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JACC REVIEW TOPIC OF THE WEEK

Telomere Length as Cardiovascular Aging Biomarker

JACC Review Topic of the Week

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ABSTRACT

Telomeres shorten with age, the major risk factor for atherosclerotic cardiovascular disease (aCVD). The observation of shorter telomeres in aCVD patients thus suggested that critical telomere shortening may contribute to premature biological aging and aCVD. Therefore, telomere length often is suggested as a causal aCVD risk factor, a proposal supported by recent Mendelian randomization studies; however, epidemiological research has shown disappointingly low effect sizes. It therefore remains uncertain whether telomere shortening is a cause of aCVD or merely a consequence. The authors argue that elucidating the mechanistic foundation of these findings is essential for any possible translation of telomere biology to the clinic. Here, they critically evaluate evidence for causality in animal models and human studies, and review popular hypotheses and discuss their clinical implications. The authors identify 4 key questions that any successful mechanistic theory should address, and they discuss how atherosclerosis-associated local telomere attrition may provide the answers. (J Am Coll Cardiol 2018;72:805–13) © 2018 by the American College of Cardiology Foundation.

While postulating the theory of DNA replication-associated telomere attrition in 1971, Alexey Olovnikov dubbed telomeres "the heel of Achilles" of DNA, immediately recognizing their potential impact on aging (1). At the cellular level, his prediction was shown to be accurate about 20 years later: telomeres indeed shorten with age and cell division (2,3), explaining the limited proliferative lifespan of normal somatic cells (4). Unsurprisingly, the detection at the turn of the new millennium of telomere shortening in association with atherosclerosis (5) led many to propose a causal role in cardiovascular aging as well, especially when telomere length (TL) was also linked to lower cardiovascular survival (6).

As a result, TL received intense attention as putative risk factor for atherosclerotic cardiovascular disease (aCVD), especially given that TL can be considered a "mitotic clock" that chronicles an individual's biological age rather than the calendar age. More specifically, the inherent promise of TL is that it may pinpoint the timing of disease onset. A decade of research, however, demonstrated only a weak association of TL with aCVD. Moreover, animal studies indicated a complex relationship between telomerase activity, telomere attrition, and atherosclerosis development, casting doubt on any putative causal relationship. Nonetheless, the causality hypothesis was soon revived by genomics research identifying genetic determinants of TL that also predict cardiovascular risk (7,8).

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ABBREVIATIONS AND ACRONYMS

aCVD = atherosclerotic cardiovascular disease

DKC = dyskeratosis congenita

GWAS = genome-wide association study

qPCR = quantitative polymerase chain reaction

SNP = single nucleotide polymorphism

TERC = telomerase RNA component

TERT = telomerase reverse transcriptase

TL = telomere length

Although causality is back at the center of mechanistic theories regarding telomeres and cardiovascular aging, most theories do not explain the low effect size in epidemiological studies, together with other counterintuitive findings. Mechanistic insight is, however, indispensable for establishing TL as a clinically relevant aCVD risk factor and for a meaningful interpretation of commercial TL tests. In this viewpoint paper, we discuss historical and alternative hypotheses and their potential impact on patient care. The main pieces of evidence for or against each hypothesis are summarized in **Table 1**. Moreover, we outline several concepts and anomalies that remain controversial or unresolved in the field, highlighting them as key questions (**Table 2**). Yet, first, we provide a brief introduction of TL biology.

TELOMERES, TELOMERASE, AND REPLICATIVE SENESCENCE

Telomeres are the nucleoprotein complexes capping chromosome ends. In humans, they consist of a hexameric repetitive DNA sequence bound by a specific set of nucleoproteins, the shelterin complex (9) (**Figure 1**). The canonical telomere sequence is $(TTAGGG)_n$, and although variant repeats exist, their position in the telomere and mechanism of generation are only now beginning to be elucidated (10). In healthy humans, average TL ranges from 5 to 12 kilobase pairs. At the proximal end, the telomere is separated from the rest of the chromatin by the subtelomere, a dynamic patchwork of sequence blocks that are often conserved between chromosomes. At the distal end, the telomere terminates in a single-stranded G-rich overhang that recoils into the double-stranded telomere DNA, forming a complex D-loop–T-loop structure (**Figure 1**). This structure distinguishes telomeres from double-strand breaks and is lost upon critical telomere attrition, which thus induces p53-mediated replicative senescence (9).

As originally postulated by Olovnikov (1), telomere shortening is caused by the end-replication problem, which is the inability of DNA polymerase to replicate the very end of the lagging strand. However, another major source of telomere attrition is oxidative stress (11). In vitro, replicative senescence arises when somatic cells stop dividing upon reaching a donor-specific number of divisions, known as the Hayflick limit (4). This limit was attributed by Olovnikov to critical telomere shortening, which was verified by the observations that telomeres indeed shorten in vitro (2) and that the Hayflick limit can be overcome by ectopic telomerase expression (12).

Telomerase is a ribonucleoprotein consisting of a reverse transcriptase (TERT) and its primer, the telomerase RNA component (TERC), which elongates individual telomeres, for example, to extend TL for the next generation. Though expressed in normal germ cells and to a lesser extent in stem cells, telomerase shows very limited expression in most somatic tissues; exceptions include blood mononuclear cells in children (13) and specific cells of the cardiovascular system (14). On the other hand, tumor cells reactivate telomerase expression to bypass replicative senescence and gain immortality (15), although telomeres can also be lengthened by an alternative mechanism (16).

Further studies evaluating the impact of telomere biology in vivo finally led to the wide acceptance of TL as a mitotic clock and anticancer mechanism (16). A crucial piece of evidence was the observation that germline telomerase complex mutations in humans often lead to bone marrow failure diseases, such as dyskeratosis congenita (DKC), which features critically short telomeres and heterogeneous geriatric-like phenotypes including aberrant skin pigmentation, nail dystrophy, leukoplakia, and premature graying (17). The increasing disease severity over generations, with progressively shorter telomeres, underscores the causal role of telomere biology in DKC and therefore its in vivo relevance (18). However, the fact that aCVD is not a common DKC feature despite critically short telomeres suggests a complicated relation between telomere attrition and aCVD (**Table 2**).

EPIDEMIOLOGICAL TL STUDIES OF (CARDIOVASCULAR) AGING: TISSUES, METHODS, AND EARLY RESULTS

As critical telomere attrition was put forward as one of the causes of physiological aging (19), its putative role in aCVD was investigated, most notably in epidemiological studies. In adults, TL and the telomere attrition rate are strongly correlated between the different tissues of an individual (20). Therefore, in humans, TL has typically been measured in blood leukocytes or mononuclear cells, a straightforward choice for measuring “systemic” TL in epidemiological studies. However, despite a clear correlation in TL when comparing different tissues from the same individual, leukocyte telomeres are shorter than those of other tissues, possibly as a consequence of the higher hematopoietic stem cell proliferation rates in early life (20,21).