Telomeres and cardiovascular disease risk: an update 2013

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Leukocyte telomere length (LTL) has been regarded as a potential marker of biologic aging because it usually shortens in a predictable way with age. Recently, a growing interest in cardiovascular aging has led to a number of new epidemiologic studies investigating LTL in various disease conditions. Some methodological problems exist because there are different methods available to determine LTL, and standardization is much needed. For example, in the majority of studies, patients with early-onset coronary heart disease have been shown to have shorter LTL. In addition, patients with diabetes mellitus complications tend to have shorter LTL than control subjects. On the other hand, increased left ventricular hypertrophy or mass is associated with longer LTL, and studies investigating hypertension have reported both shorter and longer LTL than found in normotensive control subjects. There is, therefore, a need for longitudinal studies to elucidate these complicated relationships further, to provide estimations of telomere attrition rates, and to overcome analytical problems when only cross-sectional studies are used. The understanding of cardiovascular aging and telomere biology may open up new avenues for interventions, such as stem cell therapy or agents that could retard this aging process over and beyond conventional risk factor control. (Translational Research 2013;162:371–380)

Abbreviations: CAD = coronary artery disease; CHD = coronary heart disease; CVD = cardiovascular disease; EF = ejection fraction; FH = familiar hypercholesterolemia; HR = hazard ratio; IHD = ischemic heart disease; LTL = leukocyte telomere length; LVH = left ventricular hypertrophy; LVMI = left ventricular mass index; MI = myocardial infarction; q-PCR = quantitative polymerase chain reaction; RTL = relative telomere length; TBP = telomere binding protein

Telomeres form the end segment of the DNA helix and protect this end between mitosis. Ever since the Nobel Prize in 2009 was awarded to 3 American scientists for telomere research, the interest in telomere biology has grown substantially. One indication of this is the increasing number of publications found at PubMed, with currently (January 2013) almost 15,000 hits for "telomere" of which 420 are combined with "cardiovascular," 357 with "coronary," 140 with "atherosclerosis," and 169 with "diabetes." This brief review focuses on the development of telomere research in relation to cardiovascular disease (CVD) and shows how complex the field is today.

STRUCTURE AND FUNCTION OF TELOMERES

Telomeres are repetitive, noncoding DNA sequences (TTAGGG) located at both ends of each chromosome.

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The length of the telomeres in humans can reach up to 15,000 bp, ending with a single-strand overhang at the 3′ end measuring 75 bp–200 bp. The telomere complex consists of several parts: the telomeres themselves, the regulating enzyme telomerase, and a few telomere binding proteins (TBP). Together with TBPs, in the shelterin complex, the telomeres form loop structures that are believed to protect the chromosome ends from fusing with each other, hence preventing possible oncogenic development.[1] The telomere loops also protect the chromosomal DNA from being recognized as damaged, reduce the risk of apoptosis, and stabilize the DNA complex. There is also the more recently described telomere-capping Cdc13-Stn1-Ten1 (CST)-complex that comprises 3 proteins—Cd13 and Stn1, and related to the conserved telomere maintenance complex component 1 (Ten1) originally found in yeast, but also present in humans, and shown in genomewide association studies to participate in telomere maintenance.[2]

During each mitosis, the DNA polymerase replicates the DNA (including the telomeres), but is unable to replicate the last part of the lagging strand, resulting in progressive shortening of the chromosomes. This is called the end replication problem, and the DNA loss amounts to approximately 30 bp–150 bp per mitosis. Because of the non-sense DNA information on the telomeres, the cell can afford losing a few hundred base pairs during each replication without loss of coding DNA and possible oncogenic transformation. Ultimately, the ends of the telomeres become too close to the coding DNA region and the loop structures cannot be kept together. The protective function of the telomeres is lost, and this partially damaged DNA is recognized by tumor suppressors, for which the cell is either programmed for apoptosis or is irreversibly blocked during the G1 phase of mitosis, also termed replicative senescence. The senescent cells have other, more aging-related properties than dividing cells, express different proteins, and, most important, lose their ability to multiply. Critically short telomeres therefore function as a tumor suppressor mechanism.[3]

REGULATION OF TELOMERE LENGTH

Variation in leukocyte telomere length (LTL) is seen interindividually, in different tissues and even on separate chromosomes, and is genetically programmed.[1,2] The attrition rate during life is also thought to be dependent on the initial length of the telomeres. At birth, telomeres are of the same length in boys and girls; later in life, however, they are proportionately longer in women, especially in the premenopausal years compared with men the same age—an effect attributed to estrogen. Also, the TBPs are believed to exert an effect on the rate of telomere shortening and to prevent premature replicative senescence. It has been debated whether short LTL is a good marker of chronological aging.[4,5]

The more cell divisions that take place in humans (ie, the older persons become), the shorter the telomeres. First, the built-in end replication problem shortens the telomeres, but they are also exposed to reactive oxygen species, which have a predilection for the single-stranded, G-rich telomere overhang.[6] This is an important variable associated with aging and with accelerating the telomere attrition rate. Thus, LTL can be regarded as a marker of biologic aging. Unhealthy lifestyle conditions such as smoking and obesity—both of which are associated with low-grade inflammation, a greater leukocyte proliferation rate, and increased reactive oxygen species production—have frequently (but not always) been associated with shorter telomeres in peripheral blood leukocytes.[7]

LABORATORY METHODS AVAILABLE

There are some conditions with features of early biologic aging that have been associated with shorter LTL, but also some common chronic disease manifestations show the same findings. Most studies, until recently, have been cross-sectional in design and there is a lack of longitudinal studies with repeated measures of LTL. Different laboratory methods are currently available for the determination of relative LTL (quantitative polymerase chain reaction, q-PCR) or absolute LTL, including the proportion of short telomeres (Southern blot), a more costly and labor-intensive method. Other methods also exist, based on immunofluorescence (fluorescence *in situ* hybridization). This has contributed to some controversy because different studies apply different methods that sometimes do not suit each other. Recently, 1 study compared the 2 methodologies of q-PCR and Southern blot based on blind measurements on the same samples from 50 donors that were performed in 2 independent laboratories on 2 different occasions.[8] Both the q-PCR and Southern blots displayed highly reproducible results, as shown by r values more than 0.9 for the correlations between results obtained by either method on both occasions. The interassay coefficient of variation measurement for the q-PCR was 6.5%, whereas that of the Southern blot was 1.7%. The authors concluded that the relation between the results generated by Southern blot vs those generated by q-PCR deviated from linearity.[8] For a number of reasons, Southern blot is considered the gold standard, but this method is also more laborious and expensive. It is for these reasons that q-PCR is