted, however, that the use of blood samples is only valid if telomere length estimated in peripheral leukocytes is the appropriate measure for the phenotype investigated. Whether telomere length measured in peripheral leukocytes is a surrogate marker for other tissues requires more investigation. The evidence also suggests that the fourth criterion is satisfied that telomere length can be examined in other mammals (e.g., rodents and macaques), although differences in biology and ethical issues may restrict the utility of this work. However, these are less important criteria than Criteria 1 and 2. In addition, Criterion 4 is less important because Criterion 3 is satisfied, as validation studies can easily be performed in humans.

Longitudinal Studies

Longitudinal studies provide stronger opportunities for unraveling the relationship between telomere length and human aging (77) because there is wide inter-individual variability in telomere length for individuals of the same chronological age in the population (36). Telomere length inter-individual differences reflect inherited and environment differences and may obscure any relationships between telomere length and biological aging. Within-individual

rates of telomere length change are therefore likely to provide better data than cross-sectional measures of telomere length, which reflect differences between individuals. Longitudinal studies also require fewer participants (36). Currently, there are population studies that are measuring telomere length and other aging-related parameters longitudinally, and in the future will offer opportunities to address this question more comprehensively (39). Such studies already suggest that the rate of telomere attrition is highly variable in the population. Interestingly, an increase in intra-individual telomere length for a minority of participants at follow-up (ie, with increasing chronological age) has also been observed in three independent studies (27,39,78). However, this may not represent an increase in overall telomere length but rather could reflect the loss of cells with shorter telomeres. The implication of such lengthening for aging processes remains unknown.

Factors That May Influence Telomere Length

Telomere length can potentially be influenced by a wide range of factors, including paternal age at time of offspring's birth (e.g., 79–82), levels of inflammation (e.g., 30,83), tobacco smoking (e.g., 84,85), physical activity (e.g., 86), sex (e.g., 32,87–89), socioeconomic status (e.g., 90,91), body mass index (e.g., 22,92,93), multivitamin intake (59), dietary antioxidants (e.g., 91), alcohol consumption (e.g., 21), hormone replacement therapy (59), and race (e.g., 94,95). However, the findings have not always been consistent across studies and may reflect differences in methodologies and samples used.

Many studies do not consider these additional factors, which may confound analyses. Furthermore, single measurements of a particular parameter may not indicate the burden of its accumulated effects over the life span. For example, a sole measure of inflammation is unlikely to provide an indication of the cumulative burden of inflammation over the lifetime. Thus, longitudinal measurements of all measures would be ideal.

Leukocyte Telomere Length in Different Cell Types

Most studies report leukocyte telomere length. This is a heterogeneous mixture of cell types of different ages that may differ in their telomere lengths. Furthermore, the proportion of cell types within a sample may change with aging (36) and from other processes, such as infection. There is also considerable telomere length heterogeneity within an individual cell (96), and mainstream methods lack the resolution to determine the length of specific chromosome telomeres.

Whether telomere length in peripheral blood samples correlates with telomere length in other tissues requires clarification. These relationships may even change across the life span. Leukocyte telomere length may be more relevant to the biological systems that leukocytes are intimately associated with, such as the immune and cardiovascular systems. Indeed, there is some evidence that telomere length may be implicated in immunosenescence as shown by (a) increased susceptibility to infections in the progeria syndrome, dyskeratosis congenita, which has dysfunctional telomeres (16,97) and (b) an increased mortality rate from infectious diseases for individuals with the shortest telomeres (18). Similarly, shorter leukocyte telomere length has been associated with cardiovascular disease (98,99).

Measurement Methods

The current mainstream methods of telomere length measurement differ in the information they provide. The two main methods are the telomere restriction fragment method (TRF), which estimates the size of enzymatically cleaved telomere fragments (100), and a quantitative real-time polymerase chain reaction technique (101). Both methods give an average telomere length for a sample but do not discriminate between telomere lengths of different cell types nor do they determine the telomere lengths of individual chromosomes. The TRF method estimates telomere length in absolute terms but is insensitive, requires large amounts of DNA, may not detect very short telomeres, and includes a variable amount of subtelomeric sequence due to genetic variation within this sequence (101,102). However, it can provide some information about the distribution of telomere lengths within a sample. Assessment of the distribution may be an important parameter as in vitro evidence suggests that it is the shorter telomeres that trigger replicative senescence (45,50,51). Estimates of the shortest telomere/s may therefore be a more informative measure than mean telomere length. For example, Kimura and colleagues (24) found a significant relationship between mortality and mean telomere length derived from the lower 25%–50% of the telomere length distribution but not for the more commonly used parameter of mean telomere length. This type of analysis is not possible with the quantitative real-time polymerase chain reaction method, which does not provide information about the telomere length distribution nor does it give an absolute estimate in base pairs. However, this method has the advantage of requiring relatively small amounts of DNA (<10-fold compared with the TRF method) and is more amenable to high throughput.

Methods that can estimate the length of the shortest telomeres with high resolution and sensitivity include quantitative fluorescence in situ hybridization (Q-FISH) (103) and single telomere length analysis (41). Quantitative fluorescence in situ hybridization, however, is restricted to dividing metaphase cells. Single telomere length analysis measures the telomere length of specific chromosomal arms, but at present is limited to only a few chromosomes. A recent development in this method examines the load of short telomeres rather than the length of specific single chromosomal arms (42) and in the future may provide further insights into

the relationships between ultrashort telomeres and cellular senescence. As these methods are labor intensive and have other limitations, further methodological developments are required before these methods can be used routinely in large epidemiological studies.

Recently, Aviv (104) has questioned the ability of the mainstream methods (TRF and quantitative real-time polymerase chain reaction) to find the same result in epidemiological studies and has called for an "impartial evaluation" (p. 979). Although numerous studies have used the quantitative real-time polymerase chain reaction method, there are still researchers who require additional ". . . data to establish its precision" [(96) p. 565]. Therefore, a side-by-side analysis of the mainstream methods of telomere length estimation in an epidemiological study would assist in confirmation that these methods are comparable.

Measurement Error

The reproducibility of telomere length measurement is dependent upon the methodology used and the expertise of the laboratory. Variability of telomere length measurement, as determined by the interassay coefficient of variation for telomere length, is usually less than 10% (e.g., 24), although not all researchers provide this information. Low coefficients of variation for telomere length estimates tend to be observed in dedicated telomere laboratories, such as 1.4% for the TRF method (105). If the variability in telomere length is high, statistical power is reduced (106) and the ability to detect significant differences in telomere length is reduced. Minimizing measurement error should be a priority for telomere length studies.

Moreover, longitudinal measurement of telomere length may pose its own challenges due to the inherent variability in the estimates, the methods used, and other sources of variability between waves of measurement, such as changes in equipment and reagents.

Telomere Length as a Biomarker of Aging Over the Life Span

It is possible that telomere length acts as a biomarker of aging at certain stages of the life span but not others (64). Longitudinal or cross-sectional narrow-age range cohort study designs spread across the life span are useful to address this question (107,108). A related issue is the difficulty of ascertaining whether an aging-related measure such as telomere length is an indicator of normal aging processes or is a marker of prodromal aging-related disease (64). Due to the lack of knowledge concerning the aging process, it can be difficult to differentiate aging from aging-related disease processes and to measure them independently (7,12). This is further complicated by the lack of knowledge regarding the importance and impact of common life experiences, such as viral infections, on telomere length and whether this is a potential confound in aging-telomere studies. However, only a few telomere studies have considered such potential confounders (e.g., 110). If the information is collected, then such short-term events may be adjusted for in analyses. Future studies should address the importance of acute events on telomere length.

A limitation of this review is that there is no consensus about validation criteria for a biomarker of aging. Criteria proposed by other researchers, such as that put forward by Butler and colleagues (7), could have been used herein to evaluate whether current evidence fulfills these criteria. However, there are common elements in the proposed criteria of different researchers and research groups, including the American Federation of Aging criteria, such as a biomarker should predict performance on age-sensitive functional tests and must be able to be sampled repeatedly and measured using nonlethal methods.

Conclusions

The evidence supporting the hypothesis that telomere length is a biomarker of aging is equivocal, and more data are required from studies that assess telomere length, aging-related functional measures, and collect mortality data. An area for future work is the clarification of which telomere length measure is the most informative and useful marker (e.g., mean, shortest telomere, longitudinal change). Nevertheless, in the near future, longitudinal designs will provide important information about within-individual telomere length dynamics over the life span. Such studies will also elucidate whether the relationships between telomere length and aging-related measures vary across the life span.

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