[Frontiers in Bioscience 15, 35-45, January 1, 2010]

The emerging role of telomere biology in cardiovascular disease

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1. ABSTRACT

A striking variability exists in the susceptibility, age of onset and pace of progression of cardiovascular diseases. This is inadequately explained by the presence or absence of conventional risk factors. Differences in biological aging might provide an additional component of the observed variability. Telomere length provides a potential marker of an individual's biological age, shorter telomeres reflect a more advanced biological age. Telomere length at birth is mainly determined by genetic factors. Telomere attrition occurs as a consequence of cellular replication and can be accelerated by harmful environmental factors such as oxidative stress. When telomeres reach a critical threshold the cell will enter senescence and becomes dysfunctional. Telomeres are remarkably shorter in patients with aging associated diseases, including coronary artery disease and chronic heart failure. In addition, numerous conventional cardiovascular risk factors are associated with shorter telomere length. If telomeres can be proven to be not only associated but also causally involved in the pathogenesis of cardiovascular disease it might provide exciting new avenues for the development of future preventive and therapeutic strategies.

2. INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death and chronic heart failure (CHF) is the main cardiovascular discharge diagnosis in the United States (1,2). In particular after the necessity of hospital admission, CHF is associated with a high mortality rate and is a growing economic burden for society (3,4). Both the incidence and prevalence of coronary artery disease (CAD) and CHF drastically increase with chronological aging (defined by the date of birth). Nevertheless, there exists a striking variability in the susceptibility, age of onset and pace of progression of both CAD and CHF. This variability cannot completely be attributed to the presence of conventional risk factors. Although chronological age is important, we also have to consider biological age as a contributing factor in the development of CVD. Unfortunately, the pace of biological aging and its inter-individual variation is not easily quantified. Telomere length fulfils a number of criteria to be considered a robust biomarker of biological aging. Telomere length is largely heritable and is affected by biological processes fundamentally involved in aging, including the number of cell divisions and exposure to external stressors (5,6,7). In this review we will focus on the properties and functions of

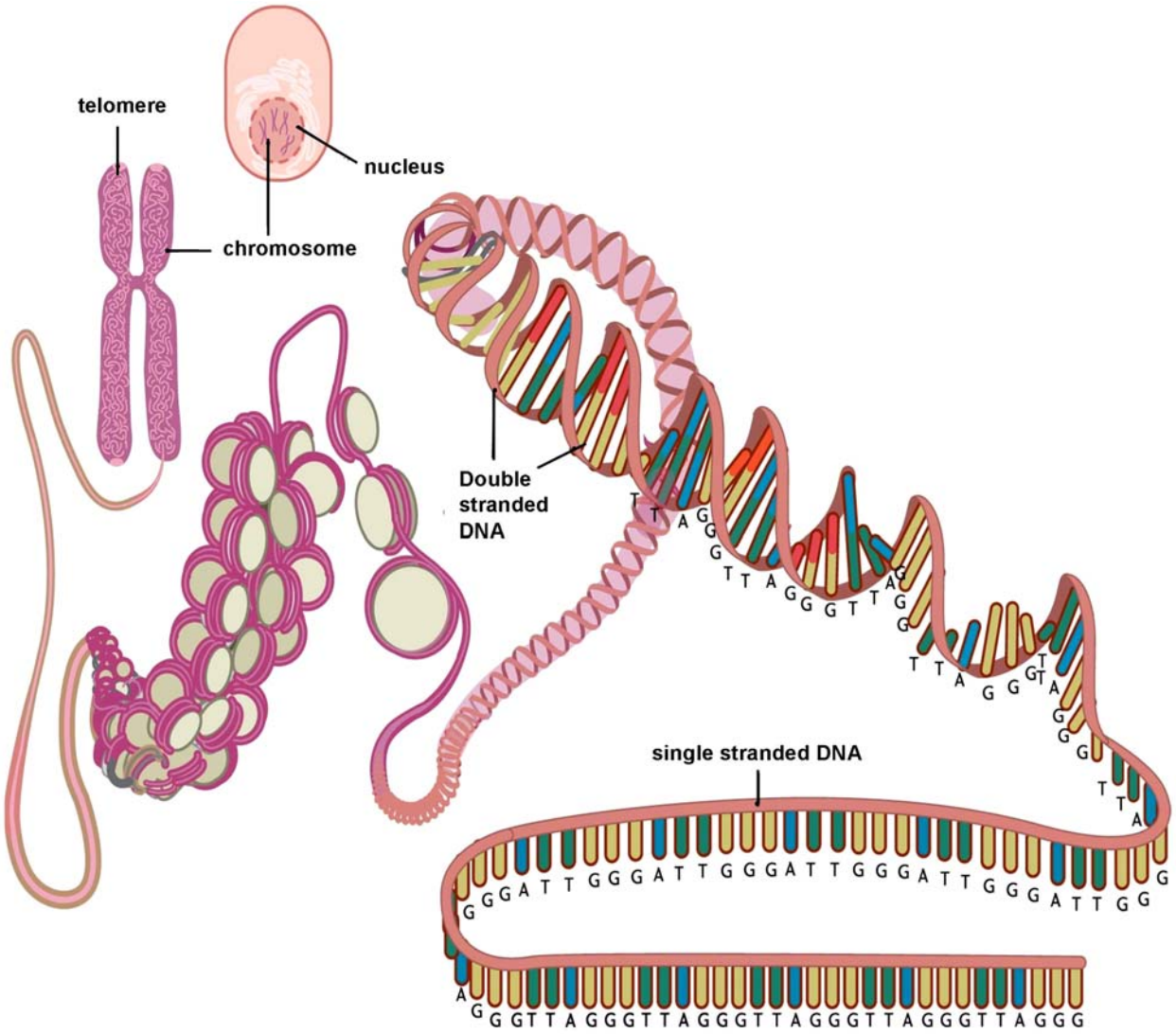

Figure 1. Localisation of the telomere in the cell. The telomere is located at the distal end of the chromosome. Reproduced with permission (85)

telomeres, describe the process of telomere shortening and elongation and the association of telomere length with cardiovascular diseases, cardiovascular risk factors and prognosis. This review provides a timely fundament for our hypothesis that telomere length is an appropriate marker of biological age and might even be involved in cardiovascular disease manifestations. We will conclude this review with potential future preventive and therapeutic strategies targeting telomere biology.

3. TELOMERES AND TELOMERASE

3.1. Telomeres

Telomeres are located at the distal ends of our chromosomes (Figure 1). Their primary function is to maintain genomic stability by protecting the integrity of the coding DNA sequence (8). Telomeres consist of numerous repeats of a specific nucleotide sequence; TTAGGG in vertebrates. In conjunction with several telomere specific and essential proteins the telomere can form a complex three dimensional structure, named the T-loop, (Figure 2) which conceals the terminal single stranded end of the chromosome (9). This is important as it prevents it from being recognised as double stranded DNA breaks consequently leading to activation of DNA-repair mechanisms (10). The following non-homologous end joining will cause chromosomal instability and will lead to senescence or apoptosis (9). The stability of the T-loop is largely dependent on the integrity of the associated telomere specific proteins (Figure 2). Telomere repeat binding factor (TRF) 1 and TRF 2 directly bind to the TTAGGG sequence and facilitate the formation of the protective T-loop. In the absence of TRFs telomeres will lose their protective T-loop structure (11). Furthermore, the capability to form this protective T-loop is influenced by the length of the single strand overhang at the end of the

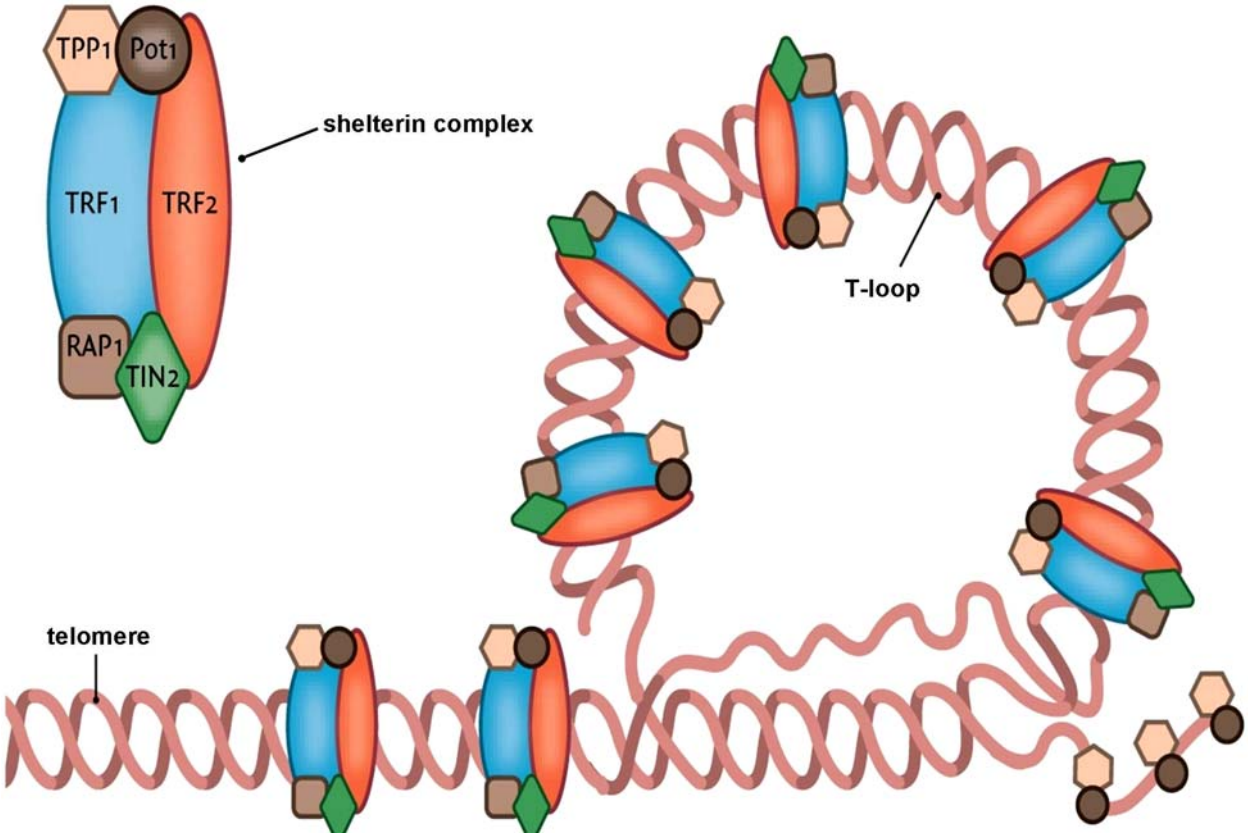

Figure 2. T-loop and the shelterin complex. The extreme end of the telomere is single stranded and ends in the T-loop. In this configuration the telomere is not recognized as a DNA break. Essential for the formation of the T-loop are the shelterin complexes. Shelterin consists of the following proteins: 'TTAGGG repeat binding factor' (TRF) 1 and 2, 'TRF1 interacting nuclear protein 2' (TIN 2), 'repressor activator protein 1' (RAP 1) and the 'protection of telomere 1' (POT 1)-TTP1 heterodimer which can form several complexes in different configurations. Reproduced with permission (85)

telomere itself. However, in the presence of high levels of TRF2 even short telomeres can form T-loops (10,12,13).

It is estimated that telomere length is for 80% dependent of the parental telomere length and the age of the father (5,14). Remarkable in this respect is that a higher paternal age at conception is associated with longer telomeres in the offspring. It is tempting to speculate that this is related to the duration of telomerase activity and the selection of cells with long telomeres in the male germ-line stem cells (15).

During life telomeres get shorter due to the so called end replication problem. This problem is caused by the inability of DNA polymerase to completely replicate the lagging DNA strand resulting in loss of telomeric base pairs during every mitosis. Besides the end replication problem there are also external factors which cause telomere attrition. For example, oxidative stress and smoking are associated with increased telomere attrition (6). Telomere length reflects both the replicative history of a cell and its exposure to detrimental factors such as oxidative stress.

When telomeres reaches a critically short length they lose their protective properties and the cell enters a non-dividing state called senescence (16). The faith of the majority of senescent cells is to enter apoptosis, although this is not necessarily true for all senescent cells. Due to morphological changes and the decreased or altered functional capacity, including the excretion of growth factors, cytokines or enzymes, a high percentage of senescent cells can disrupt tissue architecture and can result in dysfunctional tissues or even dysfunctional organs (17).

3.2. Telomerase

To avoid extinction of species there must be mechanisms to upkeep the length of telomeres of certain cells (e.g. the germ cell line). The most important mechanism involves the specialized ribonucleoprotein enzyme telomerase. This enzyme consists of two molecules of Telomerase RNA Component (TERC), two molecules of Telomerase Reverse Transcriptase (TERT) and one molecule of dyskerin (9,18). The TERC component is complementary to the telomeric DNA and functions as a template for the new to be formed telomeric repeats which are formed by the TERT component (Figure 3). The main function of dyskerin is to stabilize the telomerase complex (19). In the foetal phase, and later on in life in stem- and germ line cells, telomerase adds new TTAGGG sequences to the telomere during mitosis. The essential function of

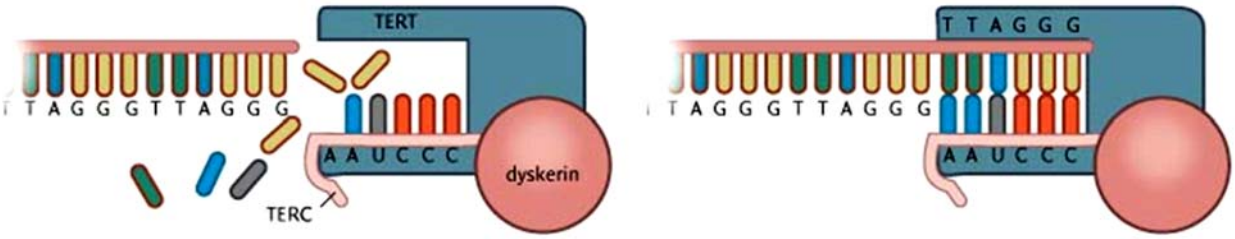

Figure 3. Telomerase. Active telomerase is formed by two RNA-complexes (TERC, one depicted here), two telomere reverse transcriptase complexes (TERT, one depicted here) and dyskerin. Dyskerin is essential for the stability of telomerase. The TERC functions as a template for the newly formed TTAGGG sequences. Reproduced with permission (85)

telomerase becomes especially evident when it fails. This is the case in the rare condition Dyskeratosis Congenita. Dyskeratosis Congenita is a progressive bone marrow failure syndrome that is characterized by abnormal skin pigmentation, nail dystrophy and leukoplakia. Most patients with Dyskeratosis Congenita become grey or get alopecia at an early age and die young mainly due to progressive bone marrow failure or malignancies (20). Telomerase deficient mice suffer progressive telomere shortening which becomes more evident in every subsequent generation. In later generations they show premature aging symptoms like infertility, grey hair or alopecia, hypertension, and decreased tissue regeneration (21). Reduced telomere length in these mice is also associated with attenuated myocyte proliferation, increased apoptosis and cardiac myocyte hypertrophy. Eventually, left ventricular failure and pathological cardiac remodelling is seen in these mice and is comparable to dilated cardiac myopathies in humans (22).

4. TELOMERES IN CARDIOVASCULAR DISEASES

4.1. Telomere length in Coronary Artery Disease

Only recently telomere biology caught the attention of cardiovascular researchers. The first study in humans on telomere length in cardiovascular disease originates from 2001 (23). In this study patients with coronary angiography proven three vessel disease were compared to patients without angiographic abnormalities. Patients with CAD had approximately 300 base pairs shorter telomere lengths. Considering the yearly attrition rate, the observed difference translates back to almost nine years difference in age (23). In a case control study with 203 cases and 180 controls it was concluded that patients with premature (before 50 years of age) myocardial infarction had telomere lengths comparable to 11.3 years older healthy controls (24). Telomere length has also been associated with the severity of disease. In a subgroup of 437 ischemic heart failure patients leukocyte telomere length was associated with the number of atherosclerotic disease manifestations. Patients with more affected vessels had shorter telomere lengths (24,25).

The association between telomere length and CAD is not limited to leukocytes but can also be observed in vascular cells. Endothelial cells in coronary arteries of patients with atherosclerosis have shorter telomeres than in patients without atherosclerosis (26). Wall biopsies of abdominal aortic aneurysms taken during surgery show shorter telomeres than healthy abdominal aortic biopsies of diseased organ donors (27). In arteries, spots with increased hemodynamic stress, display increased cellular turn-over (28). Endothelial cells at these spots (Iliacal Artery) show increased telomere attrition compared to the Internal Thoracic Artery (29). In atherosclerotic patients, the telomeres of coronary endothelial cells are shorter than those of non-affected vessels (26). Senescent human aortic endothelial cells exhibit increased levels of intercellular adhesion molecule (ICAM)-1 which stimulates the adhesion of monocytes (30). In addition senescent vascular endothelial cells show upregulation of plasminogen activator inhibitor I, have reduced production of nitric oxide (NO) and endothelial NO syntase activity (30,31). Taking this all together senescent endothelial cells seem to promote an atherogenic environment.

Whether leukocyte telomere length can be used as a good reflection of telomere status in diseased tissue can be disputed. In a recent small-scale study among 32 subjects a positive correlation has been reported between leukocyte telomere length and the telomere length of abdominal aortic wall tissue biopsies (27). These promising results will need to be confirmed in large scale studies.

4.2. Telomere length in Chronic Heart Failure

In a study conducted in 19 patients with dilated myopathy and 7 healthy age matched controls endomyocardial biopsies of the diseased hearts were characterised by shorter telomeres, increased cellular senescence and cell death (32). In an independent set of cardiac muscle biopsies of 8 failing hearts, telomere length was reduced by 25% compared to hearts of 8 healthy or 8 hypertrophic, non-failing obstructive cardiomyopathy subjects (33). In a large study leukocyte telomere length was substantially shorter in patients with CHF compared to age and gender balanced controls (25). This observation accounts for patients with ischemic as well as non-ischemic aetiology of heart failure. Moreover, the clinical severity of CHF was related to the degree of telomere shortening (25). Ejection fraction is strongly associated with telomere length in subjects without evidence of previous myocardial infarction (34). One standard deviation longer telomere length was associated with 5% higher ejection fraction. Telomere length alone accounted for 12% in the observed variability in ejection fraction in these elderly subjects (34).

Table 1. Overview of the association of cardiovascular risk factors and leukocyte telomere length

Risk factor	Association with telomere length	Reference
Gender	Females have longer telomeres Men have higher telomere attrition rate	(38,39) (39,14)
Positive family history	Offspring of fathers with premature myocardial infarction have shorter telomeres than offspring with healthy fathers	(42,43)
Diabetes	Diabetic patients have shorter telomeres than non-diabetic controls, adequate glykemic control prevents telomere shortening in type II diabetes and attenuates it in type I diabetes	(73)
	Insulin resistance and obesity are associated with increased telomere attrition	(48)
	Insulin resistance is negatively correlated with telomere length	(45)
Blood pressure	Negative correlation between telomere length and pulse pressure in men	(38)
	Hypertensive subjects have shorter telomeres than normotensives	(45)
	Telomere length is associated with circulating biomarkers of the renin-angiotensin-aldosterone system	(49)
Renal dysfunction	Telomere length correlates positively with estimated glomerular filtration rate in chronic heart failure patients	(51)
ICA-IMT	Telomere length is inversely associated with ICA-IMT after adjusting for age and gender	(47)
	Borderline significant inverse association of telomere length with ICA-IMT	(67)
Homocysteine	Increased homocysteine levels are associated with shortened telomeres	(53)
Obesity	Obesity is negatively correlated with telomere length in women	(46)
	Telomere length is inversely associated with body mass index adjusted for age and gender	(47)
Lifestyle	Telomere length is positively associated with an increased physical activity level	(78)
	Subjects with moderate physical activity levels have longer telomeres compared to both low and high levels of physical activity	(79)
Smoking	Smoking females have shorter telomeres	(46)
	Age adjusted smokers have shorter telomeres than never-smokers	(47)
Psychological stress	Psychological stress is associated with lower telomerase activity and shorter telomeres	(54,55)
	Patients with mood disorders have shorter telomeres than healthy controls	(59)

Abbreviations: internal carotid artery intima media thickness, ICA-IMT

In older patients findings between the presence and absence of cardiovascular disease is in general less well consistent related to telomere length (35,36,37). In 193 subjects over 70 years of age telomere length was not associated with the presence of CAD, but an association with aortic valve calcification was reported (36). In 190 persons over 85 years of age shorter telomere length was related to the presence of self reported heart disease and ischemic changes on electrocardiography (35). However, studies in elderly are likely to suffer from important selection biases.

4.3. Telomere length and cardiovascular risk factors

Besides the relation with cardiovascular diseases, telomere length has also been associated with a striking amount of cardiovascular risk factors. A good example is the male gender. Male gender is an important risk factor for cardiovascular disease as well as it is associated with shorter telomere length (38,39). In men the pace of telomere shortening during life is also faster than in women (40). Possibly due to the protective properties of estrogen on telomerase (41). A positive family history of cardiovascular disease is one of the most important risk factors. Offspring of parents with premature CAD already have shorter telomeres than children of parents without cardiovascular disease (42,43).

In vitro as well as *in vivo* there is clear evidence that oxidative stress reduces telomere length (44,45). Consistent is also the dose-dependent relation of smoking with reduced telomere length (46,47). In cross-sectional studies shorter telomeres have also been associated with diabetes, increasing body weight and increasing insulin resistance (45,46,48). Increased pulse pressure in men and increased carotid artery internal media thickness (ICA-IMT) is also associated with shorter telomere length (38,47). In the Framingham heart study, subjects with increased circulating biomarkers of the renin-angiotensin-aldosterone system had shorter telomeres (49). Subjects with decreased renal function are at increased risk to experience cardiovascular events and also have shorter telomeres (50,51,52). Recently an inverse correlation between plasma homocysteine levels and telomere length was found (53). Besides these physical and biochemical risk factors there are also psychological and environmental risk factors associated with telomere length. Psychological stress, chronicity of stress, depressive symptoms and decreased social status are associated with having shorter telomeres (54,55,56,57,58,59). In a recent large survey among 1,502 subjects self-perceived early aging was associated with abdominal obesity, poor self-rated health, lower education and shorter telomere length (60). An overview of the associations between telomere length and cardiovascular risk factors can be found in Table 1.

Interestingly, the relation between CAD and telomere length cannot be fully explained by classical risk factors (24,61). This suggests that the relation of telomere shortening on cardiovascular disease is not only through classical pathways, but might be an independent factor as well. Taken together, these data supports the hypothesis that telomere shortening is involved in the pathogenesis of cardiovascular diseases (43).

5. THE PROGNOSTIC VALUE OF TELOMERE LENGTH

When telomere length is considered a biomarker of biological aging and associates with cardiovascular pathology the question arises whether telomere length conceals prognostic value as well. The first study aimed to answer this question was undertaken in 143 patients without selection on presence or absence of diseases. Adjusted for age, and after 20 years of follow-up, patients with telomere lengths shorter than the median had a three times higher risk of cardiovascular death than patients with telomeres longer than the median. Subjects with telomere length in the lowest quartile had an eight times increased risk of dying from infectious diseases than persons from the three higher quartiles (62).

In a case-control study a three times increased risk of getting a premature myocardial infarction (before 50 years of age) was observed in persons with telomere lengths shorter than average compared to persons with telomere lengths in the highest quartile (24). In another case-control study, nested in the West of Scotland Primary Prevention Study (WOSCOPS), 485 patients who reached the primary endpoint (myocardial infarction or cardiovascular death) after a follow-up of 5 years where compared to 1058 matched controls who did not reach the endpoint. Patients with shorter telomeres had an almost doubled risk of reaching the primary endpoint than patients with long telomeres (61). In 870 patients with stable CAD it was observed that patients in the lowest quartile of telomere length had a 1.9 increased risk of dying compared to patients in the highest quartile after adjusting for age, clinical, inflammatory and echocardiographical risk factors (63). In the elderly this association is again less clear. In 812 persons aged 73-101 years old and in 598 persons over 85 years of age no association between telomere length and survival was observed (64,65). However in 195 stroke survivors over 75 years of age longer telomeres were associated with a better survival and in 412 healthy persons over 65 years of age with a median age of 74.4 years telomere shortening was associated with an increased risk for myocardial infarction and stroke (66,67). Again in the elderly telomere length may be a less accurate marker of biological age due to an important selection bias or confounders.

6. FUTURE PERSPECTIVES AND CONCLUSIONS

6.1. Potential Intervention strategies

If telomere biology is proven to be involved in the development and progression of cardiovascular disease it will pave the way for new therapeutic or preventive strategies. For example, statins have, besides their many influences on conventional risk factors, proven to have a protective effect on telomeres by preventing the loss of the essential TRF-2 protein in endothelial progenitor cells (68,69,70,71). In addition, statins promote DNA repair and prevent telomere shortening and senescence in cultures of vascular smooth muscle cells (72). In diabetic patients adequate glycemic control prevents additional telomere shortening (73). A different possible target for therapy can be through telomerase. Human telomerase can also be regulated by erythropoietin (74). Evidence is accumulating for a beneficial role of erythropoietin in endothelial function, independent of increasing haemoglobin levels (75,76). Increased levels of telomerase could be involved in this process. Changes in telomerase activity can also be achieved by comprehensive changes in lifestyle (77). In short these changes included a low-fat diet, moderate aerobic exercise, stress management and food supplements and resulted in an increased telomerase activity in peripheral blood mononuclear cells after a period of three months (77). Increased levels of physical exercise are by itself associated with longer telomeres and can thus potentially have a decelerating effect on biological aging (78,79).

Besides pharmacological and lifestyle interventions, gene modification can also provide new opportunities. Over-expression of telomerase can counteract telomere dependent replicative senescence (80). Transfecting cells with the human TERT increases telomerase activity resulting in delay of replicative senescence (80). *In vitro* immortalized TERT overexpressing swine umbilical vein epithelial cells produced normal levels of NO, endothelin and prostacylin indicating their biological functioning and metabolic capacities are similar to mortal cells (81). Porquine ventricular endocardial endothelial cells (EEC) also exhibit phenotypic and functional characteristics similar to primary EEC (82). Bovine microvascular endothelial cells exhibit an endothelial phenotype similar to that of wild-type endothelial cells. Specifically, they had the typical cobblestone morphology, expressed endothelial cell-specific markers and vascular endothelial growth factor receptor-2 (VEGFR-2). Besides, they expressed receptors for low density lipoprotein (LDL) and were able to form tubular structures (83).

In humans several trials have been undertaken to evaluate the safety and efficacy of infusion of bone marrow derived mononuclear cells (84). The results this far look promising however there are still some concerns. One of them is the viability and the proliferative potential of the cells transfused (84). Homologues transplantation of cells of various tissues and progenitor cells, of which the telomeres have been elongated *in vitro*, may in the future help to more effectively repair damaged endothelium or alter the remodelling processes leading to CHF. However one should remember that telomere dependent senescence also has a protective function, namely to prevent cells from unlimited cell divisions. A possible solution for this potentially dangerous process is to bring selective expression of telomerase under control by a specific substance sensitive promotor. In the presence of this substance telomeres can then be elongated *in vitro* and after having them transfused back, telomerase expression will cease again.

6.2. Conclusions

Exponential increasing evidence is suggesting that telomere length is associated with cardiovascular diseases. Shorter telomeres are not only associated with the presence of cardiovascular risk factors and established cardiovascular diseases but also the degree of telomere shortening is related to the severity of the disease (25). In addition, telomere length also predicts the occurrence of clinical manifestations of cardiovascular disease and outcome. The major limitation of most previous studies present lays in its cross-sectional nature. The key question, whether or not telomere shortening is causally involved in the development and progression of cardiovascular diseases, remains a target for future studies. In these studies telomere dynamics over time need to be related to the development of cardiovascular diseases and events. In addition, this design will give more insight in other potential cardiovascular risk factors or modifiers of telomere length. With a longitudinal design interactions of risk factors or efficacy of interventions on telomere length and clinical outcome can be identified better. The aforementioned telomerase deficient mice with short

telomeres could serve as a model to study the vulnerability of having short telomeres to specific factors. These factors can be genetic, for example deficient DNA repair mechanisms, pathologic, for example diabetes and hypertension or environmental factors like smoking, stress or diet. Combining these different scientific approaches more insight can be gained in the causal role telomere shortening potentially plays in cardiovascular disease.

7. ACKNOWLEDGEMENTS

This work was supported by the Innovational Research Incentives Scheme program of the Netherlands Organisation for Scientific Research (NWO VENI, grant 916.76.170 to P. van der Harst) and the Netherlands Heart Foundation (grant 2006B140). P. van der Harst is a research fellow of the Netherlands Heart Foundation (grant 2006T003) and the Interuniversitair Cardiologisch Instituut Nederland (ICIN). D.J. van Veldhuisen is an Established Investigators of the Netherlands Heart Foundation (grant D97-017).

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Abbreviations: cardiovascular disease: CVD, chronic heart failure: CHF, coronary artery disease: CAD, telomere repeat binding factor (TRF), telomerase RNA component: TERC, telomerase reverse transcriptase (TERT),

endocardial endothelial cell (EEC), internal carotid artery internal media thickness (ICA-IMT)

Key Words: Telomere, Telomerase, Heart Failure, Atherosclerosis, Prognosis, Review

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