Telomere Length Dynamics in Vascular Disease: A Review


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KEYWORDS
Telomere; Telomerase; Vascular; Cardiac

Abstract
Introduction: Telomeres are specialised DNA-protein complexes which cap the ends of linear chromosomes serving to maintain DNA integrity during cell division. Telomere length naturally shortens with successive cell divisions and represents a cellular marker of biological age. This paper aims to provide an overview of telomere biology and review the evidence for any association between vascular surgical conditions and short telomere length.

Methods: A systematic review of the literature was performed using the search terms 'telomere' and 'vascular'.

Results: Considerable associations between a shorter mean telomere length and coronary heart disease have been observed. This finding extends to vascular disease risk factors including age, sex, smoking, obesity, hypertension and diabetes. Vascular diseases including abdominal aortic aneurysm, peripheral vascular disease and carotid disease were also associated with shorter telomere lengths but evidence was limited to a small number of studies. There were no reports of short telomere length associated with varicose veins or arteriovenous malformations suggesting a novel area for further investigation.

Conclusion: Multiple associations between short telomere length and vascular disease characterised by atherosclerosis suggest a possible link between telomere attrition and disease mechanisms. Further studies are warranted to validate and define the role of telomeres in vascular disease pathogenesis.

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Introduction

Telomeres are specialised DNA-protein complexes present at the ends of linear chromosomes in eukaryotes. They safeguard gene integrity during mitosis by preventing DNA degradation at chromosomal termini. Telomere length shortens with each cell division and can be likened to a ‘mitotic clock’ reflecting cellular turnover. This has given rise to concepts of telomere length as a surrogate marker of age at both the cellular level and organism level, as well as a potential biomarker of age-related diseases. Shortening of telomeres to a critical length induces ‘replicative senescence’ — the cessation of mitosis due to a finite number of cell divisions (the ‘Hayflick limit’). This mechanism appears to prevent genomic instability and development of malignancy in aged cells by limiting the number of cell divisions. Studies relating to coronary heart disease have demonstrated short telomere length in peripheral blood leukocytes to be an independent risk factor for disease and potentially a superior predictor of disease onset than chronological age. Furthermore, correlations between telomere lengths with specific stages of disease progression observed in cancer studies have revealed their potential prognostic and diagnostic value. The aim of this review was to determine whether this evidence exists for vascular conditions including aortic aneurysms and carotid artery disease.

Methods

The Medline and Embase databases were searched from 1965 to October 2009 using the search terms ‘telomere’ and ‘vascular’ and limits ‘English language’ and ‘Human’. Additional articles were obtained manually from reference lists and the latest ‘Telomere Biology’ supplement in Nature. Articles were screened as per PRISMA guidelines (Fig. 1). Sixty-nine articles were included in the final qualitative synthesis. Following an overview of telomere biology, we present salient findings from the literature under subheadings pertaining to different vascular conditions.

Telomere biology

Structure and function

Telomeres confer a protective role for each chromosome by preventing enzymatic erosion, non-homologous recombination and end–end fusion of chromosomal DNA. Such events can induce chromosomal instability, apoptosis and cell death. Human telomeres consist of three components, (i) tandem repeats of guanine-rich non-coding DNA sequences, predominantly TTAGGG in 5′–3′ direction, (ii) shelterin—a complex of six proteins adherent to telomeric DNA and (iii) telomerase, a DNA polymerase. Telomeres are arranged in to duplex loops which comprise a double-stranded telomere loop (T-loop) and a single-stranded (D-loop) (Fig. 2). Proteins forming the ‘shelterin complex’ bind to and protect the duplex loop. They prevent false recognition of telomeres as double-stranded DNA breaks which would otherwise be vulnerable to aberrant processing by DNA repair complexes. The two major shelterin proteins TRF-1 and TRF-2 (telomere repeat-binding factors) possess binding domains with high specificity for the telomere-repeat sequences explaining why shelterin is abundant at chromosome ends. TRF-1 is involved in telomeric DNA remodeling and negative regulation of telomere length. The removal of TRF2 initiates DNA damage repair pathways which can result in deleterious fusion of chromosomal ends. Human repressor activator protein (hRAP1) is a binding-partner of TRF2 and can extend telomeres.
when overexpressed. TRF1-interacting protein 2 (TIN2) is thought to mediate TRF1 activity and collectively both can inhibit DNA lengthening by telomerase. The two remaining shelterin proteins, pot-1 (protection of telomeres) and tpp1 (tripeptidyl peptidase I) are binding partners thought to both augment and inhibit telomerase function. Unlike the shelterin proteins which constantly reside at the telomere, numerous non-shelterin accessory proteins are intermit-tently recruited to the telomere and thought to influence telomere length control and repression of the DNA damage response. Examples include tankyrase (tank-1, tank-2) which may release TRF1 from the telomere and cause DNA elongation. In addition, Ku protein, a heterodimer of 2 polypeptides (Ku70 and Ku80), is integral to the non-homologous end-joining DNA repair pathway which is important to telomere maintenance. Knockout studies of the Ku86 gene have shown human cell lines to exhibit higher rates of double-stranded DNA breaks and telomere loss culminating in cell death. Telomerase synthesizes telomeric DNA and prevents telomere attrition (erosion). It

<table>
<thead>
<tr>
<th>Tissue type (healthy tissue unless specified)</th>
<th>Attrition rate (mean base pair loss/year)</th>
<th>n</th>
<th>Age ranges (years)</th>
<th>p-value of regression</th>
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<tr>
<td>Vascular tissues</td>
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<tr>
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<td>47</td>
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<td>twins</td>
<td>2–95</td>
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<td>Iliac arteries intima</td>
<td>102 ± 13 (SD)</td>
<td>4</td>
<td>21–58</td>
<td>0.03</td>
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<td>Internal thoracic artery intima</td>
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<td>4</td>
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<tr>
<td>Iliac vein endothelium</td>
<td>47 ± 4 (SD)</td>
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<td>14–49</td>
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<td>(non-diseased autopsy specimens)</td>
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<td>Myocardium</td>
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<td>168</td>
<td>0–104</td>
<td>0.17</td>
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<tr>
<td>(Non-diseased autopsy specimens)</td>
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<tr>
<td>Non-vascular tissues</td>
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<td></td>
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<tr>
<td>Oesophageal mucosa</td>
<td>60</td>
<td>177</td>
<td>0–102</td>
<td>Not given</td>
</tr>
<tr>
<td>Thyroid</td>
<td>91</td>
<td>46</td>
<td>0–98</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Parathyroid (normal operative and autopsy specimens)</td>
<td>92</td>
<td>21</td>
<td>0–83</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Large Bowel Mucosa (matched normal mucosa from colorectal Ca patients-operative specimens)</td>
<td>59</td>
<td>129</td>
<td>0–9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hepatic (non-diseased autopsy specimens)</td>
<td>55</td>
<td>94</td>
<td>0–101</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Figure 2  The duplex structure of a telomere consisting of DNA forming a T-loop and D-loop. Several shelterin proteins bind specifically to the telomeric DNA and facilitate telomere end protection and length control. Shelterin complex core proteins- TRF1, 2 - Telomeric repeat-binding factors; RAP1- Human repressor activator protein 1; TIN2 - TRF1-interacting protein; Accessory Binding proteins- Ku- the Ku70/Ku86 heterodimer, tank-1, tank-2- tankyrase; MRE11/RAD50/NBS1-MRN DNA detection and repair complex, hnRNPs - heterogeneous nuclear ribonucleoprotein.

Figure 3  Illustration of the 'end replication problem' during unidirectional DNA replication. 1. Double stranded DNA 'unzips' in to a replication fork to allow DNA polymerase to synthesize in the 5’→3’ direction. 2. Leading strand is straightforwardly synthesized as continuous DNA from parental DNA. 3. Lagging strand can only be synthesized in discontinuous fragments with requirement of a primer. 4. Gap left by primer removal at the chromosomal terminus (telomere) cannot be repaired.
is integral to telomere maintenance and regulates cellular lifespan. While absent in most somatic cells, low levels exist in highly proliferative cell lines including skin and intestinal mucosa. Conversely, telomerase expression is upregulated in germline, embryonic and stem cells and more so in human cancer cells, contributing to cell immortalisation. Telomerase is composed of a RNA component (TERC) which serves as a template for DNA synthesis and a reverse transcriptase (TERT) which catalyses the elongation. Mutations and overexpression of these subunits may have pathological implications having been observed in conditions involving stem-cell failure, idiopathic pulmonary fibrosis and carcinogenesis.

Telomere length and assessment

Each round of DNA replication results in a shorter telomere by 30–100 nucleotides. This is consequence of the 'end replication problem' that arises from the inability of DNA polymerase to replicate the 5' end of the lagging strand after degradation of an RNA primer (Fig. 3). This loss of telomeric DNA can be regenerated by telomerase. Human telomere length varies between tissues from 12 to 16 kilobase (kb) pairs in comparison to 20–50 in mice. It may vary between chromosomes belonging to the same cell and between individuals of the same age. During intrauterine life till birth however, telomere length appears to be consistent between leukocytes, liver, lung, muscle and myocardium. Loss of this synchrony in adulthood may be attributable to variable cellular proliferation rates as one matures. Indeed, it is evident that the rate of telomere attrition varies in different tissues, and accounts for 30–150 bp loss per annum (Table 1). Strong associations observed between peripheral blood leukocyte (PBL) telomere length in fathers and their children, may suggest paternal inheritance.

Women’s telomeres have been reported to be 3.5% longer than men’s after adjustment for age in one study (n = 143). Despite similar PBL telomere lengths in newborn males and females, age-dependent telomere attrition occurs at a higher rate in males. However, beyond the age of 60 there is no such difference and women maintain longer telomeres into old age. Others could not replicate these gender differences in PBL telomere length in elderly cohorts (79 years).

Telomere length is thus far considered an emerging marker of cellular ageing and age-related disease and has been measured by an array of techniques, most commonly by Southern Blot analysis of terminal restriction fragments (TRF). Other techniques including hybridization protection assays and fluorescence in situ hybridization are gaining popularity.

Evidence of shortened tissue telomere length patterns observed in both malignant and benign diseases including colorectal carcinoma, colonic polyps and gastric adenomas have revealed their potential value as biomarkers.

Telomere length and aortic disease

Latest telomere studies suggest that biological ageing of the vasculature may play a role in abdominal aortic aneurysm aetiology. We implemented the Southern Blot technique to compare peripheral blood leukocyte (PBL) telomere length in abdominal aortic aneurysms (n = 190) with that in age-sex matched controls (n = 183). Significantly shorter telomere length was detected in AAA, evident by a mean difference of 189 bp (95% CI 77 bp–301 bp, P = 0.005). This relationship persisted after controlling for confounding risk factors with multiple regression analysis. Individuals in the lowest quartile of mean telomere length exhibited a 2-fold risk of AAA relative to those with a mean telomere length in the highest quartile (odds ratio 2.30, 95% CI 1.28–4.13, P = 0.005). Secondly, our data demonstrated a significant negative correlation between AAA diameter and telomere length. Previously, Wilson et al. compared PBL ‘telomere content’ from AAA (n = 20, n = 12 controls) in a separate cohort of patients by qPCR. Significantly reduced telomere content was apparent in AAA against controls (0.82, SD 0.06 and 1.27, SD 0.10 respectively, p < 0.001). The mean telomere content of aneurysm wall biopsies was compared in these cases (obtained during elective AAA repair) with unmatched controls (normal cadaveric aorta). Telomeric DNA was reduced in AAA vs. controls after adjusting for age and gender (2.17, 95% CI 1.77–2.56 vs. 2.80, 95% CI 2.32–3.28, P < 0.05). Furthermore, significant positive correlation of PBL telomere content with tissue telomere content was apparent for both cases and controls (partial correlation coefficient 0.62, P < 0.001). This correlation suggests that such PBL telomere measurements hold potential as accessible markers of vascular ageing in the aorta, in individuals with or without aneurysmal disease. Earlier, Okuda et al. assessed the telomere length in biopsies from non-aneurysmal aorta against the severity of any atherosclerotic lesions present (n = 44). Although telomere length in distal aortic biopsies shortened as atherosclerotic grade increased, this association was not significant after adjustment for age (p = 0.06). The group did demonstrate however, a significantly higher rate of age-dependent telomere attrition in proximal aorta in comparison to distal aorta (intima p = 0.003, media p = 0.004). Although there is no obvious explanation for this, the authors suggest that local factors such as shear wall stress may influence such findings.

Peripheral arterial disease and venous disease

Leukocyte telomere length has been measured in patients with chronic heart failure, with or without other atherosclerotic manifestations including peripheral vascular disease (age 40–80, cases n = 620, controls n = 183). The presence or past history of peripheral vascular disease (PVD), defined as “claudication”, was an independent predictor of a short telomere length (age-adjusted regression, p = 0.001). The ambiguous definition of PVD however, requires cautious interpretation of results. These findings failed to be replicated by the prospective Cardiovascular Health Study (age >65, n = 419), where PVD was defined as an ABI of <0.9. Twenty-two cases of PVD were identified (7.9 year follow-up) and did not correlate with a shorter leukocyte telomere length (p = 0.51). Zee et al. have conducted the only telomere study in venous disease.
<table>
<thead>
<tr>
<th>Technique</th>
<th>Principles</th>
<th>Merits</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southern Blot analysis of terminal restriction fragments&lt;sup&gt;8&lt;/sup&gt;</td>
<td>Genomic DNA is digested by restriction enzymes into fragments which are separated by electrophoresis. These are hybridized to labelled probes specific for telomere sequences. The hybridized terminal restriction fragments are quantified by densitometry and reflect an approximation of telomere length.</td>
<td>Universal standard of comparison to newer methods. Provides mean telomere length of all chromosomes.</td>
<td>Overestimates telomere length since includes subtelomeric sequences of unknown length. Labour intensive (~5 days). Requires pure unfragmented input DNA. Radiolabelling procedures unavoidable. Provides limited detail of telomere length at the chromosomal or cellular level.</td>
</tr>
<tr>
<td>Hybridization protection assays&lt;sup&gt;68&lt;/sup&gt;</td>
<td>Oligonucleotide probes labelled with a highly chemiluminescent acridinium ester specifically bind to telomere sequences. Resultant luminometer readings allow quantification of telomere repeats.</td>
<td>Mean telomere length obtained without including subtelomeric regions - more accurate than Southern blotting. Less stringent input DNA purity required. Expedient protocols (&lt;1 h). Radiolabelling procedures not required.</td>
<td>Provides limited detail of telomere length at the chromosomal or cellular level.</td>
</tr>
<tr>
<td>Fluorescence in situ hybridization (FISH)&lt;sup&gt;69&lt;/sup&gt;</td>
<td>Telomere repeat-specific peptide nucleic acid probes labelled with fluorescent dye emit signals quantifiable by digital imaging systems. Small sized probes allow effective cellular penetration.</td>
<td>Allows precise telomere repeat number quantification in individual cells. Viable cells from culture required but only &lt;30. Enhanced precision variants available e.g. quantitative-FISH.</td>
<td>Labour intensive. High requirement for technical expertise.</td>
</tr>
<tr>
<td>Flow cytometry FISH — 'Flow-FISH'&lt;sup&gt;21&lt;/sup&gt;</td>
<td>FISH is supplemented by use of antibody staining i.e. 2 probes, which allows targeting of specific cell lines within a sample.</td>
<td>Telomere length determination possible in single cells. High sensitivity, accuracy and reproducibility due to use of 2 probes.</td>
<td>Moderate/high complexity protocols ~30 h protocol. Large number of cells required.</td>
</tr>
<tr>
<td>Quantitative-polymerase chain reaction (qPCR)&lt;sup&gt;8&lt;/sup&gt;</td>
<td>Oligonucleotide primers specifically anneal to telomere repeat sequences and allow amplification and quantification in a thermal cycler. Comparison to a single reference gene allow telomere length to be expressed as telomere repeat copy number/single gene copy number ratio.</td>
<td>More rapid than Southern Blot and requires less input DNA. Multiplex high-throughput e.g. 384-well format protocols available — suitable for epidemiological studies.</td>
<td>Provides a relative reading of telomere length as opposed to absolute telomere length — therefore difficulty comparing to other methods.</td>
</tr>
</tbody>
</table>
incidentally developed venous-thromboembolism \( (n = 108, \text{DVT } 60\%, \text{PE } 40\%) \) were compared with age–sex matched controls \( (n = 108) \). No significant difference in telomere length was detected between cases and controls \( (p = 0.62). \)

**Telomeres and carotid disease**

The Cardiovascular Health Study conducted ultrasound measurements of common carotid and internal carotid intima-media thickness \( (n = 419) \).\(^{41}\) Although shorter leukocyte telomere length was observed with increasing carotid stenosis, this failed to reach significance \( (p = 0.07) \). Benetos et al. compared leukocyte telomere length against incidence of carotid plaques in an exclusively hypertensive cohort \( (n = 163) \).\(^{42}\) Carotid plaques were detected by ultrasound in 73 patients \( (44\%) \), whom collectively exhibited a significantly shorter telomere length than those without plaques \( (n = 90 \text{ controls}, 8.21 \text{ kb vs. } 8.43 \text{ kb}, p = 0.03) \). There was no difference in mean lipid levels \( (\text{HDL, LDL, total cholesterol}) \) between the two groups and statistical significance remained after excluding fifty-five patients on lipid-reducing drugs. This suggests that a shorter leukocyte telomere length may be a superior predictor of carotid plaque development in hypertensive patients and questions the role of telomeres in plaque pathogenesis. Subsequently, Adaikalakoteswari et al. compared leukocyte telomere length in type-2 diabetics with \( (n = 30) \) or without evidence of carotid plaques and femoral plaques on ultrasound \( (n = 30) \).\(^{44}\) Telomere length was shorter in diabetics with plaques \( (5.39, \text{SEM } 0.2 \text{ kb}) \) compared to those without \( (6.21, \text{SEM } 0.2 \text{ kb}) \) and collectively these were significantly shorter than those of non-diabetic controls \( (8.7, \text{SEM } 0.5 \text{ kb}) \). However, they have failed to specify what proportion of the group of ‘diabetics with plaques’ had either femoral or carotid plaques.

**Telomeres and vascular risk factors**

**Hypertension and pulse pressure**

In the Framingham Heart Study, hypertensive male subjects \( (n = 171) \) exhibited significantly shorter age-adjusted leukocyte telomere length compared with their normotensive peers \( (n = 156) \).\(^{45}\) The Cardiovascular Health Study however, could not replicate this in a mixed male and female cohort \( (p = 0.82) \).\(^{47}\) Arterial pulse pressure widens with age since systolic pressure gradually rises and diastolic pressure plateaus. This prompted Jeanclos et al. to hypothesize that pulse pressure may represent a phenotype of biological age. They investigated leukocyte telomere length against pulse pressures in a twin population \( (n = 98 \text{ pairs}) \).\(^{46}\) Significant inverse correlation of telomere length with pulse pressure was noted, suggesting those with shorter telomeres exhibit a wider pulse pressure \( (p = 0.0032) \). Two studies suggest that telomere shortening may occur in arterial segments susceptible to increased hemodynamic stress. Okuda et al. identified a higher telomere attrition rate in infrarenal aortic specimens relative to the proximal abdominal aorta \( (n = 51 \text{ matched biopsies}) \).\(^{39}\) In addition, Chang and Harley showed iliac artery endothelial cells have shorter telomeres than those in internal thoracic arteries \( (n = 13 \text{ matched biopsies}) \).\(^{27}\) These data collectively imply that shorter telomere length, associated with increased endothelial cell turnover, may be a consequence of higher shear stresses in the vasculature. Replacement of functional cells with senescent endothelial cells, a process accelerated by exposure to reactive oxygen species, has been implicated in the pathogenesis of atherosclerosis.\(^{27,45,47}\)

**Smoking, obesity and diabetes**

Valdes et al. reported that the mean leukocyte telomere length of obese women \( (\text{BMI } \geq 30, n = 119) \) was found to be 240 bp shorter than in lean women \( (\text{BMI } \leq 20, n = 85, p = 0.026) \).\(^{48}\) In turn, a cohort study of both sexes \( (n = 70, 57\% \text{ female}) \) demonstrated annual leukocyte telomere length to significantly fall in those with an increasing BMI, independent of age over a 10-year period \( (p \leq 0.001) \).\(^{49}\) Valdes et al. demonstrated a dose-dependent relationship between leukocyte telomere shortening and smoking.\(^{48}\) Each pack-year smoked equated to loss of an additional 5 bp \( (18\%) \) of the average yearly loss in age-adjusted telomere length. The dose-dependent effects of smoking were replicated by Morla et al. in a cohort of male smokers with and without COPD \( (50 \text{ smokers, } 26 \text{'never-smokers'}).\(^{50}\) Telomere shortening correlated with cumulative exposure to tobacco, independent of concomitant COPD. These results may collectively implicate smoking and obesity with pro-ageing effects. However, these findings were not reproduced by two studies of elderly individuals which failed to correlate shorter telomeres with number of cigarettes smoked \( (p = 0.30) \) against controls \( (n = 30) \) compared to controls \( (n = 30) \).\(^{51,52}\) The mean ages of each cohort were considerably higher in these studies, at 81 \( (n = 812) \) and 79 \( (n = 598) \). Recent evidence of telomere instability in individuals over 85 years old may explain the discrepancies between these studies with dissimilar demographics.\(^{52}\) Studies on South Asian populations have shown shorter leukocyte telomere lengths in individuals affected by non-insulin dependent diabetes \( (\text{NIDDM, } n = 40) \) and impaired glucose tolerance \( (n = 30) \) compared to controls \( (n = 40) \).\(^{44,53}\) Jeanclos et al. previously demonstrated the same findings for insulin dependent diabetes mellitus \( (\text{IDDM}) \) but not for NIDDM in Caucasian men \( (n = 54 \text{ vs. } n = 106 \text{ controls}) \).\(^{54}\) Others have shown association between short leukocyte telomeres and diabetes, fasting insulin and fasting glucose and higher attrition rates in increasing insulin resistance.\(^{41,45,49}\) Conversely, Sampson et al. could only demonstrate shorter telomere length specifically in monocytes, not lymphocytes, in those with NIDDM \( (n = 21) \) against controls \( (n = 29) \).\(^{55}\)

**Telomeres and coronary heart disease**

Several studies have described short telomere length to carry predictive value for coronary heart disease \( (\text{CHD}) \) onset and CHD-specific mortality. The largest prospective study amongst these included that of Brouilette et al.
<table>
<thead>
<tr>
<th>Condition/tissue</th>
<th>Telomere source</th>
<th>Telomere measurement</th>
<th>Main findings</th>
<th>cases/controls</th>
<th>P-value</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>AAA</td>
<td>Leukocytes</td>
<td>TRF*-Southern Blot analysis</td>
<td>Reduced telomere length in AAA Telomere shortening correlated with increasing AAA diameter</td>
<td>190/183</td>
<td>0.005</td>
<td>Atturu et al. 36, 37</td>
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<td></td>
<td>Leukocytes</td>
<td>Telomere/genomic DNA ratio – qPCR</td>
<td>Reduced telomere content in AAA Telomere shortening in AAA</td>
<td>20/12</td>
<td>&lt;0.001</td>
<td>Wilson et al. 38</td>
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<td>AAA proximal sac vs. healthy aorta</td>
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<td>20/12</td>
<td>&lt;0.05</td>
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<td></td>
<td>Matched aortic intimal and medial biopsies</td>
<td>TRF – Southern Blot analysis</td>
<td>Increased telomere attrition rate in distal aorta vs. proximal aorta for both tunica intima and tunica media</td>
<td>44/44</td>
<td>0.003</td>
<td>Okuda et al. 39</td>
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<tr>
<td>Peripheral vascular disease (PVD)</td>
<td>Leukocytes</td>
<td>Telomere to single reference gene (T/S) ratio – qPCR</td>
<td>Reduced telomere length in patients with chronic heart failure and claudication vs. those without claudication</td>
<td>620/183</td>
<td>0.001</td>
<td>Van Der Haarst et al. 40</td>
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<tr>
<td>Carotid disease</td>
<td>Leukocytes</td>
<td>TRF – Southern Blot analysis</td>
<td>No association between telomere length and PVD - ABPI &lt; 0.9</td>
<td>22/n/a</td>
<td>0.51</td>
<td>Fitzpatrick et al. 41</td>
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<tr>
<td></td>
<td>Leukocytes</td>
<td>TRF – Southern Blot analysis</td>
<td>Reduced telomere length in hypertensives with carotid plaques vs. those without plaques</td>
<td>73/90</td>
<td>0.03</td>
<td>Benetos et al. 43</td>
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<tr>
<td></td>
<td>Leukocytes</td>
<td>TRF – Southern Blot analysis</td>
<td>Reduced telomere length in type 2 diabetics with carotid and femoral plaques vs. those without plaques</td>
<td>30/30</td>
<td>0.0001</td>
<td>Adaikalakoteswari et al. 44</td>
</tr>
</tbody>
</table>

*TRF— terminal restriction fragment.
whom detected shorter leukocyte telomere lengths in patients whom developed CHD compared to controls in a 4.9-year follow-up study (cases 484, controls 1058). The risk of developing CHD was doubled in patients with telomere lengths in the middle and lowest tertiles. Brouilette et al. had previously shown that telomere length in the lowest 2 quartiles carried at least a 2-fold risk of developing premature MI (203 cases, 180 controls). A prospective study following healthy individuals aged 60–97 years assessed the impact of baseline leukocyte telomere length on subsequent cause-specific mortality (n = 143). Individuals in the lower half of telomere length distribution displayed a 3.18-fold higher risk of CHD-specific mortality than those in the higher distribution. Comparable results have arisen from a ten-year prospective analysis of 419 individuals (aged ≤ 73). This revealed a 3-fold risk of myocardial infarction in those with shorter telomeres. Most recently (2008), replication of these results has been achieved by a larger prospective case-control study (337 cases, 337 controls), limited however, to an entirely male cohort. Starr et al. recruited 190 participants and found PBL telomere lengths were significantly shorter in patients symptomatic of CHD (n = 63) and those with ischaemic ECG changes (n = 75). Telomere length studies in coronary endothelium have been limited due to difficulty obtaining sufficient DNA from inherently small target vessels. However, Ogami et al. were able to compare telomeric DNA content in diseased coronary arteries (n = 11) against normal arteries from autopsy specimens (n = 22). Significantly lower telomere content existed in the group with diseased arteries.

**Discussion**

Multiple studies support an association between short telomere length and increased predisposition to cardiovascular morbidity including myocardial infarction and hypertension. This association extends to vascular risk factors including obesity, smoking and diabetes. However, only a small sub-set of studies show direct-association with vascular surgical conditions such as aortic aneurysm, carotid stenosis and PVD (Table 3). The few studies contesting such associations involved exclusively elderly cohorts (>85 years). An explanation for the latter negative findings may include that telomere attrition may increase vascular disease risk up to a certain point, beyond which other risk factors including chronological age may invoke a greater additional risk.

Aside from one study relating to venous thromboembolism, there were no telomere studies of venous disorders such as varicose veins and arterio-venous malformations suggesting a novel area for further investigation. Overall, the associations reported above suggest that premature vascular ageing and associated disease may potentially be discernable by a shorter telomere length. There remains insufficient evidence however, to validate this association and determine whether short telomeres are a cause or consequence of disease. Cellular senescence being a major feature of atherosclerotic plaques, lends support to the hypothesis that shorter telomeres may be linked to conditions characterised by atherosclerosis. Evidence of short telomeres with differential expression of senescence-associated markers exists in vascular smooth muscle cells (VSMC) derived from atherosclerotic plaque specimens compared to normal VSMC. Accelerated replacement of healthy tissue with senescent tissue may link telomere attrition to disease development since the senescent phenotype has been linked to endothelial dysfunction and plaque instability. Other proposed mechanisms linking telomere attrition to vascular disease include; (1) oxidative DNA damage — particularly in the presence of smoking and hypertension (known states of high oxidative stress); (2) telomere attrition due to higher rates of leukocyte proliferation as part of a global inflammatory response accompanying atherosclerotic disease; (3) reduced telomerase activity; and (4) association of short leukocyte telomere length with elevated plasma homocysteine (a risk factor for vascular disease). Accurate delineation of true changes in telomere length will require prospective longitudinal studies where serial measurements are scaled against incidence and progression of vascular disease over time e.g. AAA incidence and rate of expansion. This requires standardised outcome measures of vascular morbidity such as objective angiographic evidence of PVD as opposed to subjective reports of claudication symptoms in previous studies. One such study in progress is the Asklepios Study, a population study prospectively monitoring leukocyte telomere length and onset of cardiovascular events in a disease-free cohort (n = 2524).

Outcome measures of interest to vascular surgeons include detection of phenotypes including carotid and femoral stenosis as well as haemodynamic measures of vascular function. The non-specific modulation of telomere length by a multitude of modifiable and non-modifiable risk factors such as age, gender, smoking and obesity however, suggest a poor specificity and limited potential as a biomarker of disease.

**Conflict of interest**

None declared.

**References**


