Review

Markus Herrmann*, Irene Pusceddu, Winfried März and Wolfgang Herrmann

Telomere biology and age-related diseases

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Abstract: Telomeres are the protective end caps of chromosomes and shorten with every cell division. Telomere length has been proposed as a biomarker of biological age and a risk factor for age-related diseases. Epidemiologic studies show an association between leukocyte telomere length (LTL) and mortality. There is solid evidence that links LTL with cardiovascular disease. Short telomeres promote atherosclerosis and impair the repair of vascular lesions. Alzheimer's disease patients have also a reduced LTL. Telomeres measured in tumor tissue from breast, colon and prostate are shorter than in healthy tissue from the same organ and the same patient. In healthy tissue directly adjacent to these tumors, telomeres are also shorter than in cells that are more distant from the cancerous lesion. A reduced telomere length in cancer tissue from breast, colon and prostate is associated with an advanced disease state at diagnosis, faster disease progression and poorer survival. By contrast, results regarding LTL and cancer are inconsistent. Furthermore, the majority of studies did not find significant associations between LTL, bone mineral density (BMD) and osteoporosis. The present manuscript gives an overview about our current understanding of telomere biology and reviews existing knowledge regarding the relationship between telomere length and age-related diseases.

Keywords: age-related diseases; biological age; senescence; telomere length; telomeres.

***Corresponding author: Prof. Dr. med. habil. Markus Herrmann,** Department of Clinical Pathology, Bolzano Hospital, Lorenz-Boehler-Str. 5, 39100 Bolzano, Italy, E-mail: markus.herrmann@medunigraz.at; and Clinical Instituite for Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Auenbruggerplatz 15/1 8036 Graz, Austria
Irene Pusceddu: Laboratory of Clinical Pathology, Hospital of Bolzano, Bolzano, Italy
Winfried März: Medical Clinic V (Nephrology, Hypertensiology, Rheumatology, Endocrinology, Diabetology), Medical Faculty of Mannheim, University of Heidelberg, Mannheim, Germany; Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria; and Synlab Academy, Synlab Holding Deutschland GmbH, Mannheim, Germany
Wolfgang Herrmann: Department of Clinical Chemistry, University of Saarland, Homburg, Germany

Introduction

Biological age describes the functional status of the body relative to its chronological age. For over half a century researchers have tried to estimate the biological age of individuals using test batteries [1–4]. These test batteries assess a range of physiological, psychological and cognitive functions. Today, blood biomarkers are increasingly used as they provide complementary information that help to predict an individual's biological age better.

Deterioration of genomic integrity and genomic instability are key aspects of aging [5, 6]. Telomeres represent the protective end caps of telomeres that are of critical importance for genomic integrity and stability. The continuous shortening of telomeres with increasing age has attracted a lot of interest in recent years [7]. Telomere length has been considered a 'biological clock' that is useful for the establishment of an individual's biological age [8]. In addition, the measurement of telomere length may help to estimate the risk for age-related disease. The present review provides an overview about the structure and function of telomeres, the relationship between telomere length and age-related diseases, and analytical aspects.

Telomeres – structure and function

Telomeres, from Greek telos 'end' and meros 'part', are nucleoprotein structures located at both ends of a chromosome. They are composed by a non-coding, repetitive DNA sequence and associated proteins that form the shelterin complex. Telomeric DNA is double-stranded for most of its length with a short, G-rich, single-stranded overhang at the 3′-OH end. With the help of the shelterin proteins telomeric DNA folds into a complex three-dimensional structure, which is important for telomere function [7, 8].

Telomeres are evolutionary conserved and were first identified in 1938 by Hermann Müller. Subsequently, the Nobel-prize winners Müller and McClintock identified the protective function of telomeres [9, 10]. The first human telomeres were isolated in 1988 by Moyzis et al. [11].

Milestones in the discovery of telomere biology are shown in Figure 1.

In humans, telomeres span several kilobase pairs (kbp) and are composed by thousands of repeats of the hexanucleotide TTAGGG [7, 8] (Figure 2). They have two pivotal functions. On one side telomeres are involved in the protection of chromosomal ends, as they prevent unwanted recombination and degradation. On the other side, telomeres play an important role during DNA replication as they prevent the loss of coding DNA. The three-dimensional folding of telomeres protects the free 3′-OH end of each DNA strand from being recognized as a double strand break (DSB). Dysfunctional telomeres activate the DNA damage response (DDR) cascade and drive cells into senescence [8, 13, 14]. It has been shown that the removal of telomeres from yeast chromosomes causes a dramatic loss of DNA [15].

The proteins of the shelterin-complex are involved in the control of telomere length by regulating the access of telomerase to the G-strand overhang and by protecting it from degradation [13, 14]. In addition, with the help of shelterin proteins end-to-end fusions of chromosomes are prevented. The shelterin complex is composed of six telomere-specific proteins: TRF1 (Telomeric Repeat Binding Factor 1), TRF2, RAP1 (Repressor/Activator Protein 1), TIN2 (TRF1 Interacting Nuclear Factor 2), POT1 (Protector of Telomeres 1) und TPP1 (POT1-TIN2 organizing protein) [16]. With the help of the shelterin complex the 3′G rich single-stranded DNA overhang at the very end of all telomeres can fold back and anneal with double-stranded telomeric DNA forming a loop structure known as T-loop (Figure 2). The closed configuration of the T-loop hides the free 3′-OH end at the very end of each DNA-strand thus avoiding it from being recognized as a DSB [7, 8]. Because of its shape and function the T-loop can be seen as a protective cap that defines the natural end of a chromosome. The thymidine- and guanine-rich hexanucleotide of human telomeres is believed to form a quadruple helix [7, 8]. First, the 3′-OH strand overhang pairs with itself by forming abnormal GG-double bonds. The resulting double strand pairs again with itself forming a quadruple helix in which the guanine residues participate in Hoogsten bonds [7, 8].

Every division of a somatic cell is accompanied by a loss of 50–200 bp of telomeric DNA resulting in a

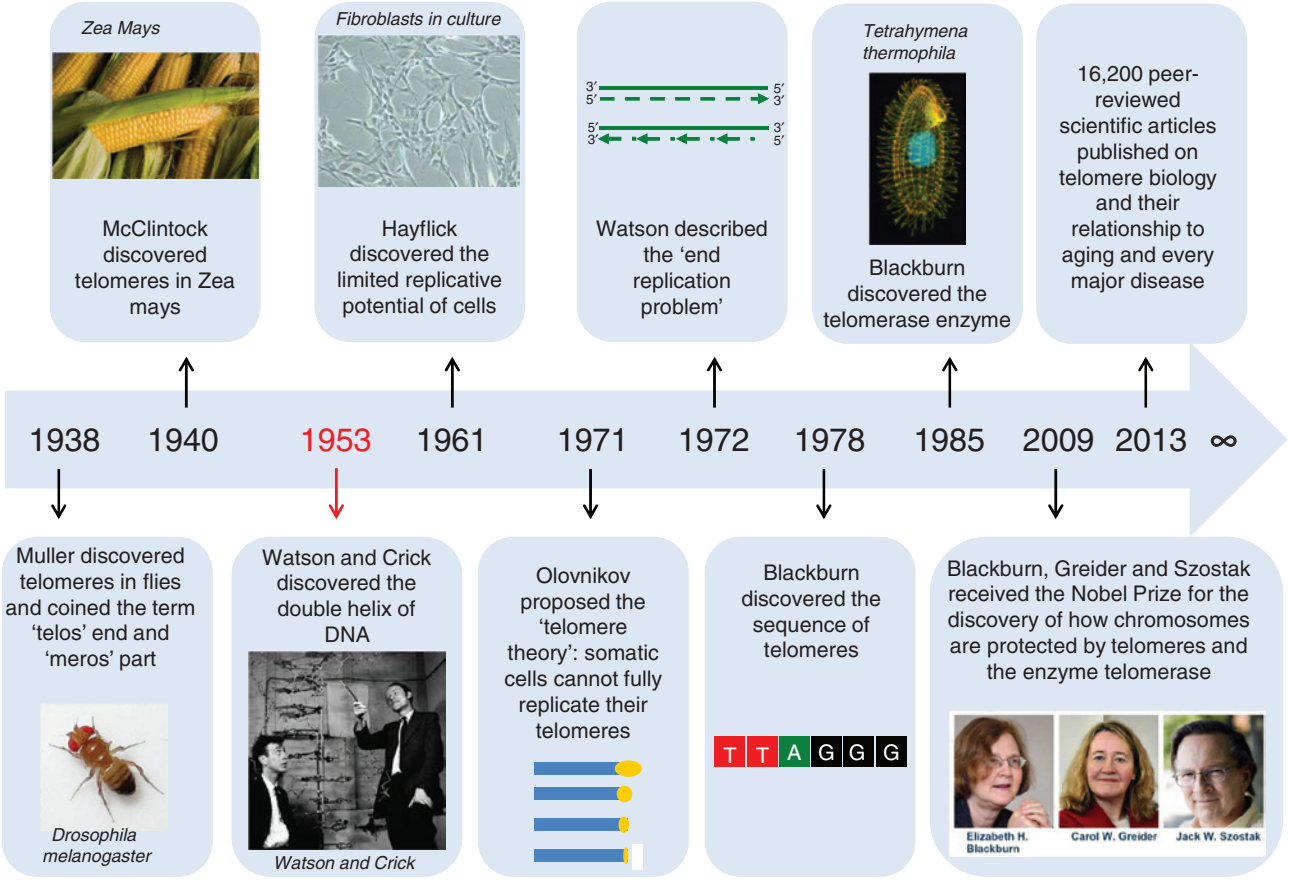

Figure 1: Milestones in telomere biology discovery.

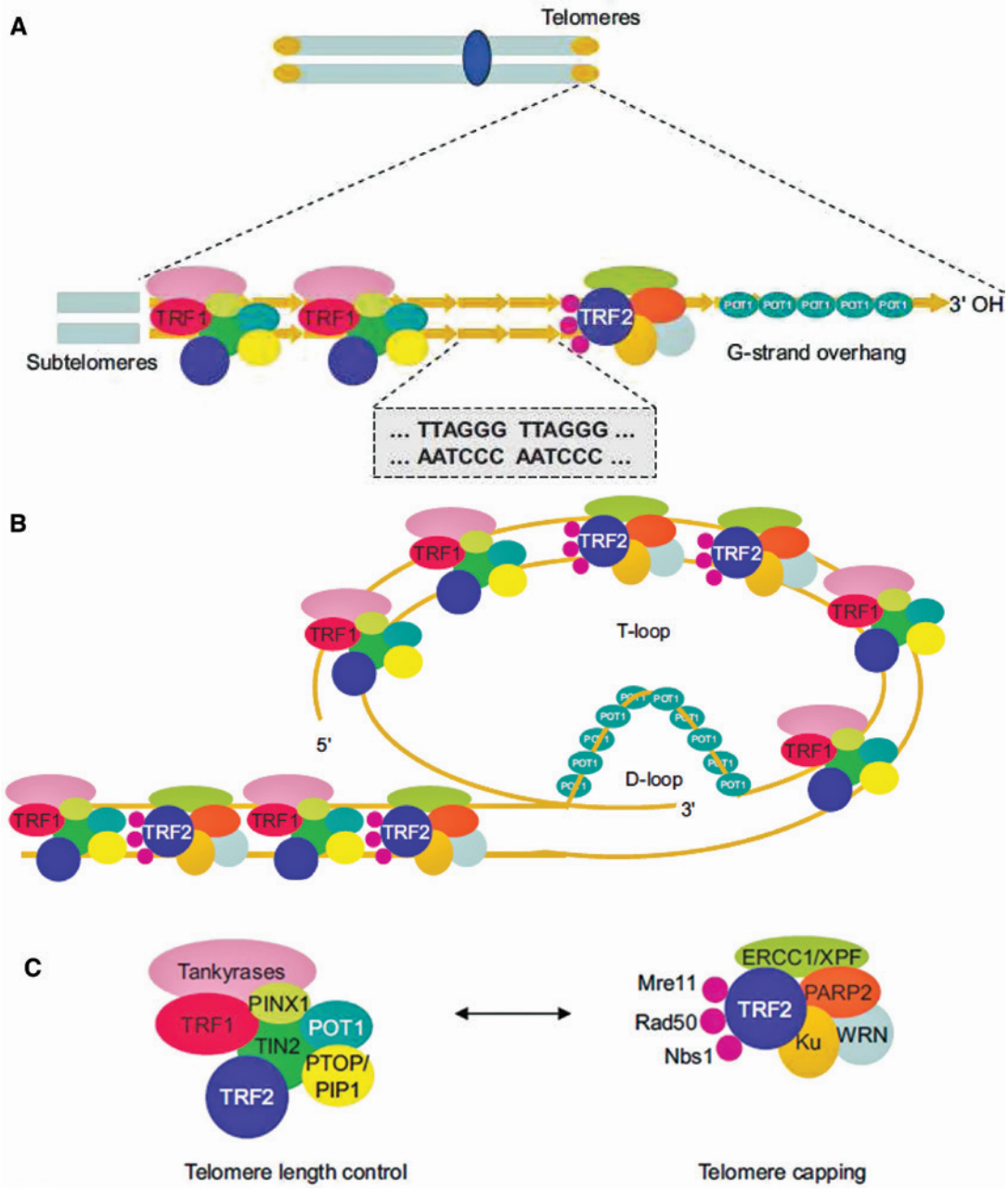

Figure 2: Telomere structure.
(A) Telomeres are composed by a double strand region of –TTAGGG– repetitions and by a single strand region called G-strand overhang. Two protein complexes are bound to telomeres, the telomere repeat binding factor 1 (TRF1) complex and the telomere repeat binding factor 2 (TRF2) complex. (B) The G-strand overhand can fold back and invades the double strand region leading to the formation of T-loop and D-loop structures. The resulting 3D conformation protects the 3′OH end of the chromosome. (C) Composition of the two main telomere-associated protein complexes. The TRF1 complex is involved in telomere length control, whereas the TRF2 complex functions as protective end cap of telomeres. Modified from Pusceddu et al. [12].

continuous shortening of telomeres. The incomplete DNA replication at the 5′-end of all newly synthesized daughter strands is due to a phenomenon called end-replication problem [7, 8, 17]. DNA replication always occurs in 5′ → 3′ direction and is catalyzed by DNA polymerase. However, the enzyme can only add nucleotides to a free 3′-OH end of an existing nucleotide. At the very beginning of a DNA strand there is no such 3′-OH group to which DNA polymerase can add nucleotides. Therefore, RNA primers that provide a 3′-OH group are needed to start DNA synthesis. Once replication is complete, primers are removed and the resulting gaps are filled with DNA. However, at the 5′-end

of each daughter strand, the space formerly occupied by the primer cannot be filled with DNA, as a free 3′-OH group is lacking [17, 18].

In the absence of telomeres, there would be inevitable loss of genetic information from the leading strand with every mitosis. As telomeres are a non-coding sequence, but are composed by repetitive elements, telomere shortening avoids the loss of genetic information. The consequence of telomere shortening is that somatic cells can undergo only a defined number of divisions before telomeres become critically short and lose their protective properties [7, 13, 14]. When a critical telomere length of approximately 4 kbp is reached, cells cannot divide anymore and undergo senescence or apoptosis. Consequently, telomere shortening limits the life span of cells and is an effective tumor suppressor mechanism. When cells with critically short telomeres continue to divide, such as in some cancer cells, chromosomes become instable. Because of the physiologic telomere shortening in somatic cells telomere length can be considered as a mitotic or molecular clock [19].

Telomerase

In contrast to most somatic cells, hematopoietic stem cells, keratinocytes in the basal layer of the epidermis, uterine endometrial cells, germ cells and various tumors avoid telomere shortening by activation of telomerase [20, 21]. Telomerase is a ribonucleoprotein with reverse transcription activity, which adds *de novo* telomere hexanucleotide repeats to the chromosomal ends [7, 13, 14]. Telomerase contains a highly conserved reverse transcriptase (human telomerase reverse transcriptase, hTERT), an associated template RNA (telomerase RNA component, TERC) and a key auxiliary protein known as Dyskerin. Inducing telomerase activity in primary human fibroblasts by retroviral gene transfer is sufficient to counteract telomere erosion and to prevent cells from entering senescence [7, 13, 14]. The resulting maintenance of telomere length immortalizes most human cell types. In some mammalian cancer cells and immortalized cell lines, telomeres are extended in a telomerase-independent manner, called ALT (Alternative Lengthening of Telomeres). It has been suggested that ALT mechanisms rely on the homologous DNA recombination between telomeric sequences [7, 13, 14].

For many years telomeres were viewed as transcriptionally inert. However, transcription of the C-strand of telomeres by RNA polymerase II produces long UUAGGG-containing transcripts, known as TERRA (telomeric repeat-containing RNA) that seems to have structural functions [7, 8].

Epigenetics – DNA and histone methylation

In addition to the 3D conformational status and the shelterin complex, a third mechanism known as epigenetics, regulates the function of telomeres. Epigenetics include DNA methylation, predominantly at CpG islands and histone modifications, such as methylation and acetylation. In humans, three main DNA methyltransferases (DNMTs) are responsible of the methylation status of DNA. DNMT1 functions as a maintenance DNMT that copies parental strand DNA methylation onto the daughter strand after replication. DNMT3a and DNMT3b function as *de novo* DNMTs. Human telomere repeats cannot be methylated because they lack CpG sequences, which are the substrates for DNMTs. However, the subtelomeric region contains a high proportion of CpG dinucleotides, which are heavily methylated. For example, DNMT knockdown mice models are characterized by hypomethylation of subtelomeres and abnormal elongation of telomeres. Reintroduction of DNMT3a and DNMT3b in these cells results in the re-methylation of subtelomeric domains and decreased telomere recombination [22–24]. In addition, methylation is not equally distributed throughout the genome indicating that DNA methylation regulates distinct biologic functions. Furthermore, telomeres and subtelomeres are bound by nucleosomes that are enriched in trimethylated histone 3 Lysine 9 (H3K9). With the help of Histone-Methyltransferases (HMT) and Histone-Demethylases (HDM) histones can be methylated and demethylated at lysine and arginine residues. Cells that lack the HMT are characterized by decreased levels of histone trimethylation and by aberrant telomere elongation. Moreover, it has been shown that progressive telomere shortening leads to epigenetic changes, both in telomeric and subtelomeric regions [22–24]. However, most of these studies were performed in mice models and the real effect in humans needs to be further elucidated.

Telomere length – influencing factors

It is well established that telomeres shorten with age. This phenomenon is the result of the end-replication problem [25]. Mean LTL at birth is 11 kbp and declines to less then 4 kbp in elderly individuals. A lot of factors have been related to LTL, such as gender, physical activity, smoking,

body mass index (BMI), alcohol consumption, hormone replacement therapy, dietary antioxidants, vitamins, trace elements, chronic inflammation, socioeconomic status, perceived stress levels, and paternal age [25–41]. For example, telomeres are longer in women, as the consequence of higher estrogen levels, that increase telomerase activity and have antioxidant effects. Similarly, psychological stress affects cellular aging through oxidative stress and telomerase activity. Highly stressed women are characterized by lower telomerase activity and higher oxidative stress compared to women with a low stress level [39–41]. In addition, regular physical activity has also been associated with decreased levels of oxidative stress and inflammation, which affect telomere shortening. For example, moderate and vigorous-intensity activity were associated with increased LTL, and telomerase activity was higher among trained athletes and after 3-month of lifestyle intervention, which included the association of moderate aerobic exercise [41].

Finally, several nutritional factors like vitamins (including folate, nicotinamide, vitamin A, B12, D, C and E), minerals (including magnesium, zinc and iron) and other bioactive dietary components (like omega-3 fatty acid, polyphenols and curcumin), are able to directly or indirectly influence LTL trough several mechanisms. Recent studies have shown consistent associations between LTL and the availability of B and D vitamins [12, 27–38]. Preliminary results from own studies demonstrate that serum folate and its metabolites correlate with LTL (Figure 3) [29]. In agreement with this observation also HCY, a functional marker of folate and vitamin B12 availability, is consistently correlated with LTL (Figure 3). Antioxidant activity, DNA methylation and prevention of DNA damage are the most important mechanisms trough which these nutritional factors slow down telomere attrition [12, 27]. In summary, a healthy lifestyle with a diet rich in fruits and vegetables combined with exercise, lower BMI and no smoking is associated with longer telomeres.

Telomeres and age-related diseases

Short telomeres and telomere dysfunction have been linked to numerous disorders. Several genetic disorders with mutations in genes involved in the expression and regulation of shelterins, telomerase or other telomere-related proteins, have been described [26, 42]. All these diseases are characterized by an accelerated rate of telomere shortening. For example, aplastic anemia, dyskeratosis congenital, idiopatic pulmonary fibrosis are all characterized by mutations in TERC, TERT or dyskerin [26, 42]. In epidemiologic studies, individuals with shorter telomeres are characterized by a higher mortality rate, which is nearly twice as high as in those with longer telomeres (Figure 4) [43]. For example, Mons et al. [45] analyzing more than 12,000 subjects of two population-based studies (ESTHER and Nurses' Health Study), identified

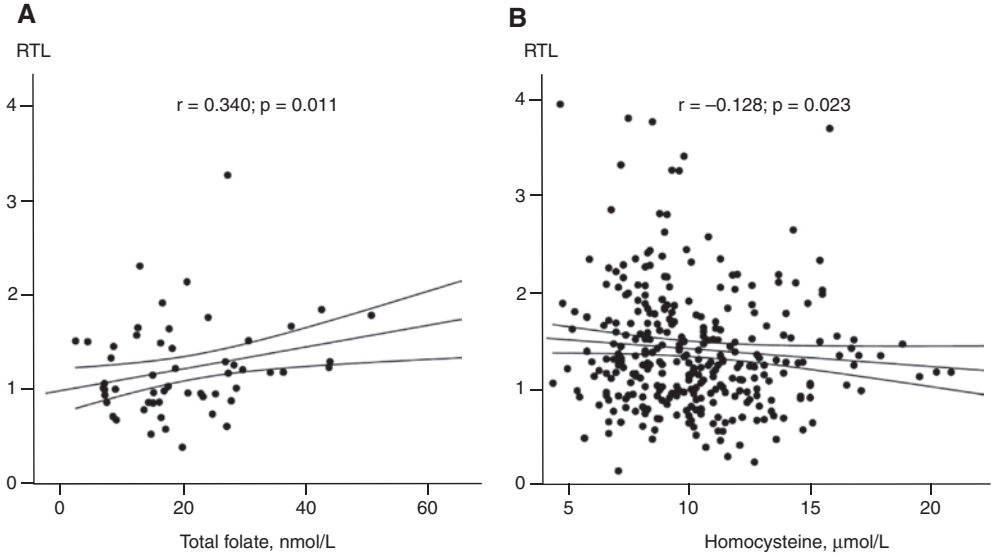

Figure 3: Relative telomere length and markers of B vitamin status.
(A) Correlation between relative telomere length and total folate in 53 elderly subjects of the KNOVIB study [29]. (B) Correlation between relative telomere length and homocysteine in 336 subjects of the STVS study (not published).

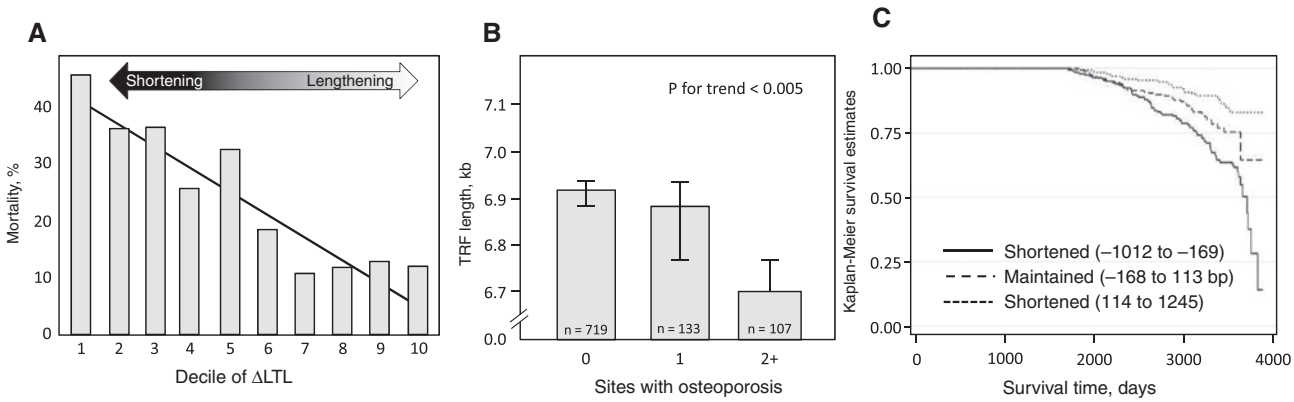

Figure 4: Telomere length, mortality and age-related disease.
(A) Mortality by decile of 5-year change in telomere length (p for trend <0.001) [43]. (B) Telomere restriction fragment (TRF) length among healthy women aged 50 and older with clinical osteoporosis at one and two or more sites. Means and standard errors are adjusted for age, smoking status, body mass index, HRT and menopausal status [44]. (C) Survival in 608 patients with coronary heart disease, stratified by change in telomere length during the previous 5 years (overall log rank test $p < 0.0001$) [43].

that subjects with shorter telomeres (1st quintile) were characterized by higher hazard ratio for all-cause mortality (1.66, 95%CI 1.09–2.53, $p = 0.018$) compared to those with higher telomere length (5th quintile). In addition, a reduced LTL may indicate an existing or an elevated risk for future age-related disease, such as cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM), neurodegenerative diseases, osteoporosis and premature aging syndromes [42, 43, 46–49].

Osteoporosis

Similar to many other tissues, telomeres shorten with age in bone (osteoblasts) and mesenchymal stem cells (MSCs). In addition, premature aging syndromes, such as Werner syndrome (Wrn) and congenital dyskeratosis, are characterized by telomere dysfunction and osteoporosis. Because of these facts it has been hypothesized that telomere shortening might contribute to the aging of bone. A first large scale epidemiologic from Valdes et al. [44] showed a significant correlation between LTL and bone mineral density (BMD) at the spine and forearm. In this study, longer telomeres were associated with a reduced risk of clinical osteoporosis. The study included 2150 unselected women from a population-based twin cohort (age range 18–79 years). In women over the age of 50, the presence of osteoporosis was associated with shorter telomeres. The difference in LTL between women with and without osteoporosis was equivalent to five years of telomeric aging (Figure 4). In a small prospective observational study in elderly men (age range 71–86 years) LTL correlated with bone loss over time at different distal forearm sites [50]. Animal studies in genetically modified mice suggest that replicative aging of osteoblast precursors promotes bone loss and the occurrence of senile osteoporosis [51]. However, several other human studies have not been able to show significant relationships between LTL, BMD and osteoporosis [52–54]. Sanders et al. [54] studied 2750 community-dwelling women from the health, aging, and body composition study for 7 years without finding a significant relationship between LTL, BMD, bone loss, prevalent and incident fractures. These results are supported by a similar study in 1867 community-dwelling Chinese women that did not find significant relationships between LTL, BMD and bone loss [53]. Furthermore, *in vitro* studies with human osteoblasts and the measurement of LTL from osteoporotic and control subjects do not support the concept of premature cellular aging and accelerated telomere shortening in patients with senile osteoporosis [55]. In conclusion, existing human data are inconsistent and the majority of studies did not find associations between LTL, BMD and osteoporosis.

CVD

CVD is among the most frequent age-related diseases and the number one cause of death. Several studies have shown that a high rate of telomere attrition is associated with an elevated risk of coronary artery disease, myocardial infarction, heart failure and stroke [47]. The large scale prospective WOSCOPS study demonstrated a 44% increase in coronary artery events during 5.5 years of follow-up in individuals in the first quartile of LTL (shortest

telomeres) compared to individuals in the fourth quartile (longest telomeres) [56]. Individuals with early myocardial infarction have also been found with a reduced LTL compared to age matched healthy controls [57]. The LTL of patients who suffered a myocardial infarction is comparable to control individuals that are approximately 10 years older [57, 58]. GWAS studies have identified seven SNPs that are responsible for interindividual variations in LTL. The presence of these alleles is associated with an increased CVD risk [59]. In a recent meta-analysis Codd et al. [59] calculated a 21% increase in CVD risk per one standard deviation reduction in LTL. LTL has also the potential to predict mortality in CVD patients [60]. In addition, LTL is related to the severity of CVD and disease progression.

Chronic inflammation and oxidative stress are the principal drivers in atherosclerosis. They cause accelerated telomere loss per cell replication and premature cellular senescence in endothelial cells, vascular smooth muscle cells (VSMC) and blood leukocytes [61]. The reduction of telomere length in VSMCs in human atherosclerotic plaques is correlated with the severity of the disease. In addition, VSMCs in atherosclerotic plaques show signs of oxidative DNA damage and express typical senescence markers such as senescence-associated galactosidase, cyclin-dependent kinase inhibitors p16 and p21, decreased expression of cyclin D and cyclin E, and hypophosphorylation of the retinoblastoma protein [62]. Senescent VSMCs possess a limited proliferative capacity and an increased amount of matrix-degrading enzymes, which promote the thinning of fibrous caps and plaque rupture [63]. In agreement with this finding it has been shown that in patients with acute coronary syndrome short leukocyte telomeres are associated with the presence of highly unstable atherosclerotic plaques and an increased proinflammatory activity [64]. In an animal model long telomeres have been protective against age-dependent cardiac disease caused by NOTCH1 haploinsufficiency [65].

Accelerated telomere shortening does not only promote atherosclerosis, it also impairs the repair of vascular lesions. Bone marrow-derived mononuclear cells are important progenitors during the re-endothelialization process of blood vessels. Functional exhaustion and an impaired proliferative capacity of endothelial progenitor cells due to accelerated telomere shortening contributes to delayed re-endothelialization after vascular injury and stent implantation [66].

Common risk factors for CVD, such as hypertension and T2DM, are also related to LTL. Reduced telomere length together with telomere uncapping was found in patients with hypertension [67, 68]. The risk of being diagnosed with T2DM is also higher in individuals with shortened telomeres [48]. Furthermore, telomere shortening seems to be associated with the progression of T2DM and the number of diabetic complications, such as retinopathy, nephropathy, neuropathy and peripheral vascular disease.

Human and animal studies also suggest a critical role of telomerase in cardiovascular aging. TERT protein and telomerase activity are present in cardiomyocytes [69] and blood vessels [70]. Low TERT activity reduces vascular protection and promotes senescence, which ultimately leads to vascular dysfunction. Experimental studies have shown that eNOS and TERT interact with each other. Moreover, statins also influence TERT expression and telomerase activity in the vascular wall and in cells of the immune system [71].

Alzheimer's disease (AD)

AD is the most common neurodegenerative disease associated with aging. Several studies showed shorter telomeres in blood leukocytes of AD patients than in controls [72–76]. A recent meta-analysis of 13 studies demonstrated a significant difference in LTL between 860 AD patients and 2022 controls. The authors concluded that there is consistent evidence of shorter telomeres in AD patients [74]. Panossian et al. [75] showed that the telomere length of T cells, but not of B cells or monocytes, correlate with AD disease status, measured by mini mental status exam (MMSE). Telomere length in T cells inversely correlated with serum levels of TNF-α (a clinical marker of disease status), with the proportion of CD8+ T cells lacking expression of the CD28 co-stimulatory molecule, and with apoptosis. Interestingly, Lukens et al. [73] found similar telomere length in the cerebellum of AD patients and controls despite a significant difference in leukocyte TL. A recent study from Tedone et al. [76] suggests that telomere length in PBMCs may be helpful in predicting disease progression. Late onset AD patients with slow disease progression had shorter telomeres than those with fast disease progression or healthy elderly controls. In addition, AD patients with fast disease progression showed an impaired response to stimulation by amyloid ß and IL-10.

In contrast to AD, Parkinson's disease (PD), another age-related neurodegenerative disease, is not consistently related to telomere length. Forero et al. [74] analyzed eight studies and did not find a significant difference in LTL between 956 PD patients and 1284 controls.

Cancer

Cancer is also considered an age-related disease, as its risk increases with age. Several studies have investigated the relationship between telomere length and cancer risk or prognosis [77–79]. Telomeres measured in tumor tissue from breast [80, 81], colon [82] and prostate [83] appear to be shorter than in healthy tissue from the same organ and the same patient. In healthy tissue directly adjacent to these tumors, telomeres are also shorter than in cells that are more distant from the cancerous lesion [84]. There is good evidence that a reduced telomere length in cancer tissue from breast colon and prostate is associated with an advanced disease state at diagnosis, faster disease progression and poorer survival [81, 85]. Another observation is that patients with the highest variation of telomere length between individual cells have the highest risk of death. Although these findings are interesting the measurement of telomere length in tumor tissue harbors substantial practical limitations that prevent its use as a biomarker in clinical practice. In order to overcome these issues LTL has been proposed as a surrogate marker for telomere shortening in the entire body. A number of studies found an association between accelerated telomere shortening in leukocytes and an increased risk of incident cancer. For example, in the normative Aging Study serial telomere measurements were performed in nearly 800 cancer free individuals. The annual telomere shortening rate in subjects that developed cancer during the follow-up was double as high as in individuals without cancer [86]. In the 10-year prospective Bruneck study patients with short telomeres at baseline had substantially higher risk for incident cancer and cancer specific mortality [46]. However, the results of prospective studies are inconsistent. For example, in a very large population study from Norway with >47,000 participants short telomeres were associated with incident cancers [87]. This effect disappeared after adjustment for potential confounders. There are also studies that found significantly longer telomeres in blood leukocytes from cancer patients when compared to cancer free subjects [88]. Meta-analyses might help to better understand the data from existing studies, but results are inconclusive. A meta-analysis of retrospective studies shows an association between short telomeres and an increased risk for most solid organ tumors [89]. By contrast, a recent meta-analysis of prospective studies does not confirm this result [90].

The controversial results might be explained by the induction of telomerase at a specific point in time during tumor genesis. The Normative Aging Study has shown a faster decline of LTL in cancer patients than in cancer free subjects [86]. However, as cancer diagnosis comes closer, the shortening of telomeres slows down and ultimately they become longer again. It has been suggested that critically short telomeres contribute to cancer initiation which then, in turn, activates telomerase.

Although there is substantial evidence for an association between LTL and age-related diseases, neither a conclusive causative link nor a predictable association can be established. Longitudinal studies as well as assessment of other markers of telomere biology are needed to further clarify the role of telomeres and telomerase in aging and the development of age-related diseases.

Leukocyte telomere as a surrogate marker for telomere shortening in the entire body

Measurement of telomere length in tumor tissue harbors substantial practical limitations that prevent its use as a biomarker in clinical practice. The most important issue is that the analysis can only be performed after a tumor has been diagnosed. Furthermore, an invasive biopsy is necessary to obtain a sample. Because of the invasive sample collection, serial measurements are not feasible. In order to overcome these obstacles, the measurement of LTL in blood leukocytes has been proposed as a surrogate marker. In blood leukocytes, telomeres shorten with an average annual rate of 30–35 bp. As this places leukocytes in the middle of all tissue types, it has been proposed that LTL might be a suitable surrogate marker for telomere shortening in the entire body.

LTL is often used in clinical studies. However, doubts remain to what extent this parameter reflects the situation in other organs and tissues. Until today, only few studies have explored the relationship between telomere length in different cell types and results are not so clear [91–95]. For example, Dlouha et al. [91] measured telomere length in 12 different human tissues (peripheral blood leukocytes, liver, kidney, heart, spleen, brain, skin, triceps, tongue mucosa, intercostal skeletal muscle, subcutaneous fat and abdominal fat) from 12 cadavers. Telomere length was significantly higher in blood compared to liver, brain, muscles, skin, spleen and mucosa but was not different compared to adipose and renal tissue. In addition, the largest telomere length variability was observed in leukocytes and kidney, and the smallest

telomere length variability was observed in brain, heart and skin [91]. In another study, Friedrich et al. [93] reported a linear relationships between telomere length measured in blood, skin and synovia of nine elderly patients. In this study, significantly shorter telomeres were found in leukocytes compared to skin and synovia [93]. However, in this study, age of patients ranged from 73 to 95 years. Telomere length from leukocytes and from skin correlated negatively with age [93]. Richard et al. [94], who analyzed telomere length in aortic biopsies and paired blood leukocyte from 20 patients, identified that shorter blood telomeres reflect shorter aortic telomeres, indicating that telomere attrition in blood leukocytes is indicative of similar changes in the vascular well. Altogether these findings could reflect the expected life span of different cell types. One aspect to consider is that senescent cells are eliminated by apoptosis so that only a few or no senescent cells persist *in vivo*. On the other site, the stem cell compartment of a tissue is enriched in cells with longer telomeres, as they express telomerase and therefore avoid telomere shortening. Indeed, longer telomeres and higher telomerase activity were found in several stem cell compartments, including hair follicle, skin, small intestine, cornea and testis [95]. Finally, it has also been speculated that a common genetically controlled mechanism regulates telomere length differently in various tissues [93].

In summary, available data suggest that telomere length is correlated between different tissues in the same individual and that peripheral blood telomere length could be used as a surrogate marker of telomere length in other organs [91–95].

Analytical aspects

Telomeres can be measured in all solid organ tissues and blood leukocytes. Although the analysis of telomere length in the tissue of interest is most representative, the invasive sample collection procedure and other practical aspects often render this approach not feasible for routine use. Because of the simple and non-invasive specimen collection, blood leukocytes are the most widely used matrix for clinical and research purposes. Telomere length can be measured with a range of different methods [95–103]. Generally, methods that use isolated DNA have to be distinguished from those using intact cells. Southern blotting and quantitative-polymerase chain reaction (Q-PCR) are the analytical principals that are applied for the analysis in isolated DNA. The Southern blot technique was the first method that has been developed and is still considered the gold standard for the measurement of telomere length [96]. In the first step, DNA is digested by endonucleases that do not cut within the telomeric TTAGGG sequence. Subsequently, the digested DNA is separated by gel electrophoresis. Telomeres are visualized by labeled DNA probes that are complementary to the telomere sequence. The longer the telomere, the more probes can bind, resulting in a higher signal intensity [96]. The obtained signal represents the average telomere length of all chromosomes from cells in the sample. It provides an absolute value of telomere length expressed in base pair, requires a substantial quantity of DNA (i.e. micrograms), is time consuming and is not suitable for large population-based studies. However, as the analytical principle of this hybridization-based method, it implies a decreasing signal as telomere become shorter. There is a threshold telomere length below which no detectable signal will be generated. The inability to detect very short telomeres limits the utility of this method in studies of cellular aging, where short telomeres are of particular interest [96]. The Q-PCR-based assay requires the amplification of the telomeric region and of a single copy gene (SCG) [97, 98]. Telomere length is quantified as the amount of telomeric sequence compared to SCG and is expressed as a relative telomere length (RTL) or telomere/SCG (T/S) ratio [97, 98]. As the Southern blot method, it is not possible to evaluate telomere length of individual cells or chromosomes. However, the Q-PCR-based technique is fast, highly sensitive and requires low amounts of DNA (i.e. nanograms). Because of these characteristics, it is often chosen for large population-based studies [97, 98]. At the cellular level, telomere length can be evaluated by quantitative fluorescence *in situ* hybridization (Q-FISH) [100] or by flow cytometry (flow-FISH) [101]. Q-FISH can be combined with a conventional FISH, allowing the evaluation of chromosome-specific telomere length. Similarly to the Southern blot-based method described above, Q-FISH is also a hybridization technique, which implies the existence of a threshold telomere length below which no detectable signal is produced. As Q-FISH is performed on cells in metaphase, the analysis is limited to cells that proliferate *in vitro*. Consequently, not all cells are suitable to be analyzed with this method. The confocal telomere Q-FISH method (Telomapping) allows the quantification of telomere length in tissue samples or biopsies [95].

More work is needed for the harmonization of telomere length assessment between laboratories, including the development of a common standard reference, of internal and external quality control programs and of reference ranges stratified for age and gender.

Conclusions

Telomeres and telomerase play an important role during the process of aging. Short telomeres in various tissues and in blood leukocytes are associated with age-related diseases, such as CVD, Alzheimer's disease and cancer. Although a number of studies have proposed the measurement of LTL as a predictor of incident age-related diseases, disease progression and mortality, it is still far from being used in clinical practice. Analytical issues (lack of standardization) and pathophysiological aspects that are insufficiently understood are the main obstacles that impede the widespread use of telomere length measurement. More work is needed for the harmonization of telomere length assessment between laboratories, including the development of a common standard reference, of internal and external quality control programs and of reference ranges stratified for age and gender. In addition, a range of preanalytical aspects need to be clarified. For example, under what circumstances the measurement of LTL should be ordered? When should the blood sample be collected? So far, the evaluation of telomere length will be reasonable only in research programs, clinical trials, longitudinal and cross-sectional clinical studies.

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