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Telomere Length in Epidemiology: A Biomarker of Aging, Age-Related Disease, Both, or Neither?

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Telomeres are nucleoprotein caps flanking DNA. They are shortened by cell division and oxidative stress and are lengthened by the enzyme telomerase and DNA exchange during mitosis. Short telomeres induce cellular senescence. As an indicator of oxidative stress and senescence (2 processes thought to be fundamental to aging), telomere length is hypothesized to be a biomarker of aging. This hypothesis has been tested for more than a decade with epidemiologic study methods. In cross-sectional studies, researchers have investigated whether leukocyte telomere length (LTL) is associated with demographic, behavioral, and health variables. In prospective studies, baseline LTL has been used to predict mortality and occasionally other adverse health outcomes. Conflicting data have generated heated debate about the value of LTL as a biomarker of overall aging. In this review, we address the epidemiologic data on LTL and demonstrate that shorter LTL is associated with older age, male gender, Caucasian race, and possibly atherosclerosis; associations with other markers of health are equivocal. We discuss the reasons for discrepancy across studies, including a detailed review of methods for measuring telomere length as they apply to epidemiology. Finally, we conclude with questions about LTL as a biomarker of aging and how epidemiology can be used to answer these questions.

aging; biomarker; oxidative stress; senescence; telomere

Abbreviations: CI, confidence interval; FISH, fluorescence in situ hybridization; HR, hazard ratio; LTL, leukocyte telomere length; PCR, polymerase chain reaction; T/S ratio, ratio of telomere repeat copy number to single-copy gene copy number.

INTRODUCTION

Telomeres are repeating nucleoprotein caps flanking nuclear DNA. With replication of nuclear DNA during mitosis, telomere length progressively shortens because the replication machinery cannot copy the absolute ends of DNA. This is termed the "end-replication problem" (1). Telomeres also shorten with oxidative stress (2). With critically short telomeres, the cell exits the cell cycle and becomes senescent (1, 3). This process protects against unbridled cellular division, which can lead to cancer. Telomeres have been recognized as a fundamental aspect of cellular biology, most recently with the awarding of the 2009 Nobel Prize in Physiology or Medicine to Elizabeth Blackburn, Carol Greider, and Jack Szostak for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase.

As an indicator of oxidative stress and cellular senescence (2 processes thought to be regulators of aging), telomere length has been postulated as a biomarker of human aging (4, 5). Epidemiology has become the central science for studying this hypothesis because telomere length is highly species specific, so results from animal models do not necessarily translate to humans. For more than a decade, epidemiologists have measured telomere length, most often average leukocyte telomere length (LTL), in cohort studies and have studied its association with demographics, behaviors, indicators of health, and other molecular markers. Widely conflicting results have produced more questions than answers about the value of telomere length as a biomarker of aging. Interpretation of data has been colored by debates over the precision and accuracy of methods for measuring telomere length.

Herein, we summarize and interpret the epidemiologic literature on telomere length as a biomarker of aging. To guide our evaluation, we used the following criteria that often are referred to for evaluating candidate biomarkers of

aging: 1) biological plausibility, 2) ability to be tested repeatedly and accurately without harming the person, and 3) ability to predict the rate of aging (6–8). After this evaluation, we provide a detailed description of methods for measuring telomere length, specifically as they apply to epidemiology. The review concludes with questions about telomere length as a biomarker of aging and how epidemiology can be used to answer these questions.

CRITERION 1: BIOLOGICAL PLAUSIBILITY

Telomere length in mice

Although telomeres are present in all eukaryotes, telomere length varies widely among eukaryotic organisms. This affects how useful particular model systems are for studying telomere length as it relates to human aging. For example, yeast organisms have telomeres that are as short as 300 base pairs (9). Consequently, yeast cells are more amenable to studies of telomere structure and dynamics. Telomeres in inbred mice are much longer (20,000–150,000 base pairs) than telomeres in humans, and mice have a higher baseline expression of telomerase (1, 10). In terms of comparing mouse aging with human aging, mice also have a higher rate of somatic expansion and a shorter lifespan. Nevertheless, mice are the most flexible model system for studying aging in vertebrates. Therefore, to use mice to study telomere length as it might relate to humans, the telomerase-deficient mouse was created through knockout of the murine telomerase RNA component gene (*TERC*) (11). These mice have markedly shorter telomere length than typical inbred mice, and their telomeres degrade at a much faster rate. Numerous studies in $Terc^{-/-}$ mice have demonstrated that they have much higher rates of chromosomal end-to-end fusions, limited viability after several generations, male and female infertility, embryonic death due to defective closure of the neural tube, small size, severe intestinal atrophy, spleen atrophy, reduced proliferation of B and T lymphocytes, impaired germinal center function, reduced angiogenic potential, reduced proliferation of bone marrow-derived stem cells, heart dysfunction, and reduced proliferation of neural stem cells (10). These mice also display a lower incidence of cancer. Rescue with addition of telomerase (comparison to $Terc^{+/-}$ mice) restores homozygous knockout mice to a relatively normal phenotype with far less chromosomal instability (12). The large effect that telomere length might have on longevity was demonstrated recently when researchers knocked in telomerase activity to aged telomerase-deficient mice with short telomeres (13). Before knock-in, these mice exhibited very short telomeres and marked age-related degeneration across tissues. Knock-in of telomerase lengthened telomeres, reduced DNA damage signaling and associated cellular checkpoint responses, allowed resumption of proliferation in quiescent cultures, and eliminated degenerative phenotypes across multiple organs, including testes, spleens, and intestines. Somatic telomerase reactivation reversed neurodegeneration with restoration of proliferating Sox2(+) neural progenitors, Dcx(+) newborn neurons, and Olig2(+) oligodendrocyte populations. These studies provide strong evidence that a minimum telomere length is necessary for maintenance of viability and proper development in vertebrates. Nonetheless, they belie the difficulty in using model organisms to study telomere length because the telomerase-deficient mouse displays phenotypic characteristics that are far from normal mouse or human aging.

Human telomere biology

Currently, determinants of telomere length are poorly understood. At birth, telomere length is highly heterogeneous, ranging from roughly 5,000 to 15,000 base pairs. Linkage analyses and genome-wide association studies have identified few possible loci influencing LTL, most notably those close to *TERC*, which appears mechanistically plausible because it encodes the template RNA component of telomerase, the enzyme that elongates telomeres (14–19). Telomeres also can be lengthened by homologous recombination during mitosis and chromosomal end-joining, although the latter introduces genomic instability. Previous reports have shown that LTL is heritable (14, 15, 20–22) and modified by paternal age at conception (23–26).

Of note are hereditary diseases caused by mutations in the telomerase complex. Patients with these diseases, including dyskeratosis congenita, bone marrow failure syndromes, and idiopathic pulmonary fibrosis, can be considered to suffer from premature aging because they all display chromosomal instability and accelerated cellular senescence, particularly in tissues that proliferate frequently (27). The clinical characteristics most common to these 3 conditions are compartment-specific or complete bone marrow failure, which could be seen as a more extreme form of age-associated senescence of hematological progenitor cells, and cancer, which is also age associated. Although some mutations, such as those in *TERC* and the human telomerase RNA gene (*hTR*), are associated with all of these diseases, presentation is nonetheless distinct. For example, the age of onset for dyskeratosis congenita is before age 60 years (typically between ages 10 and 30 years). Onset of bone marrow failure syndromes occurs at all ages, and onset of idiopathic pulmonary fibrosis is usually after age 40 years. Short telomeres appear in all patients with dyskeratosis congenita, in some patients with bone marrow failure syndromes, and in an unknown proportion of patients with idiopathic pulmonary fibrosis. Other aspects of these diseases, such as pulmonary fibrosis, nail dystrophy, and leukoplakia, are not seen with normal aging. Thus, these diseases do not exactly mirror normal aging, so it is difficult to use them as models to study a potential impact of short telomeres on aging in the general population.

Werner's syndrome provides stronger evidence for a mechanistic role for telomere length in aging. Werner's syndrome is characterized by a mutation in the Werner syndrome, RecQ helicase-like gene (*WRN*), which encodes a DNA helicase and exonuclease important for DNA replication, repair, and telomere maintenance. Patients have short telomeres and display normal development until lack of a pubertal growth spurt, which is accompanied by hypogonadism, short stature, flat feet, cataracts (bilateral in nearly

100% of patients), dermatological pathology, graying or thinning hair, type II diabetes mellitus, osteoporosis, soft tissue calcification, premature atherosclerosis, and cancer (28). By their 30s and 40s, patients look like they are several decades older. In general, patients with Werner's syndrome display a phenotype that appears more like accelerated aging than that of patients with dyskeratosis congenita, bone marrow failure syndromes, and idiopathic pulmonary fibrosis. Nonetheless, Werner's syndrome is not entirely comparable to normal aging. In particular, the dermatologic pathology (tight skin, hyperkeratosis, and ulceration) and types of cancer (mesenchymal cancers such as sarcoma, multiple synchronous cancers, and rare cancers) experienced by Werner's patients are not similar to the dermatological changes (skin wrinkling, sagging, and thinning) and cancers (predominantly epithelial tissue of the colon, prostate, and female reproductive organs) seen with normal aging. Therefore, Werner's syndrome suggests but does not prove that telomere length plays a role in normal aging.

What biological processes, then, might telomere length reflect? A great deal of in vitro and in vivo evidence indicates that telomere length is affected by 2 processes in particular: cellular replication and oxidation. With regard to replication, as stated previously, the DNA replication machinery is unable to copy the extreme ends of chromosomes during mitosis. Thus, telomeric DNA is progressively lost with each cell division. It is estimated that roughly 50 base pairs are lost during cell division because of the end-replication problem (29). Most age-associated shortening occurs during rapid somatic expansion, as occurs during growth from birth through puberty (4, 30). With critically short telomeres, the cell exits the cell cycle and becomes senescent (1, 3). It is notable that cells also can senesce because of telomere-independent signaling, such as propagated DNA damage response, altered mitogenic signaling, and cyclin-dependent kinase inhibitor signaling, among others (31). Thus, telomere length can reflect the growth rate or remaining replicative potential of a population of cells.

Telomere length also shortens with increasing oxidative stress (2, 4). In fact, in human diploid fibroblasts cultured under normal conditions, the major determinant of telomere shortening appears to be single-stranded breaks in DNA caused by oxidative stress (32). The large effect of oxidation on telomeric DNA occurs because of a specific deficiency in base excision repair (32, 33). Single-stranded breaks result in telomere shortening during replication (34), most likely because of temporary stalling of the replication machinery (35). In vitro, oxidative stress increases the rate of telomere shortening by an order of magnitude (32, 36, 37). Reduction of oxidative stress decreases the rate of telomere shortening and postpones replicative senescence (34). Depending on where the single-stranded break occurs, the lesion can cause either no effect on telomere length or up to a 1,000-base pair loss of telomeric DNA (29). Modeling studies that predict that oxidative stress is the major contributor to telomere shortening match experimental results (2, 29).

In sum, evidence suggests that, of the basic biological processes, telomere length most likely reflects somatic growth before the end of puberty and cellular senescence and oxidative stress after puberty. It is unknown what biological pathways telomere length might indicate about aging beyond cellular senescence and oxidative stress. It is possible that short telomeres induce senescence, which upregulates secretion of inflammatory factors systemically that promote aging. In this case, short telomere length could be a cause of inflammation and aging more than a consequence of these processes. Because phenotypic associations with rapidly shortening telomeres in humans are observed in patients with distinct genetic syndromes, it is unknown whether more modest rates of shortening in the general population are also associated with aging phenotypes.

CRITERION 2: METHODS TO MEASURE TELOMERE LENGTH IN HUMANS—HARM, EFFICIENCY, REPRODUCIBILITY, AND ACCURACY

To properly interpret results from an epidemiologic study, one must understand the methodological approaches to measuring telomere length, particularly their efficiency, reproducibility, and functional reasons for underestimating or overestimation of LTL. In population-based studies, telomere length is measured almost exclusively as LTL. Measurement protocols dictate that leukocytes are retrieved from blood, so patient harm consists of only that associated with a typical blood draw. It should be acknowledged that, although there is synchrony in telomere length among hematopoietic cells (38), LTL might not be a perfect surrogate for telomere length in other tissues (1). Furthermore, LTL represents the average telomere length across leukocytes, which are a heterogeneous cell population composed of granulocytes and lymphocytes. These subpopulations have different average telomere lengths. Lymphocytes have shorter telomeres than granulocytes at all ages, because granulocytes are related more closely to hematopoietic stem cells, which are less differentiated (39). In addition to the absolute length, these subpopulations differ in their rates of telomere shortening. In both populations, there is a biphasic rate of telomere decline, with accelerated decline in childhood and old age and a linear decline in adolescence and adulthood. However, during phases of more accelerated decline (childhood and late adulthood), lymphocytes appear to have a steeper rate of decline than that of granulocytes (39–41). Fluctuations in these cell subpopulations and changes in replicative activity of specific subpopulations could greatly affect the average LTL and thus could contribute to intraindividual variation.

Several platforms exist for measuring LTL. Measurement reproducibility and accuracy are platform dependent. The original technique, Southern blot (42), remains the "gold standard." Cawthon (43) introduced an assay using quantitative polymerase chain reaction (PCR) and recently an updated multiplexed version with higher throughput (44). Variations of the quantitative PCR method also have been described (45). The third notable method involves coupling individual cell sorting using flow cytometry and metaphase DNA staining with fluorescence in situ hybridization (FISH) (46), which was modified into a high-throughput quantitative FISH technique (47). Single-telomere length analysis was developed to measure the length of telomeres

on specific single-chromosomal arms (48). Each platform has different strengths and weaknesses and measures a different part of the telomere complex. Furthermore, measurement error is introduced in multiple places (e.g., within- and between-gel error for Southern blot; within- and between-well and -plate error for quantitative PCR), but this error rarely is incorporated into statistical analysis. Because of heterogeneity in results from population-based studies of LTL, there is considerable debate over how the measurement platform could affect findings and weaken comparability of results across platforms (5). Moreover, Southern blot and quantitative PCR, the methods used chiefly in epidemiology, return mean telomere length. It is unknown whether cellular senescence is preferentially induced in the presence of one critically short telomere or in the presence of shorter average telomere length, so it is also unknown whether these platforms even measure the parameter of telomeres that is most biologically relevant.

Southern blot

The Southern blot technique for measuring telomere length was developed by Kimura et al. (42) chiefly to examine LTL in epidemiologic studies. Nonetheless, it can be modified to measure telomere length in any nucleated cell type with intact DNA if telomeres are within the detectable limits of the technique. The technique uses traditional gel electrophoresis and labeled probe hybridization to the telomere sequence, followed by gel imaging (42). The protocol notes that a skilled technician could process approximately 130 samples per week.

Strengths of Southern blot include its reproducibility (coefficient of variance is typically <2%) and expression of values in absolute base-pair units (though measurements are in terminal restriction fragments because of the use of restriction enzymes to digest the DNA). It also allows determination of telomere length distribution, but this parameter of the telomeres is seldom used in epidemiology because it requires accurate measurement of the width of the gel bands relative to reference DNA, which is difficult and prone to human error. Weaknesses of Southern blot include the need to obtain a considerable amount of DNA (3 μg per sample) and to measure both the telomeric and subtelomeric regions. Inclusion of the subtelomeric region could result in artificial inflation of the mean LTL. Cawthon (43) has commented that differences in the subtelomeric region can also cause variation in terminal restriction fragment lengths when different restriction enzymes are used, which has been observed with direct comparison of results after digestion with *Hin*fI/*Rsa*I, the most common restriction enzyme used for Southern blot, versus *Hph*I/*Mn*I (21).

Quantitative polymerase chain reaction

Cawthon's original quantitative PCR assay was created to provide a high-throughput technique to measure LTL (43). This method measures the relative average LTL in genomic DNA by determining the ratio of telomere repeat copy number to single-copy gene copy number (T/S ratio) in experimental samples relative to a reference sample. The mean LTL in base pairs is calculated by multiplying the T/S ratio by the coefficient from a regression equation that describes the relation between the T/S ratio and a set of samples, the absolute length of which was measured with Southern blot (43). In the original description of the assay, the overall coefficient of variance was 5.8%, and the linear correlation between the T/S ratio and terminal restriction fragment length was 0.677 ($P = 1.5 \times 10^{-24}$). Cawthon (43) stated that average LTL differing by as little as 11.4% could be resolved by using this technique at 95% confidence. Recently, Cawthon (44) introduced a multiplex version of the quantitative PCR assay to eliminate variance in the T/S ratio introduced by pipetting different quantities of DNA into separate wells, as well as to reduce cost and increase efficiency by requiring half the number of reactions. T/S ratios obtained via the multiplexed assay are more highly correlated with terminal restriction fragment lengths ($R^2 = 0.844$). The intraassay coefficient of variance is 5.22%, and the interassay coefficient of variance is 3.13% (44).

Polymerase chain reaction has the advantages of requiring far less DNA (50 ng per sample) and achieving true high-throughput performance. In general, the variance of quantitative PCR is believed to be >2%, and in the literature it is often quoted as 5%–10%. Because LTL is highly heterogeneous between individuals of the same age and because rates of LTL shortening vary by an unknown amount, this higher variance can weaken the ability to resolve significant differences between mean LTLs (42). For example, in cross-sectional studies of LTL, the observed difference in mean LTL between case and control groups is commonly from several dozen to several hundred base pairs (42, 49). With an assay coefficient of variance of 2%, individuals with a baseline LTL of 5,000–15,000 base pairs could register a difference in LTL of 100–300 base pairs simply because of measurement error, which could easily obscure observed differences in mean LTL between cases and controls. Furthermore, stability of the reference gene is not guaranteed, and there is no agreed-upon reference gene despite frequent use of albumin or β-globin (42–44). For these reasons, quantitative PCR has been questioned as an accurate technique for telomere epidemiology despite having higher throughput and lower cost.

Flow cytometry plus FISH and single-telomere length analysis

Baerlocher et al. (46) developed the original flow cytometry plus FISH technique to allow measurement of mean telomere length with metaphase staining from any subpopulation of circulating peripheral cells isolated by use of flow cytometry as a first step. Thus, flow cytometry plus FISH has the distinct advantages of returning telomere length for individual cells of a distinct cell type and the distribution of telomere lengths of that cell type. Furthermore, flow cytometry plus FISH uses internal reference controls and highly specific nucleic acid probes to hybridize to the telomeric repeats. This improves accuracy compared with other platforms that are prone to error due to including

Table 1. Association of Telomere Length With Age, Sex, Race, Health Risk Factors, Age-Related Chronic Disease, and Markers of Inflammation and Oxidation

First Author, Year (Reference No.)	Sample	Study	Variable	Measurement Method	Association	*P* Value
Benetos, 2001 (53)	193 Caucasian men and women, aged 54–58 years, not on hypertensive medication		Age, years	Southern blot	$r = -0.45$ (men), $r = -0.48$ (women)	<0.0001, <0.0001
Brouilette, 2003 (55)	203 cases with MI before the age of 50 years; 180 controls		Age, years	Southern blot	N/A	<0.0001
Benetos, 2004 (54)	163 men, aged 58–65 years, with chronic treated essential hypertension		Age, years	Southern blot	$r = -0.25$	<0.01
Demissie, 2006 (57)	327 Caucasian men, aged 40–89 years	FHS	Age, years	Southern blot	$r = -0.41$	<0.0001
Bekaert, 2007 (52)	2,509 Caucasian men and women, aged 35–55 years, without overt CVD	Asklepios Study	Age, years	Southern blot	−26 bp	<0.0001
Fitzpatrick, 2007 (58)	419 men and women, aged 65–93 years	CHS	Age, years	Southern blot	−31 bp	<0.001
Hunt, 2008 (21)	1,742 Caucasians and 711 African Americans, aged 19–93 years	NHLBI-FHS, BHS	Age, years	Southern blot	−20 bp (Caucasian), −29 bp (African American)	<0.0001, <0.0001
Nordfjäll, 2008 (59)	989 Caucasians, aged 48–68 years	MDCC, NS-MONICA	Age, years	PCR	$r = -0.002$ (men), $r = -0.230$ (women)	0.96, <0.001
Cherkas, 2008 (56)	2,401 Caucasian twins, aged 18–81 years	TwinsUK	Age, years	Southern blot	$r = -0.38$	<0.001
Batty, 2009 (51)	1,542 Caucasian men, aged 45–64 years, without MI	WOSCOPS	Age, years	PCR	−8.5 bp	0.001
Diez Roux, 2009 (60)	981 Caucasian, African-American, and Hispanic men and women, aged 45–84 years	MESA	Age, years	PCR	−0.003 T/S (men), −0.007 T/S (women)	0.0002, <0.0001
Sanders, 2009 (61)	2,750 Caucasian and African-American men and women, aged 70–79 years	HABC	Age, years	PCR	$r = -0.065$ (men), $r = -0.055$ (women)	0.017, 0.038
Tang, 2010 (62)	963 Chinese men and 904 Chinese women, aged ≥65 years		Age, years	PCR	Negatively correlated in men but not women	0.037, 0.78
Benetos, 2001 (53)	193 Caucasian men and women, aged 54–58 years, not on hypertensive medication		Male	Southern blot	−280 bp (age adjusted)	0.016
Bekaert, 2007 (52)	2,509 Caucasian men and women, aged 35–55 years, without overt CVD	Asklepios Study	Male	Southern blot	−172 bp	<0.0001
Hunt, 2008 (21)	1,742 Caucasians and 711 African Americans, aged 19–93 years	NHLBI-FHS, BHS	Male	Southern blot	FHS: −120 bp (Caucasian), −260 bp (African American); BHS: 10 bp (Caucasian), −130 bp (African American)	FHS: <0.0001; BHS: 0.26
Nordfjäll, 2008 (59)	989 Caucasians, aged 48–68 years	MDCC, NS-MONICA	Male	PCR	Shorter in men	<0.0001
Diez Roux, 2009 (60)	981 Caucasian, African-American, and Hispanic men and women, aged 45–84 years	MESA	Male	PCR	−0.041 T/S	0.0005

Table Continues

Table 1. Continued

First Author, Year (Reference No.)	Sample	Study	Variable	Measurement Method	Association	*P* Value
Tang, 2010 (62)	963 Chinese men and 904 Chinese women, aged ≥65 years		Male	PCR	−550 bp	<0.001
Hunt, 2008 (21)	1,742 Caucasians and 711 African Americans, aged 19–93 years	NHLBI-FHS, BHS	Caucasian race	Southern blot	FHS: −180 bp (men), −320 bp (women); BHS: −500 bp (men), −680 bp (women)	All <0.0001
Chen, 2009 (63)	472 Caucasian and 190 African-American men and women, aged 26–48 years	BHS	Caucasian race	Southern blot	−541 bp (men), −517 (women)	<0.001, <0.001
Diez Roux, 2009 (60)	981 Caucasian, African-American, and Hispanic men and women, aged 45–84 years	MESA	Caucasian race	PCR	−0.041 T/S (vs. African American), −0.044 (vs. Hispanic)	0.025, 0.015
Valdes, 2005 (65)	1,122 Caucasian women twins, aged 18–76 years	TwinsUK	BMI[a]	Southern blot	−0.077 TRF	0.031
Bekaert, 2007 (52)	2,509 Caucasian men and women, aged 35–55 years, without overt CVD	Asklepios Study	BMI	Southern blot	−4 bp (men), −4 bp (women)	0.41, 0.35
Fitzpatrick, 2007 (58)	419 men and women, aged 65–93 years	CHS	BMI	Southern blot	−10 bp	0.13
Hunt, 2008 (21)	1,742 Caucasians and 711 African Americans, aged 19–93 years	NHLBI-FHS, BHS	BMI	Southern blot	$r = -0.071$	0.002
Nordfjäll, 2008 (59)	989 Caucasians, aged 48–68 years	MDCC, NS-MONICA	BMI	PCR	$r = -0.041$ (men), $r = -0.106$ (women)	0.35, 0.021
O'Donnell, 2008 (64)	1,062 Caucasian men and women, aged 33–86 years	FHS	BMI	Southern blot	$r = -0.08$	0.01
Batty, 2009 (51)	1,542 Caucasian men, aged 45–64 years, without MI	WOSCOPS	BMI	PCR	−1 bp	0.79
Diez Roux, 2009 (60)	981 Caucasian, African-American, and Hispanic men and women, aged 45–84 years	MESA	BMI	PCR	0.001 T/S	0.58
Yang, 2009 (70)	Chinese, 379 controls and 388 patients with hypertension, aged 30–80 years		BMI	PCR	NS (controls), NS (cases)	0.61, 0.24
Valdes, 2005 (65)	1,122 Caucasian women twins, aged 18–76 years	TwinsUK	Smoking, pack-years	Southern blot	−0.11 TRF	0.045
Bekaert, 2007 (52)	2,509 Caucasian men and women, aged 35–55 years, without overt CVD	Asklepios Study	Smoking, pack-years	Southern blot	−20 bp (men), 6 bp (women)	0.16, 0.75
Fitzpatrick, 2007 (58)	419 men and women, aged 65–93 years	CHS	Smoking, pack-years	Southern blot	−2 bp	0.27
Nordfjäll, 2008 (59)	989 Caucasians, aged 48–68 years	MDCC, NS-MONICA	Smoking, pack-years	PCR	$r = 0.084$ (men), $r = -0.115$ (women)	0.46, 0.23
O'Donnell, 2008 (64)	1,062 Caucasian men and women, aged 33–86 years	FHS	Current smoking	Southern blot	−130 bp	0.02
Batty, 2009 (51)	1,542 Caucasian men, aged 45–64 years, without MI	WOSCOPS	Ever smoking	PCR	35 bp	0.17

Table Continues

Table 1. Continued

First Author, Year (Reference No.)	Sample	Study	Variable	Measurement Method	Association	*P* Value
Diez Roux, 2009 (60)	981 Caucasian, African-American, and Hispanic men and women, aged 45–84 years	MESA	Smoking, pack-years	PCR	−0.0007 T/S	0.054
Bekaert, 2007 (52)	2,509 Caucasian men and women, aged 35–55 years, without overt CVD	Asklepios Study	Alcohol, units/week	Southern blot	−8 bp (men), −7 bp (women)	0.63, 0.68
Batty, 2009 (51)	1,542 Caucasian men, aged 45–64 years, without MI	WOSCOPS	Alcohol, units/week	PCR	3 bp	0.93
Bekaert, 2007 (52)	2,509 Caucasian men and women, aged 35–55 years, without overt CVD	Asklepios Study	Physical activity, MET × times/week)	Southern blot	25 bp (men), −3 bp (women)	0.15, 0.81
Cherkas, 2008 (56)	2,401 Caucasian twins, aged 18–81 years	TwinsUK	Physical activity, questionnaire	Southern blot	−88 to −213 bp (depending on comparison)	<0.05
Diez Roux, 2009 (60)	981 Caucasian, African-American, and Hispanic men and women, aged 45–84 years	MESA	Leisure MET-minutes (1,000 seconds)	PCR	−0.009 T/S	0.13
Sanders, 2009 (61)	2,750 Caucasian and African-American men and women, aged 70–79 years	HABC	Weekly physical activity, kcal	PCR	$r = -0.036$ (men), $r = -0.029$ (women)	0.19, 0.28
Bekaert, 2007 (52)	2,509 Caucasian men and women, aged 35–55 years, without overt CVD	Asklepios Study	Total cholesterol, 10 mg/dL	Southern blot	−2 bp (men), 1 bp (women)	0.75, 0.84
Nordfjäll, 2008 (59)	989 Caucasians, aged 48–68 years	MDCC, NS-MONICA	Total cholesterol, 10 mg/dL	PCR	$r = 0.026$ (men), $r = 0.070$ (women)	0.58, 0.13
Yang, 2009 (70)	Chinese, 379 controls and 388 patients with hypertension, aged 30–80 years		Total cholesterol, 10 mg/dL	PCR	NS (controls), NS (cases)	0.97, 0.18
Bekaert, 2007 (52)	2,509 Caucasian men and women, aged 35–55 years, without overt CVD	Asklepios Study	HDL cholesterol, 10 mg/dL	Southern blot	−20 bp (men), 55 bp (women)	0.15, 0.64
Nordfjäll, 2008 (59)	989 Caucasians, aged 48–68 years	MDCC, NS-MONICA	HDL cholesterol, 10 mg/dL	PCR	$r = 0.111$ (men), $r = 0.072$ (women)	0.053, 0.41
Chen, 2009 (63)	472 Caucasian and 190 African-American men and women, aged 26–48 years	BHS	HDL cholesterol, 10 mg/dL	Southern blot	−31 bp (childhood), −44 bp (adulthood)	0.024, 0.058
Yang, 2009 (70)	Chinese, 379 controls and 388 patients with hypertension, aged 30–80 years		HDL cholesterol, 10 mg/dL	PCR	Controls inverse association, cases NS	0.046, 0.24
Bekaert, 2007 (52)	2,509 Caucasian men and women, aged 35–55 years, without overt CVD	Asklepios Study	LDL cholesterol, 10 mg/dL	Southern blot	−0.7 bp (men), 3 bp (women)	0.93, 0.70
Nordfjäll, 2008 (59)	989 Caucasians, aged 48–68 years	MDCC, NS-MONICA	LDL cholesterol, 10 mg/dL	PCR	$r = 0.040$ (men), 0.160 (women)	0.49, 0.07
Bekaert, 2007 (52)	2,509 Caucasian men and women, aged 35–55 years, without overt CVD	Asklepios Study	Triglycerides, 10 mg/dL	Southern blot	2 bp (men), 1 bp (women)	0.21, 0.83

Table Continues

Table 1. Continued

First Author, Year (Reference No.)	Sample	Study	Variable	Measurement Method	Association	*P* Value
Nordfjäll, 2008 (59)	989 Caucasians, aged 48–68 years	MDCC, NS-MONICA	Triglycerides, 10 mg/dL	PCR	$r = -0.047$ (men), $r = 0.004$ (women)	0.34, 0.95
Yang, 2009 (70)	Chinese, 379 controls and 388 patients with hypertension, aged 30–80 years		Triglycerides,10 mg/dL	PCR	NS (controls), NS (cases)	0.48, 0.051
Bekaert, 2007 (52)	2,509 Caucasian men and women, aged 35–55 years, without overt CVD	Asklepios Study	Glucose, 10 mg/dL	Southern blot	4 bp (men), −25 bp (women)	0.85, 0.29
Fitzpatrick, 2007 (58)	419 men and women, aged 65–93 years	CHS	Glucose, 10 mg/dL	Southern blot	−15 bp	0.06
Sanders, 2009 (61)	2,750 Caucasian and African-American men and women, aged 70–79 years	HABC	Glucose, 10 mg/dL	PCR	$r = -0.061$ (men), $r = -0.045$ (women)	0.025, 0.089
Yang, 2009 (70)	Chinese, 379 controls and 388 patients with hypertension, aged 30–80 years		Glucose, 10 mg/dL	PCR	NS (controls), NS (cases)	0.72, 0.87
Nordfjäll, 2008 (59)	989 Caucasians, aged 48–68 years	MDCC, NS-MONICA	Oral glucose tolerance test, 0 minutes	PCR	$r = -0.040$ (men), $r = -0.001$ (women)	0.41, 0.98
Nordfjäll, 2008 (59)	989 Caucasians, aged 48–68 years	MDCC, NS-MONICA	Oral glucose tolerance test, 120 minutes	PCR	$r = -0.202$ (men), $r = -0.063$ (women)	0.045, 0.45
Demissie, 2006 (57)	327 Caucasian men, aged 40–89 years	FHS	Homeostasis model assessment—insulin resistance score	Southern blot	$r = -0.16$	0.007
Fitzpatrick, 2007 (58)	419 men and women, aged 65–93 years	CHS	Insulin, 10 mg/dL	Southern blot	−100 bp	0.009
Nordfjäll, 2008 (59)	989 Caucasians, aged 48–68 years	MDCC, NS-MONICA	Insulin, 10 mg/dL	PCR	$r = -0.026$ (men), $r = 0.096$ (women)	0.65, 0.28
Sanders, 2009 (61)	2,750 Caucasian and African-American men and women, aged 70–79 years	HABC	Insulin, 10 mg/dL	PCR	$r = -0.064$ (men), $r = -0.069$ (women)	0.028, 0.014
Bekaert, 2007 (52)	2,509 Caucasian men and women, aged 35–55 years, without overt CVD	Asklepios Study	SBP, 10 mm Hg	Southern blot	15 bp (men), −4 bp (women)	0.34, 0.80
Fitzpatrick, 2007 (58)	419 men and women, aged 65–93 years	CHS	SBP, 10 mm Hg	Southern blot	−34 bp	0.15
Nordfjäll, 2008 (59)	989 Caucasians, aged 48–68 years	MDCC, NS-MONICA	SBP (10 mm Hg)	PCR	$r = 0.064$ (men), $r = -0.038$ (women)	0.15, 0.41
Yang, 2009 (70)	Chinese, 379 controls and 388 patients with hypertension, aged 30–80 years		SBP, 10 mm Hg	PCR	NS (controls), NS (cases)	0.09, 0.81
Bekaert, 2007 (52)	2,509 Caucasian men and women, aged 35–55 years, without overt CVD	Asklepios Study	DBP, 10 mm Hg	Southern blot	17 bp (men), −5 bp (women)	0.40, 0.82
Fitzpatrick, 2007 (58)	419 men and women, aged 65–93 years	CHS	DBP, 10 mm Hg	Southern blot	−83 bp	0.06

Table Continues

Table 1. Continued

First Author, Year (Reference No.)	Sample	Study	Variable	Measurement Method	Association	*P* Value
Nordfjäll, 2008 (59)	989 Caucasians, aged 48–68 years	MDCC, NS-MONICA	DBP (10 mm Hg)	PCR	$r = 0.013$ (men), $r = 0.010$ (women)	0.77, 0.82
Yang, 2009 (70)	Chinese, 379 controls and 388 patients with hypertension, aged 30–80 years		DBP, 10 mm Hg	PCR	NS (controls), NS (cases)	0.08, 0.69
Benetos, 2001 (53)	193 Caucasian men and women, aged 54–58 years, not on hypertensive medication		Pulse pressure, mm Hg	Southern blot	−3.7/TRF (men), 0.4/TRF (women)	0.005, NS
Nordfjäll, 2008 (59)	989 Caucasians, aged 48–68 years	MDCC, NS-MONICA	Pulse pressure, mm Hg	PCR	$r = 0.075$ (men), −0.056 (women)	0.09, 0.23
Benetos, 2001 (53)	193 Caucasian men and women, aged 54–58 years, not on hypertensive medication		Pulse wave velocity, meters/second	Southern blot	−0.5/TRF (men), −0.2/TRF (women)	0.047, NS
Benetos, 2004 (54)	163 men, aged 58–65 years, with chronic treated essential hypertension		Presence of carotid artery plaque	Southern blot	−0.22 TRF length with presence of carotid plaque	<0.05
De Meyer, 2009 (71)	2,509 Caucasian men and women, aged 35–55 years, without overt CVD	Asklepios Study	Presence of carotid artery plaque	Southern blot	−0.006/TRF (men), −0.202/TRF (women)	0.96, 0.09
De Meyer, 2009 (71)	2,509 Caucasian men and women, aged 35–55 years, without overt CVD	Asklepios Study	Presence of femoral artery plaque	Southern blot	−0.101/TRF (men), −0.251/TRF (women)	0.28, 0.02
De Meyer, 2009 (71)	2,509 Caucasian men and women, aged 35–55 years, without overt CVD	Asklepios Study	Presence of carotid or femoral plaque	Southern blot	−0.083/TRF (men), −0.243/TRF (women)	0.36, 0.01
Fitzpatrick, 2007 (58)	419 men and women, aged 65–93 years	CHS	Common carotid intima-media thickness, mm	Southern blot	−260 bp	0.10
Fitzpatrick, 2007 (58)	419 men and women, aged 65–93 years	CHS	Internal carotid intima-media thickness, mm	Southern blot	−110 bp	0.07
O'Donnell, 2008 (64)	1,062 Caucasian men and women, aged 33–86 years	FHS	Internal carotid intima-media thickness, mm	Southern blot	$r = -0.055$	0.15
De Meyer, 2009 (71)	2,509 Caucasian men and women, aged 35–55 years, without overt CVD	Asklepios Study	Femoral intima-media thickness, mm	Southern blot	−0.004/TRF (men), 0.000/TRF (women)	0.73, 0.99
De Meyer, 2009 (71)	2,509 Caucasian men and women, aged 35–55 years, without overt CVD	Asklepios Study	Carotid intima-media thickness, mm	Southern blot	−0.003/TRF (men), −0.003/TRF (women)	0.54, 0.40
Fitzpatrick, 2007 (58)	419 men and women, aged 65–93 years	CHS	Ankle-brachial index	Southern blot	270 bp	0.09
Mainous, 2010 (72)	325 adults, aged 40–64 years, free of diagnosed diabetes, coronary heart disease, stroke, and cancer		Coronary artery calcium	PCR	$r = -0.15$	0.009
Brouilette, 2003 (55)	203 cases with MI before the age of 50 years; 180 controls		MI before the age of 50 years	Southern blot	−300 bp	<0.0001

Table Continues

Table 1. Continued

First Author, Year (Reference No.)	Sample	Study	Variable	Measurement Method	Association	*P* Value
Honig, 2006 (91)	125 demented cases, 132 nondemented controls, aged 66–103 years	WHICAP	Dementia	PCR	−0.058 T/S	0.034
Honig, 2006 (91)	125 demented cases, 132 nondemented controls, aged 66–103 years	WHICAP	Apo ε4 allele, ≥1	PCR	−0.042 T/S	NS
Yaffe, 2011 (90)	2,734 nondemented Caucasian and African-American men and women, aged 70–79 years	HABC	Apo ε4 allele, ≥1	PCR	N/A	0.16
Yaffe, 2011 (90)	2,734 nondemented Caucasian and African-American men and women, aged 70–79 years	HABC	3MS	PCR	N/A	0.28
Yaffe, 2011 (90)	2,734 nondemented Caucasian and African-American men and women, aged 70–79 years	HABC	Digit Symbol Substitution Test	PCR	N/A	0.02
Valdes, 2007 (82)	2,150 Caucasian women twins, aged 18–80 years	TwinsUK	Femoral neck BMD	Southern blot	$r = 0.040$	0.052
Valdes, 2007 (82)	2,150 Caucasian women twins, aged 18–80 years	TwinsUK	Forearm BMD	Southern blot	$r = 0.054$	0.013
Valdes, 2007 (82)	2,150 Caucasian women twins, aged 18–80 years	TwinsUK	Spine BMD	Southern blot	$r = 0.058$	0.005
Sanders, 2009 (61)	2,750 Caucasian and African-American men and women, aged 70–79 years	HABC	Hip BMD	PCR	$r = -0.017$ (men), $r = -0.007$ (women)	0.53, 0.79
Sanders, 2009 (61)	2,750 Caucasian and African American men and women, aged 70–79 years	HABC	Femoral neck BMD	PCR	$r = -0.003$ (men), $r = -0.016$ (women)	0.92, 0.55
Tang, 2010 (62)	963 Chinese men and 904 Chinese women, age ≥65 years	TwinsUK	Femoral neck BMD	PCR	3 bp (men), −1 bp (women)	0.11, 0.46
Tang, 2010 (62)	963 Chinese men and 904 Chinese women, aged ≥65 years		Hip BMD	PCR	2 bp (men), 0 bp (women)	0.42, 0.99
Bekaert, 2007 (52)	2,509 Caucasian men and women, aged 35–55 years, without overt CVD	Asklepios Study	IL-6 (highest half)	Southern blot	−133 bp (men), −83 bp (women)	<0.01, >0.05
Fitzpatrick, 2007 (58)	419 men and women, aged 65–93 years	CHS	IL-6, pg/mL	Southern blot	−50 bp	0.03
Sanders, 2009 (61)	2,750 Caucasian and African-American men and women, aged 70–79 years	HABC	IL-6	PCR	$r = -0.074$ (men), $r = -0.008$ (women)	0.008, 0.77
Fitzpatrick, 2007 (58)	419 men and women, aged 65–93 years	CHS	CRP, mg/L	Southern blot	−59 bp	0.02
Richards, 2007 (80)	2,160 Caucasian women twins, aged 18–79 years	TwinsUK	CRP	Southern blot	$r = -0.05$	0.0009
Richards, 2008 (79)	1,319 Caucasian twins, aged 18–81 years	TwinsUK	CRP	Southern blot	$r = -0.02$	<0.05

Table Continues

Table 1. Continued

First Author, Year (Reference No.)	Sample	Study	Variable	Measurement Method	Association	*P* Value
Sanders, 2009 (61)	2,750 Caucasian and African-American men and women, aged 70–79 years	HABC	CRP	PCR	$r = -0.051$ (men), $r = 0.004$ (women)	0.063, 0.88
Demissie, 2006 (57)	327 Caucasian men, aged 40–89 years	FHS	Isoprostane urinary 8-epi-prostaglandin $F_{2\alpha}$	Southern blot	$r = -0.16$	0.005
Bekaert, 2007 (52)	2,509 Caucasian men and women, aged 35–55 years, without overt CVD	Asklepios Study	Homocysteine	Southern blot	Data not shown	>0.25
Richards, 2008 (79)	1,319 Caucasian twins, aged 18–81 years	TwinsUK	Homocysteine (highest tertile)	Southern blot	−111 bp	0.004

Abbreviations: BHS, Bogalusa Heart Study; BMD, bone mineral density; BMI, body mass index (weight (kg)/height $(m)^2$); bp, base pairs; CHS, Cardiovascular Health Study; CRP, C-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; FHS, Framingham Heart Study; HABC, Health, Aging, and Body Composition Study; HDL, high density lipoprotein; IL-6, interleukin-6; LDL, low density lipoprotein; MDCC, Malmö Diet and Cancer Cohort; MESA, Multi-Ethnic Study of Atherosclerosis; MET, metabolic equivalent of task; MI, myocardial infarction; 3MS, Modified Mini–Mental Status Examination; N/A, not available; NHLBI-FHS, National Heart, Lung, and Blood Institute Family Heart Study; NS, not significant; NS-MONICA, Northern Sweden Monitoring of Trends and Determinants in Cardiovascular Diseases; PCR, polymerase chain reaction; SBP, systolic blood pressure; TRF, terminal restriction fragment; T/S, ratio of telomere repeat copy number to single copy gene copy number; UK, United Kingdom; WHICAP, Washington Heights-Inwood Columbia Aging Project; WOSCOPS, West of Scotland Coronary Prevention Study.

subtelomeric sequences (Southern blot), missing some sequence because of less specific probes (quantitative PCR), altering length depending on the restriction enzyme used (Southern blot), or relying on the stability of a reference gene to measure relative LTL (quantitative PCR). Human error is reduced by automation of most pipetting and cell sorting, and systemic error can be minimized by inclusion of internal controls with known telomere lengths. The original protocol suggests that telomere lengths from 22 different individuals can be measured in 12 hours over 2–3 days. Despite returning more information than other platforms, flow cytometry plus FISH is much more expensive, is far less efficient, and requires greater technical expertise that limit its use in population-based research.

High-throughput quantitative FISH was developed by Canela et al. (47) to maintain the accuracy and flexibility of flow cytometry plus FISH while capitalizing on the efficiency achieved by plate-based technologies (e.g., quantitative PCR) and to allow measurement of telomere length from any cell type that can be grown under conventional tissue culture conditions. High-throughput quantitative FISH can analyze 96 samples with at least 1,000 nuclei in 2 hours. Because time is greatly reduced and more samples can be analyzed simultaneously, there is a reduction in error from variation in metaphase staining conditions over time and across samples (47). The technique reportedly costs less than traditional flow cytometry plus FISH. Thus far, high-throughput quantitative FISH has not been widely used in epidemiologic studies.

Baird et al. (48) described single-telomere length analysis as a viable platform for measuring telomere length in 2003. Single-telomere-length analysis was developed to measure the telomere length of single chromosomes to investigate whether individual critically short telomeres influenced cell senescence. Consequently, single-telomere-length analysis is a powerful tool to examine telomere dynamics, but it must be applied to specific chromosomes. For example, it can be used to investigate how interindividual or even interchromosomal differences in the genetic code (e.g., single-nucleotide polymorphisms), genetic structure (e.g., proximity of binding proteins), or genetic machinery (e.g., fidelity of replication and repair complexes) could affect telomere length. Universal single-telomere-length analysis is a modification to an original protocol that allows measurement of telomere load across chromosomes to determine the effect of critically short telomeres on senescence (50). Because of its low throughput, single-telomere-length analysis is more amenable to basic studies of telomere biology and has not been used in epidemiologic studies.

CRITERION 3: TELOMERE LENGTH PREDICTING THE RATE OF HUMAN AGING

Selection criteria for article retrieval

Articles with community-dwelling adult or older adult human subjects where LTL was measured in at least 100 participants were included in the present review. We conducted a review on this subject in 2010 and updated the

review annually. Because of the diversity of outcomes included in this review, updates were made by searching the literature with the term "telomere length" paired with "aging," "epidemiology," or various search terms for the outcomes examined (e.g., race, interleukin-6, cardiovascular disease, grip strength, mortality). Citations in articles were cross-referenced to obtain additional sources. Because the primary concern was examining the association of telomere length with aging phenotypes rather than clinical disease phenotypes (e.g., pulmonary vital capacity instead of chronic obstructive pulmonary disease), articles were excluded if they were exclusive studies of clinical disease. Study design was not used as a selection factor, but it is notable that a substantial amount of data has been generated from twin studies. Twin studies are most useful for characterizing the relative contribution of genes and environment to a particular phenotype. The present review does not discuss this aspect of twin studies as it applies to the potential determinants of telomere length.

Association of telomere length with age, sex, race, health risk factors, inflammation, age-related chronic disease, function, and mortality

LTL has been studied in association with many phenotypes in cross-sectional epidemiologic studies. Major findings are listed in Table 1. If LTL were truly associated with a phenotype, one would ideally observe consistency across populations, measurement methods, and statistical models. This consistency has been observed only for associations with age, gender, and race. Shorter LTL is associated with older age, male gender (21, 51–62), and Caucasian race (21, 60, 63). The strength of the association with age is highly dependent on the age range of the population. Male gender and Caucasian race appear to be associated with a mean LTL that is several hundred base pairs shorter relative to LTL in women and in African Americans or Hispanics, respectively. The causes of these large gender and race effects are unknown.

LTL exhibits a relatively similar number of positive and negative associations with other health risk factors, including smoking (51, 52, 58–60, 64, 65), alcohol consumption (51, 52, 66), physical activity (52, 56, 60, 61), socioeconomic status and education (67–69), body mass index (21, 51, 52, 58–60, 64, 65, 70), lipid levels (52, 59, 63, 70), markers of glucose metabolism (52, 57–59, 61, 70), and blood pressure (52, 53, 58, 59, 70). Likelihood of a significant association does not appear to track with any particular type of study population, measurement method, or statistical modeling. Except for age, gender, and race, significant associations with LTL are weak, with correlation coefficients generally <0.2.

LTL has been inconsistently associated with markers of subclinical cardiovascular disease, including carotid or femoral intima-media thickness (54, 58, 64, 71), ankle-brachial index (58), coronary artery calcium (72), and pulse wave velocity (53). Despite both positive and negative results that do not seem to be patterned on study population, measurement method, or statistical modeling, some have argued that there is a robust association with atherosclerosis (21, 52, 54, 55, 57, 58, 64, 73–77) and have posited a mechanism for this association (78). This mechanism hinges on inflammation, whereby with greater inflammation there would be increased demand for hematopoietic cells, which would propagate up the lineage chain until it included hematopoietic stem cells. Increased stem cell division would lead to progressively shorter telomeres in both stem cells and differentiated cells that continued to divide in the periphery (e.g., lymphocytes). In total, the hematopoietic pool would "age" as the population of cells edged closer to senescence. Thus, with age and inflammation, there would be less division of circulating hematopoietically derived cells and epithelial progenitor cells, which would then be less able to maintain the vascular wall in response to oxidative stress and plaque growth (78). This effect would be augmented by oxidative stress, which is heightened in the setting of atherosclerosis and which accelerates LTL shortening directly because of the susceptibility of the telomere nucleotide sequence to oxidation (78). Because oxidative stress and inflammation are associated with atherosclerosis and because there are consistent associations between markers of oxidation and inflammation (e.g., interleukin-6, C-reactive protein, homocysteine, isoprostane urinary 8-epi-prostaglandin $F_{2\alpha}$) and shorter LTL (52, 57, 58, 61, 79, 80), atherosclerosis and shorter LTL might be noncausally linked via oxidation's acceleration of LTL shortening. These mechanisms are hypothetical and have not been proved true.

Several age-related changes are not associated with LTL. Pulmonary function (66, 81) and bone mineral density (61, 62, 82), markers of specific tissues that decline in function and degrade in structure with age independent of disease, are likely not associated or are only weakly associated with LTL. Self-reported physician diagnosis of cataract (per 1,000-base pair LTL, odds ratio = 0.95, 95% confidence interval (CI): 0.89, 1.01) and incident cataract surgery (per 1,000-base pair LTL, hazard ratio (HR) = 1.02, 95% CI: 0.94, 1.10) were not associated with LTL in the Health, Aging, and Body Composition Study, though older adults with highly transparent lenses measured by objective means had markedly longer LTL (5,700 base pairs) than older adults with any lens opacity (4,770 base pairs) (odds ratio = 0.47, 95% CI: 0.22, 1.02) (83). Grip strength, a marker of physical function, has not been associated with LTL (66, 81, 84). An association between LTL and cognitive function is equivocal. In 382 women not diagnosed with dementia or cognitive impairment, shorter LTL was independently associated with worse episodic memory and associated learning ($P = 0.032$), recognition memory for nonverbal patterns ($P = 0.007$), and working memory capacity ($P = 0.003$) (85). These findings were corroborated by data from the Nurses' Health Study (86) and a hospital-based case-control study (87) but not by a cohort of 449 inpatients in which there was no difference in LTL between patients who were cognitively normal, had mild cognitive impairment, or were demented (88). In an Australian study with several hundred middle- and older-age participants, there were few associations between tests of cognitive function and LTL, positive associations were small in magnitude, and some were even in the opposite direction from what was hypothesized (89). A report from the Health,

Table 2. Studies of the Association of Leukocyte Telomere Length With Mortality Rate

First Author, Year (Reference No.)	Study	No.	Measurement Method	Population				Standard Deviation, years	Follow-up, years	Mean (SD)	Association
				Ethnicity	Gender	Mean Age, years	Age Range, years				
Cawthon, 2003 (94)	Utah residents	143	PCR	Caucasian	Men/ women	N/A	60–97		15		HR for death for bottom half of LTL vs. top half = 1.86 (95% CI: 1.22, 2.83); HR for cardiac death = 3.18 (95% CI: 1.36, 7.45); HR for infectious death = 8.54 (95% CI: 1.52, 47.9)
Martin-Ruiz, 2005 (100)	Leiden 85-plus Study	598	PCR	Dutch	Men/ women	89.8	85–101		14		LTL not associated with rates of all-cause, cardiac, infectious, or cancer mortality (all $P > 0.4$)
Bischoff, 2006 (101)	Longitudinal Study of Aging Danish Twins, Danish 1905 Cohort Study, Longitudinal Danish Centenarian Study	812	Southern blot	Danish	Men/ women	82	73–101		8		HR for death per 1-kb shorter LTL, men = 0.97 (95% CI: 0.83, 1.14); women = 0.93 (95% CI: 0.85, 1.03)
Harris, 2006 (66)	Lothian Birth Cohort	190	PCR	Scottish	Men/ women	79.1		0.6	5		LTL dead subjects = 6.40 (SD, 1.61); LTL alive subjects = 6.68 (SD, 1.72); HR for death not significant
Bakaysa, 2007 (96)	Swedish Twin Registry	700	Southern blot	Swedish twin pairs	Men/ women	78.8		7.8		6.9 (3.1)	HR for death for twins with shortest LTL vs. twins with longest LTL = 2.8 (95% CI: 1.1, 7.3)
Fitzpatrick, 2007 (58)	Cardiovascular Health Study	419	Southern blot	Caucasian/ African American	Men/ women	74.2		5.2	10		HR for death per 1-kb shorter LTL = 1.22 (95% CI: 0.91, 1.63)
Kimura, 2008 (95)	Danish Twin Registry	548	Southern blot	Same-sex Danish twins	Men/ women		73–94		10		Overall intrapair comparison for 204 same-sex twin pairs in which 1 or both co-twins died = 0.56, $P = 0.08$; using mode of LTL = 0.60, $P = 0.006$; using shortest 25% LTL = 0.59, $P = 0.014$
Ehrlenbach, 2009 (98)	Bruneck Study	510	PCR	Italian	Men/ women	62	53–71		10		T/S ratio of 1.37 for living subjects longer than the T/S ratio of 0.98 for deceased subjects, $P < 0.05$

Table Continues

Table 2. Continued

First Author, Year (Reference No.)	Study	No.	Measurement Method	Population				Standard Deviation, years	Follow-up, years	Mean (SD)	Association
				Ethnicity	Gender	Mean Age, years	Age Range, years				
Epel, 2009 (97)	MacArthur Health Aging Study	236	PCR	Caucasian	Men/ women	73.7	70–79		12		Odds ratio for overall mortality not significant; odds ratio for cardiovascular death of women = 2.3 (95% CI: 1.0, 5.3)
Njajou, 2009 (102)	Health, Aging, and Body Composition Study	2,721	PCR	Caucasian/ African American	Men/ women	74	70–79		10		HR for death per 1-kb shorter LTL = 1.0 (95% CI: 0.9, 1.1)
Fitzpatrick, 2011 (99)	Cardiovascular Health Study	1,136	Southern blot	Caucasian/ African American	Men/ women	73.9		4.7	8.1		HR for death of shortest quartile LTL vs. longest quartile LTL = 1.61 (95% CI: 1.22, 2.12); HR for cardiac death = 1.82 (95% CI: 0.95, 3.49); HR for infectious death = 2.80 (95% CI: 1.32, 5.94)

Abbreviations: CI, confidence interval; HR, hazard ratio; kb, kilobase pairs; LTL, leukocyte telomere length; N/A, not available; PCR, polymerase chain reaction; SD, standard deviation; T/S, ratio of telomere repeat copy number to single copy gene copy number.

Aging, and Body Composition Study, where LTL was examined in association with cognitive function in 2,734 nondemented community-dwelling older adults, provided inconclusive results (90). At baseline, longer LTL was associated with a better Digit Symbol Substitution Test score (36.4, 34.9, and 34.4 points for groups of long, medium, and short LTL; $P < 0.01$) but not with change in score. However, 7-year Modified Mini-Mental Status Examination change scores were lower among those with longer telomere length (−1.7 points vs. −2.5 and −2.9; $P = 0.01$). Furthermore, LTL is likely not associated with the apolipoprotein E (*ApoE*) genotype, the strongest genetic risk factor for Alzheimer's disease (90, 91).

Because disease can be undiagnosed and can exist in several tissues simultaneously, LTL was studied in association with an index of disease burden that had been shown previously to be a strong predictor of death and incident disability independent of age, sex, race, and diagnosed disease (92). The index of disease burden tabulates age-related chronic disease in 5 physiological systems regardless of diagnosis and includes measurement of carotid intima-media thickness, lung vital capacity, white matter grade, cystatin C, and fasting glucose. To the extent that an index across systems might capture an underlying propensity to age-related changes in all systems, a marker of fundamental aging processes such as LTL should be associated with it. LTL was associated with this index of disease burden ($\beta = -132$ (standard error, 47) base pairs; $P < 0.01$ for each standard deviation in disease burden score) but not with a count of diagnosed chronic conditions (93). This departure from studying individual diagnosed conditions might indicate that LTL could illustrate widespread incremental changes in structure or function in older adults independent of diagnosed disease, but this hypothesis requires corroboration.

Telomere length has been used in several epidemiologic studies as a predictor of lifespan or death (Table 2). Cawthon et al. (94) first reported an association between shorter LTL and increased risk of death in individuals aged ≥60 years (HR = 1.86, 95% CI: 1.22, 2.83). In that study, the increased mortality rate was specifically due to higher rates of death from heart disease (HR = 3.18, 95% CI: 1.36, 7.45) and infectious disease (HR = 8.54, 95% CI: 1.52, 47.9), although the sample size was small ($n = 143$). Since that report was published, 5 other studies have found that shorter LTL is associated with an increased mortality rate (95–99), and 5 have not (58, 66, 100–102). Of note, Epel et al. (97) found that shorter LTL was associated with cardiovascular disease-specific death in women (HR = 2.3, 95% CI: 1.0, 5.3) but not in men (HR = 1.2, 95% CI: 0.6, 2.6) and not with overall death. In most of these studies, models have been minimally adjusted, typically only for age and occasionally for sex or race. Differences in measurement methods for LTL (Southern blot vs. quantitative PCR) could contribute to inconsistency between studies. Furthermore, with increasing age, the variability in LTL in a population can decrease (100, 101). Because these studies were conducted in individuals who were on average older than 60 years, it is possible that the studies had reduced power to detect associations between LTL and death. In

sum, LTL seems to be weakly associated with overall risk of death and possibly more strongly with cardiovascular disease- or infectious disease-specific death.

Finally, several longitudinal studies have been conducted to determine predictors of change in LTL over time (Table 3) (73, 97, 98, 103–106). In these studies, time between samples of LTL ranged from 2.5 to 10 years. All except one (73) relied on quantitative PCR to measure LTL. All found that baseline LTL was the strongest and often the only predictor of the subsequent LTL measurement, and several noted that 11%–34% of the population increased in LTL during the time period (73, 98, 103, 105, 106). In patients with stable coronary artery disease, Farzaneh-Far et al. (105) found that higher omega-3 fatty acid levels were associated with less decline in LTL, although this has not been replicated.

Although studies of changes in LTL have the potential to provide the strongest evidence for what LTL might reflect in humans, those published to date have substantial methodological flaws (49, 107). In particular, the time between telomere measurements was short, which resulted in small changes in mean LTL during the follow-up period. If telomere attrition in late life is approximately 30–100 base pairs per year (49), 5 years of change would result in only 150–500 base pairs of shortening, which was generally observed in these longitudinal studies. Given that interindividual variation in LTL is approximately 5,000–10,000 base pairs (depending on the mean age of the population) and that most longitudinal studies of LTL change have used quantitative PCR for measurement (which has a higher coefficient of variation) and have had a small sample size, it is highly likely that these studies were underpowered to detect possible associations between change in LTL and outcomes because of the degree of measurement variability. In fact, it is possible that the changes in LTL observed during the follow-up periods were partly or mostly artifacts from measurement error (49). Furthermore, the change in LTL is not necessarily independent of the baseline length (108). If the change is partly conditioned on the baseline value, spurious associations can be found between the baseline length and the change, which can also obscure other associations by accounting for a large part of the variance in the change. Longitudinal studies with much longer follow-up times and larger sample sizes are necessary to convincingly determine predictors of change in LTL and what change in LTL might predict.

CONCLUSIONS AND RECOMMENDATIONS FOR DESIGN OF FUTURE EPIDEMIOLOGIC STUDIES ON TELOMERE LENGTH

Is telomere length a biomarker of aging? With regard to measurement, telomere length can be assayed with minimal harm to humans, but advances must be made in measurement methods to achieve reproducibility, accuracy, and efficiency simultaneously. With regard to biological plausibility and predicting the rate of aging, data derived from in vitro and in vivo studies strongly suggest that telomere length reflects levels of cellular senescence and oxidative stress, but data from epidemiologic and clinical studies are less conclusive. Although shorter LTL seems to be associated with older age, male gender, and Caucasian race, LTL has been inconsistently associated or not associated with other characteristics of aging or age-related chronic disease, including death. Inconsistency might be due partly to differences among study populations, measurement methods, and statistical modeling, although it also could imply that an association does not exist. Animal studies have provided important data on telomere dynamics and the impact of a minimum telomere length on organismal health, but because telomere length is highly species specific and alterations in model systems do not appear like normal aging, it is difficult to extrapolate conclusions from animal models to humans. Currently, it remains unknown whether, in human populations, telomere length is a biomarker of aging for a whole organism or a biomarker of aging in specific tissues. The data suggest that, overall, if telomere length is a biomarker of human aging, it is a weak biomarker with poor predictive accuracy compared with many traditional covariates.

Salient questions that remain unanswered include the following: What is the normal rate of telomere shortening in a human population? What is the variance in the rate of telomere shortening? Which is the most biologically relevant metric of telomere length: average length, shortest length, etc.? What risk factors accelerate telomere shortening, and what protective factors ameliorate shortening? Is the rate of telomere shortening predictive of specific age-associated outcomes or overall organismal aging measured by different constructs? If there truly is a dichotomy between aging and cancer, is telomere shortening the central regulator of this dichotomy? Is oxidation or inflammation the real connection between telomere length and aging? Answering these questions requires longitudinal measurement of telomere length, likely over decades, in well-characterized populations that measure social, behavioral, medical, and biological factors.

Because telomere length is heritable, shortens throughout life, can be influenced by unknown risk factors with unclear sensitive periods, and can affect aging differently over the life of an organism, it is an ideal candidate for life-course analysis. As it applies to chronic disease, life-course analysis is "the study of long-term effects on chronic disease risk of physical and social exposures during gestation, childhood, adolescence, young adulthood and later adult life. It includes studies of the biologic, behavioral, and psychosocial pathways that operate across an individual's life course, as well as across generations, to influence the development of chronic diseases" (109, p. 285). Life-course analysis incorporates hierarchical, temporal, and path relations among exposures, outcomes, moderators, and mediators. A single factor can be included at different points along the model. If theoretical life-course models are operationalized and tested carefully, they have the potential to represent etiology more accurately. Follow-up of existing cohorts into advanced old age would allow epidemiologists to conduct life-course analyses for aging, particularly because retrospective ascertainment of life-course data is available to construct theoretical models (109, 110). Studying telomere dynamics in human populations

Table 3. Studies of Longitudinal Change in Leukocyte Telomere Length

First Author, Year (Reference No.)	Study	No.	Measurement Method	Population				Baseline LTL, kb	Attrition Rate, base pairs/year	Years of Follow-up	Findings
				Ethnicity	Gender	Mean Age, years	Age Range, years				
Aviv, 2009 (73)	Bogalusa Heart Study	635	Southern blot	White/African American	Men/women	31	20–40	7.3 (white), 7.8 (African American)	38 (white), 48 (African American)	6 (white), 5.7 (African American)	11% of participants ↑ LTL; baseline LTL only significant predictor of attrition
Epel, 2009 (97)	MacArthur Health Aging Study	236	PCR	White	Men/women	74	70–79	4.7	Not reported	2.5	Baseline LTL strongest predictor of attrition; mortality potentially associated with attrition
Nordfjäll, 2009 (103)	Swedish cancer case-control studies	959	PCR	Swedish cancer cases and controls	Men/women		30–61	—[a]		10	34% participants ↑ LTL; baseline LTL only significant predictor of attrition
Ehrlenbach, 2009 (98)	Bruneck Study	510	PCR	Italian	Men/women	62	53–71	8.0	46	10	16% of participants ↑ LTL; baseline LTL only significant predictor of attrition
Farzaneh-Far, 2010 (104, 105)	Heart and Soul Study	608	PCR	Stable coronary artery disease	Men/women	66		5.5	42	5	23% of participants ↑ LTL; baseline LTL strongest predictor of attrition; omega-3, age, male gender, and waist/hip ratio associated with attrition
Houben, 2011 (106)	Zutphen Elderly Study	75	PCR	Dutch	Men	78	73–91	5	40	7	16% of participants ↑ LTL; baseline and follow-up LTL significantly correlated ($r = 0.51$, $P < 0.001$)

Abbreviations: kb, kilobases; LTL, leukocyte telomere length; PCR, polymerase chain reaction.
[a] —, no averages reported.

by using a life-course approach could be highly advantageous.

Another epidemiologic technique uncommonly applied to the study of telomere length is examining extreme populations, such as individuals with progeroid syndromes or centenarians. One often finds interesting associations at the extreme because there is a large difference between the mean of the general population and the mean of outliers. Gathering a small number of people at an extreme might create a more homogeneous phenotype, dampening the noise effect that can obscure associations and reduce statistical power. Progeroid syndromes (e.g., Werner's syndrome, Hutchinson-Gilford progeria syndrome, xeroderma pigmentosum) are often considered models of accelerated aging (111–114). Progeroid syndromes are useful for studying the biology of aging because distinct molecular pathways are altered in these syndromes, providing clues to underlying mechanisms of how telomeres could influence aging. The drawbacks of studying progeroid syndromes are the small numbers of patients and that aspects of their phenotypic presentation are different from those of "normal" aging.

Epidemiologists might be more poised to study telomere length in people who age slowly. These people have been described as exhibiting longevity, healthy aging, or exceptional survival, among other terms that are not necessarily interchangeable. Opportunities to study the epidemiology of healthy aging are increasing rapidly (115). The key to using these studies is defining the appropriate control group, particularly because it can be difficult to define controls from the same birth cohort as long-lived individuals. One approach is to define an internal control group with different functional capacity and disease burden compared with cases. For example, LTL was examined in 36 members of the New England Centenarian Study, specifically comparing 19 "healthy" centenarians who had physical function in the independent range and did not have hypertension, congestive heart failure, myocardial infarction, peripheral vascular disease, dementia, cancer, stroke, chronic obstructive pulmonary disease, or diabetes with 19 "unhealthy" centenarians who had physical function limitations and ≥ 2 of the above conditions. Healthy centenarians had significantly longer telomeres than did unhealthy centenarians ($P = 0.0475$) (116). Another approach is to follow general population cohorts for years, pool cohort data to achieve a sufficiently large sample of long-lived individuals, and compare those long-lived individuals with people in the pooled sample who died earlier. This approach was used in the Cohorts for Heart and Aging Research in the Genomic Epidemiology (CHARGE) Consortium to create a longevity phenotype for genome-wide association studies (117). CHARGE data were used in a genome-wide association study of LTL, which identified novel loci that could influence LTL (19). One can also compare populations specifically selected for longevity with populations not selected for longevity. For example, the Long Life Family Study used specific criteria to define longevity and recruited nearly 5,000 individuals clustered in families with exceptional longevity and their spouses. Long Life Family Study participants exhibited better health than their age-matched controls from population-based cohorts not selected for longevity, such as the Framingham Heart Study and the Cardiovascular Health Study (118). LTL has been measured in the Long Life Family Study, and analysis of LTL heritability, genetic associations, and associations with other phenotypes is underway. Finally, unique populations that are "self-selected" for longevity also can be compared with populations not "self-selected" for longevity. These unique populations include the Seventh-Day Adventists, a self-selected cohort who adhere to strict dietary, social, and religious customs, and the Okinawan Japanese, a geographically and ethnically defined cohort who also have a unique dietary, social, and religious makeup. These populations exhibit exceptional longevity compared with that of general population references (119–121). LTL has not been examined in these groups. Clearly, many opportunities exist to explore LTL among the extremes of healthy aging.

In the future, epidemiologists who study telomere length as it relates to aging will use several tools to probe human biology more sensitively. At the epicenter of the toolbox will be classic epidemiologic techniques used in artful ways, like studying the extremes of the population and doing so over extended timeframes. Furthermore, better assays will enable researchers to quantify LTL more precisely and accurately. High-throughput systems will allow epidemiologists to link large populations with deep phenotyping to variations in molecules and genes that could influence telomere length. Finally, advancing statistical techniques, such as joint modeling, longitudinal trajectory latent class analysis, mixed modeling, and life-course analysis, will shift our rudimentary representation of biology toward a more sophisticated representation that leverages the strengths of longitudinal data. With these new tools, epidemiologists will help to uncover the role of telomere length in living a healthy, long life.

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