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Telomeres and human health

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Abstract. Bojesen SE (Department of Clinical Biochemistry, Herlev Hospital, Copenhagen University Hospital, Denmark; The Copenhagen General Population Study, Herlev Hospital, Copenhagen University Hospital, Denmark; The Copenhagen City Heart Study, Frederiksberg Hospital, Copenhagen University Hospital, Denmark; Faculty of Health Sciences, University of Copenhagen, Denmark). Telomeres and human health. (Review). *J Intern Med* 2013; **274**: 399–413.

Telomeres are the tips of chromosomes and consist of proteins and hexanucleotide tandem repeats of DNA. The DNA repeats are shortened at each mitotic division of normal cells, and the telomere length chronicles how many divisions the cell has undergone. Thus, telomere length is a marker of fundamental biological pathways. It has been possible to measure telomere length for more than 20 years, and it has been established that telomere length is associated with age, sex and lifestyle factors. Here, the current knowledge of telomere length as a biomarker of disease susceptibility and mortality will be reviewed. In addition, technical difficulties and the reasons why measurement of telomeres has still not been introduced into routine clinical practice will be discussed. Findings from recent studies conducted in many thousands of individuals indicate that telomere length is not–or at best only marginally–independently associated with risk of common disorders such as cardiovascular, pulmonary and neoplastic diseases. However, in sufficiently powered studies, short telomeres are repeatedly and independently found to be associated with increased risk of early death in the general population or in subsets of individuals. This indicates that measurement of telomeres could be a valuable prognostic biomarker in many clinical settings. However, whether short telomeres are a causal factor for or simply a marker of increased risk of early death must be determined. Finally, how Mendelian randomization studies could clarify this issue, and which clinical studies might be carried out to refine this very promising biomarker for routine clinical use will be considered.

Keywords: biomarker, epidemiology, mortality, telomere length.

Introduction

In several studies, it has been shown that there is a high correlation between telomere lengths from different tissues of the same individual and that telomeres in the frequently dividing peripheral blood cells are shorter than in most other tissues [1–4]. Furthermore, the variation between individuals is partly explained by lifestyle [5, 6], gender and age [7]; telomeres are shorter in smokers than in nonsmokers [7], in obese than individuals of normal weight [8], in men than in women [9], and in older than in younger individuals [10]. Telomere length is also determined by normal individual genetic variation [11]. The heritability–that is the variation in telomere length explained by the variation in noncommunicable hereditary factors–was found in different studies to vary between 60% and 80% [11–16].

Telomeres are the tips of the chromosomes and consist of protein complexes and noncoding DNA comprising hundreds to thousands of tandem repeats of TTAGGG [17]. A protein complex composed of the shelterins protects the DNA ends from inappropriate responses from the DNA damage surveillance system in the cell [17], which would otherwise lead to recognition of double-strand DNA breaks and subsequent activation of the ATM protein and induction of senescence or apoptosis [18, 19]. Due to the unidirectional nature of DNA replication, the telomeric tandem DNA repeats are shortened at each mitotic division of normal cells. At a certain critical short length, the cell does not divide further and enters senescence [20]. Only certain specialized stem cells circumvent senescence by activating the telomerase complex [21–24]. One protein in this complex, TERT, synthesizes the TTAGGG repeats of the telomeres

through its reverse transcriptase activity. It uses an RNA template produced by TERC, another protein of the telomerase complex. In this way, the telomeric tandem DNA repeats are elongated beyond the critical short length.

Deleterious mutations in genes coding for telomerase proteins (*DKC1, TINF2, TERC, TERT, NOP10* and *NHP2*) cause the inherited disorder dyskeratosis congenita, which is characterized by dystrophic nails, patchy skin pigmentation, oral leukoplakia and increased risk of cancer [25]. Patients with dyskeratosis congenita can also develop aplastic anaemia and/or pulmonary fibrosis [26]. Furthermore, 20% of patients with isolated idiopathic pulmonary fibrosis have mutations of telomerase genes [27]. Mutations in telomerase genes can also be acquired, and mutations of *TERT* and *TERC* are detected in bone marrow cells in 10% of patients with aplastic anaemia [26].

Thus, for many reasons, telomere length is a very attractive biomarker: (i) it provides a quantitative measurement which is, theoretically, intimately related to basic biological mechanisms such as proliferation, senescence and immortalization of cells; (ii) it appears to record the number of past cell divisions; (iii) it is reproducible in the individual and highly correlated across tissues; and iv) it is associated with adverse lifestyle and other risk factors, including age and gender.

But despite the widespread interest and scientific effort (Fig. 1), measurement of telomere length has still not been introduced into general clinical practice. Here, the reasons for the lack of interest in using telomeres in the clinical setting, and changes that could lead to the measurement becoming clinical routine, are discussed.

Measurement of telomere length

The measurement of telomere length in cells is technically not simple, which is one of the major obstacles for the introduction of this measurement into clinical practice. The length of telomeres is currently measured using three fundamentally different techniques [28], each with advantages and drawbacks (Fig. 2).

Southern blotting is the oldest technique and the *de facto* gold standard for measurement. In short, the native DNA is digested with restriction enzymes specific for subtelomeric DNA sequences. After purification, the fragments are separated by size in a normal agarose gel and immobilized on a nylon membrane. The telomere fragments are hybridized with radioactive-labelled plasmids containing the tandem hexanucleotide repeat telomere sequences. Finally, the migration pattern of the labelled DNA fragments with telomeres is exposed on an X-ray film.

Fluorescence *in situ* hybridization (FISH) is the most labour-intensive technique and requires considerable laboratory equipment. Living lymphocytes are propagated in culture, arrested in metaphase and plated using standard cytogenetic techniques. Fluorescent probes hybridize with the tandem hexanucleotide repeat telomere sequences, and the telomere length is determined by quantifying the fluorescence signal. Because the chromosomes in the metaphase are separated, this technique can measure the length of the telomeres of individual chromosomes.

In quantitative polymerase chain reaction (Q-PCR), the telomeric tandem hexanucleotide repeat telomere sequences are amplified in competition with a reference gene to adjust for the amount of DNA in the reaction. The amplification of both products is monitored for each PCR cycle. The cycle number, where the amount of the amplification product exceeds baseline, is the threshold cycle, and the amount of telomere is calculated on the basis of the difference between the two threshold cycles. Thus, the Q-PCR technique actually measures the average cumulative amount of the tandem hexanucleotide repeat telomere sequences relative to the diploid genome and not the length *per se*.

All three methods are difficult to establish as a routine test, either because they are very labour intensive and require highly trained personnel (Southern blot and FISH) or because the establishment of the technique and its calibration can be very challenging (Q-PCR). In addition to the specific work-flow and metrological problems for each technique, introduction of the measurement into the clinic also suffers from the fact that telomere length is not well defined in practice, even though it is relatively well defined in theory. Southern blot was the first technique used [10] and is therefore widely considered as the standard against which other techniques should be compared. Rather than one number, this technique delivers a distribution of telomere lengths for each individual. The measured fragments also include subtelomeric

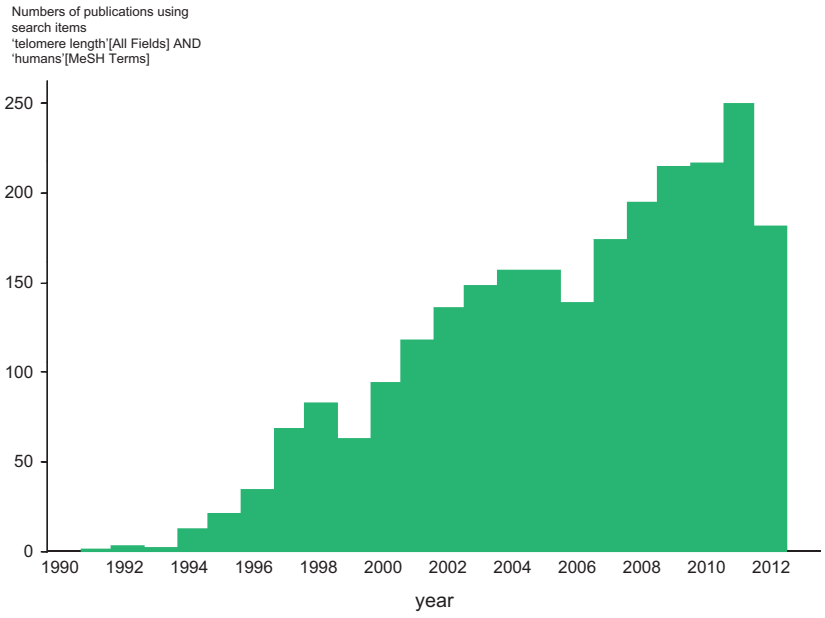
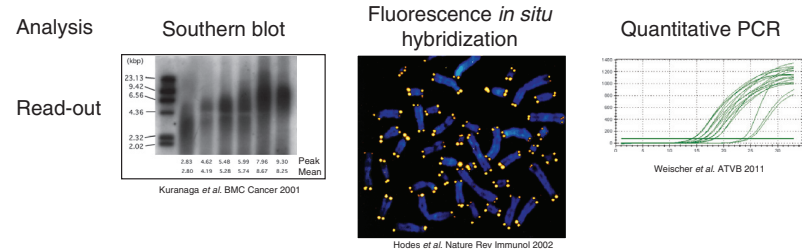


Fig. 1 *Number of publications per year using search items: 'telomere length'[All Fields] AND 'humans'[MeSH Terms] in PubMed in February 2013.*



Hallmark	Well-known technique	Analysis of individual chromosome telomeres possible	Automation. Multiple tests per individual
Speed	Very slow	Slow	Very fast
Handling	Complicated	Very complicated	Easy
Automation	None	None/some	Yes
DNA amount	High	High. Living cells necessary	Low
Precision	Low	Low	High
Calibration necessary?	*De facto* gold standard	Yes	Yes
Low/high throughput	Low	Low	High
Other	Includes subtelomeric fragments	Requires a lot of equipment and training	Difficult to establish

Fig. 2 *Techniques for telomere length measurements. The three different methods for measuring telomere length are Southern blot, fluorescence in situ hybridization and quantitative PCR. Adapted from [28].*

fragments, the length of which depends on the chromosome and the genetic variation influencing the restriction enzyme sites.

Fluorescence *in situ* hybridization (FISH) techniques to determine telomere length have evolved with improved digital picture analysing software [29] and have been extensively used, especially to measure the telomere length in individual chromosomes. The major drawback of this technique is the manual labour required, precluding high-throughput approaches. Recent technological improvements might provide a solution to this problem [28, 30], but whether FISH or derivatives thereof can be used in thousands of individuals remains unclear.

Quantitative polymerase chain reaction (Q-PCR) is the latest of the three analysis methods and was first established and later improved by Cawthon [31, 32]. It is a real-time PCR technique that follows the amplification of the telomere PCR fragment together with that from a reference gene represented twice in the genome. The fundamental disadvantage of telomere length measurement by Q-PCR techniques is the tandem repetition of hexanucleotide sequences, which normally prevents the design of primers. This problem has been largely overcome, but establishing the assay in the laboratory remains extremely challenging. Furthermore, rigorous and continuous quality control measures are necessary to avoid false results.

Telomere length as a biomarker

In the first report of measurements of telomere length in humans in 1990 [10], it was noted that telomere length in leucocytes declines with increasing age. Subsequently, it was speculated that telomere length could be a biomarker of biological cell age and thus indicate the risk of age-related disease such as cancer, cardiovascular diseases, pulmonary disease, diabetes and others.

One of the hallmarks of cancer cells is their ability to maintain telomere length [33, 34] to escape senescence and termination of cell division induced by critically shortened telomeres [35]. This suggests that long telomeres could be a marker of cancer risk. On the other hand, because short telomeres are a marker of 'wear and tear', short telomeres could also be a marker of cancer risk. This paradox has been extensively examined [36–38] including in two recent meta-analyses, both of more than 25 000 individuals [37, 38]. It was noted that 'short surrogate tissue telomere length is associated with cancer; the strongest evidence exists for bladder, oesophageal, gastric and renal cancers. Additional prospective studies with consistent methodology are needed to confirm this hypothesis' [38]. By contrast, from another recent review [36], it was concluded that 'either excessively short or long telomere length may both contribute to cancer development'. The authors also stated that further investigation is 'needed to improve our understanding of surrogate tissue telomere length as a biomarker for cancer risk and progression' [36]. In an attempt to addresses this issue, Weischer *et al.* conducted a study of cancer risk in >47 000 individuals from the general population followed for up to 20 years. They observed that 'short telomere length was not associated with cancer risk' [39]. Currently no meta-analyses of the association between telomere length and risk of disease, other than cancer, have been reported.

Table 1 shows some of the nonmalignant diseases and other phenotypes that have been associated with telomere length. In almost all studies, it was demonstrated that short telomeres are associated with increased risk of disease and/or that short telomeres are associated with adverse lifestyle and other risk factors (Table 1, Fig. 3). The association between telomere length and cardiovascular disease has been examined in numerous studies [7, 40–48], most of which showed that short telomeres are associated with increased risk of disease. For pulmonary disease, the evidence is less clear. In a study of >46 000 individuals, Rode *et al.* [49] demonstrated that the very strong associations between telomere length and chronic obstructive pulmonary disease and lung function indices were reduced to almost insignificance after adjusting for age, gender, smoking habits, body mass index and other lifestyle factors. Risk of diabetes has also been examined; the combined evidence does not suggest a strong association with telomere length [50–68], although it might be predictive of late complications of diabetes [69–71].

Telomere length and mortality

The association between telomere length and age has prompted many studies to investigate the relation between telomere length and mortality (Table 2). The findings are inconsistent, but in the two largest studies, so far of individuals from the general population, Weischer *et al.* did find a 25% increased risk of any early death amongst individuals in the deciles with the shortest versus longest telomeres [7] as well as a 43% increased risk of early death in patients with cancer from the general population [39]. Similar results were found in patients with COPD [72] or diabetes [70], although a lack of association between telomere length and mortality has also been observed in other studies (Table 2).

It is interesting that dyskeratosis congenita patients with extremely short telomeres need reduced-intensity conditioning treatment before bone marrow transplantation, compared with other patients [73–81], otherwise the risk of complications and therapy-related death is high[73].

Table 1 *Studies of telomere length as a biomarker*

Reference	Year of publication	Number of individuals	Disease/characteristic	Conclusion: are short telomeres associated with disease, adverse change of variable or adverse lifestyle?
Rode [49]	2012	46 396	Pulmonary function and disease	Yes
Weischer [7]	2012	19 838	Myocardial infarction	Yes
Surtees [100]	2012	4441	Educational attainment in women	Yes
Prescott [101]	2012	4250	Increased paternal age	Telomeres are long in daughters of old fathers
Brydon [102]	2012	434	Cynical hostility	Yes
Okereke [103]	2012	5243	Phobic anxiety	Yes
Sun [6]	2012	5862	Healthy lifestyle	Yes
Balistreri [41]	2012	300	Thoracic aortic aneurysm	Yes
Ding [43]	2012	2618	Stroke	Yes
Shen [64]	2012	4016	Type 2 diabetes	Yes
Du [104]	2012	7813	Physical activity in women	Yes
Moverare-Skrtic [105]	2012	60	Mild cognitive impairment	Yes
Hochstrasser [106]	2012	51	Alzheimer's disease	Yes
Njajou [8]	2012	2721	Obesity	Yes
Humphreys [114]	2012	102	Intimate partner violence	Yes
Elvsåshagen [107]	2011	56	Bipolar II disorder	Yes
Mainous [108]	2011	312	Marital status	Yes
Entringer [109]	2011	188	Intrauterine stress	Yes
Rollison [110]	2011	128	Myelodysplastic syndrome	Yes
Betjes [111]	2011	281	End-stage renal disease	Yes
Wolkowitz [112]	2011	35	Lifetime depression exposure	Yes
Liu [113]	2011	360	Hepatitis B-related hepatocellular carcinoma	No; long telomeres associated with increased risk of hepatitis B-related hepatocellular carcinoma
Pavanello [115]	2011	457	Drink driving	Yes
O'Donovan [116]	2011	90	Childhood trauma	Yes
Buxton [117]	2011	793	Childhood obesity	Yes
Farrag [44]	2011	55	Hypertension	Yes
Masi [118]	2011	563	Periodontitis	Yes
Kananen [119]	2010	974	Childhood chronic or serious illness	Yes
Barcelo [42]	2010	404	Sleep apnoea	Yes
Maubaret [46]	2010	1251	Coronary heart disease	Yes
Wong [120]	2010	875	Anaemia in chronic heart failure	Yes
Cassidy [5]	2010	2284	Healthy lifestyle	Yes
Risques [121]	2010	624	Activities of daily living	Yes
Zee [68]	2010	846	Type 2 diabetes	Yes

Table 1 (*Continued*)

Reference	Year of publication	Number of individuals	Disease/characteristic	Conclusion: are short telomeres associated with disease, adverse change of variable or adverse lifestyle?
Atturu [40]	2010	373	Abdominal aortic aneurysm	Yes
Pavanello [122]	2010	92	Exposure to aromatic hydrocarbons	Yes
Tamayo [123]	2010	216	Rheumatic diseases	Yes
Christensen [124]	2009	1826	Perceived age	Yes
O'Donovan [125]	2009	36	Pessimism	Yes
Mirabello [126]	2009	1049	Healthy lifestyle	Yes
Zee [47]	2009	674	Myocardial infarction	Yes
Savale [127]	2009	291	Chronic obstructive pulmonary disease	Yes
Nordfjäll [128]	2008	1502	Self-perceived early ageing	Yes
van der Harst [129]	2007	803	Chronic heart failure	Yes
Brouilette [130]	2007	1542	Myocardial infarction	Yes
Kurz [45]	2006	193	Aortic valve stenosis	Yes
Simon [131]	2006	88	Mood disorders	Yes

Search terms 'telomere length' and 'case control'.

Taken together, these findings indicate that short telomeres might be a sign of overall fragility. It is conceivable that sequential measurements of telomere length in leucocytes could be used to monitor the residual stem cell capacity in the bone marrow during chemotherapy in patients with cancer. Thus, telomere length measurement could prove to be a valuable clinical tool to influence clinical decision-making.

If telomere length is highly associated with risk factors and carries important information about past exposures (Fig. 3), why has it been so difficult to provide sufficient evidence to support introduction of the measurement into everyday clinical practice? First, one obstacle has probably been the strong association with risk factors. This may appear to be a strength that can be used as validation of the measurement. However, when telomere length reflects age, gender, smoking and other general risk factors in one parameter, how can it be determined whether telomere length alone provides independent prognostic information? And how can the possibility be excluded that the information seemingly provided by telomere length alone is in fact caused by residual confounding, even after multifactorial adjustment for age, gender, smoking, obesity and other risk factors? Secondly, the different measurement techniques and the lack of traceable standardized measurements constitute another obstacle when different studies are compared. Thirdly, use of surrogate tissue such as blood or buccal cells is the only practical method when large numbers of individuals are examined, and it is also the best way to ensure that measurement of telomere length can be introduced into clinical practice. However, it raises the concern of reverse causation: does the disease, preclinical disease or its treatment cause telomeres to shorten in the surrogate tissue?

These questions can best be answered using very large and extremely well-characterized cohorts, in which the telomere length is measured reliably and uniformly.

Copenhagen city heart study and copenhagen general population study

We examined how telomere length is associated with risks of a wide variety of diseases, as well as other factors including smoking and body mass index, using a prospective cohort based on the general population. This approach eliminates the problem of reverse causation, making the observations as generalizable as possible (Fig. 4). Telomere

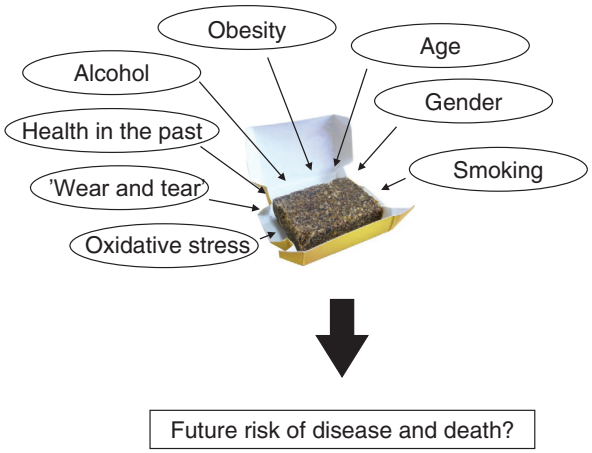


Fig. 3 *Schematic diagram showing telomere length as 'a soup cube' carrying condensed information about past exposures and future health.*

length must be measured from samples collected before diagnosis of the disease under study because the disease itself or its treatment could influence the length. Because the absolute risk of disease amongst individuals from the general population is relatively small, the study must include many thousands of participants and a long follow-up period. Therefore, Q-PCR-based techniques are most suited because of the potential for automation and the speed of the technique, and also because archive DNA already collected can be used. To enable comparison with other studies, the assay and its calibration must be well documented so the measurement is traceable. Furthermore, the recording of confounding factors and end-points must be as precise and complete as possible. Two studies in which these conditions are fulfilled are the Copenhagen City Heart Study and the Copenhagen General Population Study.

The Copenhagen City Heart Study was initiated in 1976–1978 with follow-up examinations in 1981–1983, 1991–1994 and 2001–2003; a fifth examination is currently being carried out. Participants were randomly selected from the national Danish Civil Registration System to reflect the adult Danish population ranging from age 20 to 100 years. At the 1991–94 examination, blood samples for DNA extraction were obtained and DNA is available for approximately 9000 participants. Participation rates were 73.6% in 1976–1978, 70.2% in 1982–1983, 61.2% in 1991–1994 and 49.5% in 2001–2004 [82–84].

The Copenhagen General Population Study was initiated in 2003 with enrolment ongoing, and in January 2013, participant number 95 000 was examined. Participant selection and examination is exactly as described for the Copenhagen City Heart Study. The overall participation rate was 44% [85, 86].

Covariates

Prior to examination, participants completed self-administered questionnaires regarding present and past lifestyle and health status. This included questions about cardiopulmonary, neurological and psychiatric symptoms and diagnoses, the use of tobacco and alcohol, diet, socio-economic status and reproductive history, including the use of contraceptives and hormone-replacement therapy for women. A complete list of current medications was also obtained. On the day of examination, a questionnaire was completed with the aid of the examiner, followed by physical examination and blood sampling. After obtaining consent (from >99.7% of participants), information was collected yearly from the Danish health registries and the Danish Civil registration system. In practice, this means that for all consenting participants, diagnoses leading to hospital admissions, and all pathological findings were recorded, before and after the examination.

Ascertainment of disease diagnoses and deaths

Diagnoses of invasive cancers are obtained from the Danish Cancer Registry from 1943 onwards, whilst other diagnoses from 1976 onwards are obtained from the national Danish Patient Registry recording all hospital discharge diagnoses and from the national Danish Cause of Death Registry. Information on date of death is obtained from the national Danish Civil Registration System.

Genetic determinants of telomere length

To clarify the value of the measurement, instruments are needed to determine telomere length independently of all other factors. Common genetic variants that may influence the length are distributed randomly at conception and are thus independent of later lifestyles and diseases. Telomere length is heritable [11, 12], and in contrast to the extensive mapping of the genes involved in telomeric diseases, such as dyskeratosis congenita, knowledge of common genetic variants and their

Table 2 *Studies of telomere length and mortality*

Reference	Year of publication	Number of individuals	Setting	End-point	Conclusion: are short telomeres associated with mortality?
Weischer [39]	2013	3142	General population cancer patients	All-cause mortality	Yes
Weischer [7]	2012	19 838	General population	All-cause mortality	Yes
Honig [132]	2012	1983	>65-year-old individuals	All-cause mortality	Yes
Lee [72]	2012	4272	COPD patients	Cancer mortality and all-cause mortality	Yes
Zekry [133]	2012	444	Geriatric patients	All-cause mortality	No; telomere length insignificant
Martin-Ruiz[134]	2011	852	>85-year-old individuals	All-cause mortality	No; telomere length insignificant
Willeit [135]	2011	787	Cancer-free general population	Cancer mortality and all-cause mortality	Yes
Strandberg [136]	2011	622	Elderly businessmen	All-cause mortality	No; telomere length insignificant
Fitzpatrick [9]	2011	1136	General population	All-cause mortality	Yes
Houben [137]	2011	278	>70-year-old individuals	All-cause mortality	No; telomere length insignificant
Valls [138]	2011	147	Colorectal cancer patients	All-cause mortality	Yes
Willeit [139]	2010	787	Cancer-free general population	Cancer mortality and all-cause mortality	Yes
Astrup [70]	2010	273	Type 1 diabetes patients	All-cause mortality	Yes
Njajou [140]	2009	3075	Well-functioning 70- to 79-year-old individuals	All-cause mortality	No; telomere length insignificant
Svenson[141]	2009	105	Renal cancer patients	All-cause mortality	No; long telomeres associated with mortality
Epel,[142]	2009	236	Well-functioning 70- to 79-year-old Men	Cardiovascular mortality	No; telomere length insignificant
Svenson [143]	2008	265	Breast cancer patients	All-cause mortality	No; long telomeres associated with mortality
Farzaneh-Far [144]	2008	780	Patients with coronary artery disease	All-cause mortality	Yes
Kimura [145]	2008	548	73- to 94-year-old twins	All-cause mortality	Yes
Bakaysa [15]	2007	350	63- to 64-year-old twins	All-cause mortality	Yes
Harris [146]	2006	200	1921 birth cohort	All-cause mortality	No; telomere length insignificant
Honig [147]	2006	257	General population	All-cause mortality	Yes

Table 2 (*Continued*)

Reference	Year of publication	Number of individuals	Setting	End-point	Conclusion: are short telomeres associated with mortality?
Martin-Ruiz[148]	2006	195	Stroke patients	All-cause mortality	Yes
Bischoff [149]	2006	812	73- to 101-year-old individuals	All-cause mortality	No; telomere length insignificant
Martin-Ruiz [150]	2005	598	>85-year-old individuals	All-cause mortality	No; telomere length insignificant
Cawthon [151]	2003	143	>60-year-old individuals	All-cause mortality	Yes

Search terms 'telomere length' and 'mortality'.
COPD, chronic obstructive pulmonary disease.

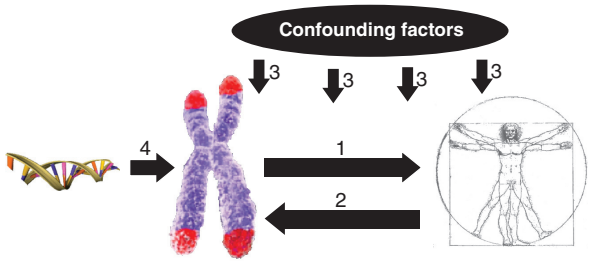


Fig. 4 *Short telomeres are associated with reduced health, but whether this association is causal (arrow 1), due to reverse causation (arrow 2) or due to the influence of confounding factors (arrows 3) is presently unknown. Genetic variants determining telomere length (arrow 4) may help to clarify this association in a Mendelian randomization approach.*

role in the regulation of telomere length in the general population is limited. Polymorphisms of *TERC* [87] and *OBFC1* [88] were identified through genome-wide association studies, and *CTC1* and *ZNF676* were identified as regulators of telomere length in a recent genome-wide meta-analysis [89].

Very recently, telomere length, breast cancer, ovarian cancer [90] and prostate cancer [91] were all found to be associated with variations in *TERT,* the gene coding for telomerase. Of note, the *TERT* variants associated with telomere length were only partly also associated with risk of these cancers and *vice versa.* Furthermore, for one variant (rs2242652), the minor allele in Europeans was associated with a significant reduction in prostate cancer risk and with significant increased risks of breast cancer and ovarian cancer, but was not associated with a change in telomere length. This indicates that the interaction between telomerase, telomere length and cancer risk is far more complex than hitherto imagined. Although the hypothesis that genetically shortened telomeres predispose to risk of cancer is attractive, this model does not fit with the observations from the *TERT* fine-mapping studies, as mentioned above [90, 91], or with the finding that telomere length was not associated with future overall cancer risk after adjusting for age in a study of 47 000 individuals from the general population [39]. There could be many explanations for this, possibly involving TERT protein functions other than pure telomere elongation. These so-called noncanonical functions of the TERT protein [92, 93] are important in cell signalling [94, 95], and TERT expression can induce cell division and decreased apoptosis in some cells, independently of telomere elongation [93].

Association and causality: Mendelian randomization

Even with highly suitable cohorts as described above, studies of associations between telomere length and risk of disease remain observational by nature, precluding any firm conclusions on causality. In theory, there are three possible explanations for an association between short telomeres and risk of disease (Fig. 4). The first is causal association. Short telomeres may indeed cause the risk of disease to increase; that is, prevention of telomere shortening by drugs or other interventions would decrease the individual's risk of the disease. The second explanation is reverse causation. The disease, preclinical disease or the treatment may cause the telomeres to shorten. Thus, isolated prevention of telomere shortening would not affect the risk of disease. Measurements of

telomere length might still, however, be extremely valuable to monitor disease progression/treatment. Thirdly, confounded association might explain the association. An unknown factor may cause both telomere shortening and increased disease risk. In this case, telomere length could act simply as a marker of disease risk and thus also be of value in the clinical setting.

Mendelian randomization is a statistical form of analysis of data from observational studies to clarify causal relationships between a specific biomarker and a specific end-point, dependent on the fact that alleles are randomly distributed in an individual at conception [96]. Therefore, the alleles are not influenced by the disease or confounding factors, which arise later in life.

By identifying genetic variants associated with telomere length (Arrow 4 in Fig. 4), an estimate of the causal association with the end-point in question can be provided by genotyping these variants in individuals with known telomere length and known diagnoses. Of note, this estimate will not be influenced by reverse causation (i.e. influence of the disease on telomere length), or confounding.

Thus, to determine the causal role of telomere length, studies are needed with precisely measured end-points and precisely measured telomere length, but also genetic variants that determine the length of the telomere. To date, four common variants have been reported: one in *TERC* (rs12696304) [87], one in *OBCF1* (rs4387287) [87] and two in *TERT* (rs2736108 and rs7705526) [90]. Thus, in view of the large heritability, many telomere length variants remain to be identified. This is the objective of the current ongoing collaboration between the Copenhagen City Heart Study [82–84] and SEARCH [97, 98]. Combined, these studies have genotyped >24 000 individuals with the iCOGS genotyping array [99] covering almost 200 000 genotypes per individual. It is hoped that this resource will be able to identify genetic variants with high frequencies and strong associations with telomere length to determine the causal role of telomere length. This could provide unconfounded estimates of whether and if so how a change in telomere length causes a change in morbidity and/or mortality.

Conclusions

Telomere length is an attractive new biomarker that is considered to mirror the state of fundamental biological pathways. Despite more than 20 years of extensive research, the measurement of telomere length has still not been introduced into everyday clinical practice, except in the management of the few patients with rare deleterious mutations in genes encoding telomerase proteins.

Many factors have probably contributed to this delay, including technical obstacles to large-scale measurements and lack of standardization across techniques and laboratories. Another barrier to introduction derives from the very characteristic that makes telomere length such an interesting biomarker: the strong associations with risk factors that are also relevant in clinical practice including age, gender and lifestyle. Therefore, it has been very difficult to demonstrate that telomere length provides independent clinical information on disease susceptibility.

Evidence from recent large studies has supported rejection of the hypothesis, with considerable statistical power, that reduced telomere length in leucocytes predicts the risk of cancer or pulmonary disease independently of age. However, in the general population and in patients with cancer, short leucocyte telomere length is associated with increased risk of early death, even after adjustment for age, gender, smoking and other risk factors. Therefore, whilst the relevance of leucocyte telomere length for predicting risk of disease in the general population seems limited, it could be a very valuable biomarker after a diagnosis of cancer or other life-threatening diseases. It is conceivable that sequential measurements of telomere length in leucocytes could be used to monitor the residual stem cell capacity in the bone marrow during intensive treatment. Thus, measurement of telomere length could prove to be a useful tool to influence clinical decision-making. Identification of sufficient genetic variants associated with telomere length will theoretically enable new statistical designs such as Mendelian randomization, in which telomere length measurements of high quality are used, to dissect the complicated association/causality relationships between telomere length, conventional risk factors and human health.

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Conflict of interest statement

No conflict of interest was declared.

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