Short Telomeres, but Not Telomere Attrition Rates, Are Associated With Carotid Atherosclerosis

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Abstract—Short telomeres are associated with atherosclerosis. However, the temporal relationship between atherosclerosis and telomere length is unclear. The objective of this work was to examine the temporal formation and progression of carotid atherosclerotic plaques in relation to telomere dynamics. In a longitudinal study, comprising 154 French men and women (aged 31–76 years at baseline), carotid plaques were quantified by echography, and telomere length on leucocytes was measured by Southern blots at baseline and follow-up examinations. Telomere attrition rates during the 9.5-year follow-up period were not different in individuals with plaques at both baseline and follow-up examinations (23.3±2.0 base pairs/y) than in individuals who developed plaques during the follow-up period (26.5±2.0 base pairs/y) and those without plaques at either baseline or follow-up examination (22.5±2.3 base pairs/y; P=0.79). At baseline, telomere length was associated with presence of carotid plaques (P=0.02) and with the number of regions with plaques (P=0.005). An interaction (P=0.03) between age and the presence of plaques was observed, such that the association between plaques and telomere length was more pronounced at a younger age. In conclusion, carotid atherosclerosis is not associated with increased telomere attrition during a 9.5-year follow-up period. Short telomere length is more strongly associated with early-onset than late-onset carotid atherosclerosis. Our results support the thesis that heightened telomere attrition during adult life might not explain the short telomeres observed in subjects with atherosclerotic disease. Rather, short telomeres antecedes the clinical manifestation of the disease. **(*Hypertension*. 2017;70:420-425. DOI: 10.1161/HYPERTENSIONAHA.117.09354.)**

Key Words: adult ■ atherosclerosis ■ follow-up studies ■ telomere ■ telomere shortening

Two recent reviews and meta-analyses showed that short leukocyte telomere length (LTL) is associated with atherosclerotic cardiovascular disease (ACVD),[1,2] which is largely aging related. Short LTL is also associated with aortic stiffness,[3] an aging phenotype, and with diminished survival.[4] With some exceptions,[5,6] previous research has shown that individuals with atherosclerotic plaques of the carotid artery had comparatively short LTL.[7–11]

Indolent inflammation and oxidative stress are key determinants in aging-related vascular injury, contributing to atherosclerosis and arterial stiffness.[12,13] For this reason, the prevailing view on the association of short LTL with ACVD has been that during adult life[14–16] chronic inflammation and oxidative stress heighten the pace of age-dependent LTL shortening in tandem with the development of ACVD. Clinical studies have emphasized this concept by linking short LTL with atherosclerosis risk factors, such as smoking, sedentary lifestyle, and high body mass index (BMI).[17–19] However, recent findings suggest that the contribution of LTL attrition during adult life to the variation in LTL across the population is comparatively small to the interindividual variation in LTL at birth[20] and LTL attrition during the first 2 decades of life.[21,22] Since short telomeres may precede the clinical manifestation of atherosclerosis, telomere length, as expressed in LTL, may be an active determinant in rather than a passive marker of arterial aging, perhaps because short telomeres might diminish replicative potential and compromise vascular repair.[23]

The Strong Heart Family Study reported that individuals with short LTL had a higher risk of developing carotid atherosclerosis during a period of 5.5 years,[9] whereas the Bruneck Study observed that short LTL was associated with progression to advance stages of carotid atherosclerosis during a 5-year period.[11] However, no study has performed sequential LTL measurements for a relatively long follow-up period to establish whether the rate of LTL attrition is higher in individuals with carotid atherosclerosis than in their peers. The present longitudinal study was designed to fill this gap, focusing on the relationship between LTL dynamics and carotid atherosclerotic plaques (CAPs).

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Subjects and Methods

Subjects

This research draws on the ERA study (Evolution de la Rigidité Artérielle) that examined determinants of arterial aging.[24] The ERA study was approved by the research ethics committee, and all participants signed an informed consent form. Participants were from a Parisian cohort that had been followed at the Centre d'Investigations Préventives et Cliniques. Among these, 156 participants had sequential LTL measurements. The follow-up examination was performed 9.5 years later (2007–2008). Two subjects were excluded because of technical problems related to the echographic quantification of CAPs at the follow-up examination. Thus, the study comprised 154 participants (31% women), whose age range was 31 to 76 years (58±10 years; mean±SD) at baseline examination (1998–1999).

Carotid Artery Measurements

Carotid artery echography was performed at baseline and follow-up examinations. The same device (Aloka SSD-650, with a 7.5-MHz linear array transducer, processing and storage of B-mode images with the software M'ATHS, Metris, France) was used on both occasions.[25] Intima-media thickness (IMT) and presence of CAP were measured as previously described.[7] Presence of CAP was examined in 2 regions (common carotid and bifurcation) of the right and left carotid arteries; the combined CAP score for these regions, therefore, ranged from 0 (no CAP in either left or right carotid artery) to 4 (CAPs in 2 regions of the left and right carotid arteries).

The relationship between LTL dynamics and carotid atherosclerosis was studied by stratifying the cohort into 3 CAP groups: Gr no/no, participants without CAP at both baseline and follow-up examinations; Gr no/yes, participants with CAP only at the follow-up visit, that is, individuals who developed CAP during the follow-up period; and Gr yes/yes, participants with CAP at both baseline and follow-up examinations.

Participants were further grouped based on the number of carotid regions with CAPs (Gr 0=no CAP; Gr 1=CAP in 1 region; Gr≥2=CAPs in ≥2 regions). This CAP grouping applied either to the baseline or to the follow-up examination.

In both baseline and follow-up examinations, supine blood pressure was measured in the right arm using a manual sphygmomanometer. After a 10-minute rest period, systolic and diastolic blood pressures (SBP and DBP) were measured 3× with a 5-minute interval between measurements, and the average of the last 2 measurements was used for the statistical analyses. Height and weight parameters were used for BMI measurements. Smoking status (current and ex-smokers versus nonsmokers) was determined using a questionnaire.

LTL Measurements

LTL measurements were performed from blood drawn at baseline and follow-up examinations. White blood cell DNA was extracted from whole blood after red cell osmotic lysis by a salting out method as previously described.[26] All DNA samples were tested for integrity using a 1% (wt/vol) agarose gel. LTL was measured by Southern blots of the terminal restriction fragments, as previously described.[27] Briefly, DNA samples were digested (37°C) overnight with restriction enzymes *Hinf*I and *Rsa*I (Roche Diagnostics GmbH, Germany). Digested DNA samples and DNA ladders were resolved on 0.5% (wt/vol) agarose gels. After 23 hours, the DNA was depurinated, denatured, neutralized, and transferred onto a positively charged nylon membrane (Roche) using a vacuum blotter (Biorad, Hercules, CA). Membranes were hybridized at 65°C with the digoxigenin-labeled telomeric probe after which the probe was detected by the digoxigenin luminescent detection procedure (Roche) and exposed on charge-coupled device camera (Las 4000, Fuji). Measurements were performed in duplicate on separate gels with analyses based on the average of these 2 measurements. The baseline and follow-up samples from each individual were run in adjacent lanes. The interassay coefficient of variation for the duplicate measurements (on different gels) was 1.2%.

Statistical Analysis

For variables presenting a normal distribution, descriptive values are expressed as mean±SD, or otherwise, as median, interquartile range, and percentages. LTL attrition was calculated from the difference between LTL at baseline and LTL at follow-up, divided by the duration of the follow-up.

The relationships of LTL attrition with LTL at baseline, LTL at follow-up, age, BMI, SBP, DBP, heart rate, and IMT were determined using Pearson correlation coefficients. The effect of age (a continuous variable) on LTL–CAP association was tested with the interaction term age × presence of CAPs. This applied to age in which the CAP has been first detected, that is, at the baseline examination for Gr yes/yes and at the follow-up examination for Gr no/yes. For Gr no/no, we used the age and LTL at the baseline examination for this analysis.

ANOVA and ANOVA trend tests were used for categorical variables (sex, smoking status, and CAP groups). Significance was tested using the post hoc tests between groups were Tukey–Kramer or Kruskal–Wallis test depending on the distribution (continuous variables) and χ^2 (discrete variables). A $P<0.05$ was considered statistically significant. Statistical analyses were performed using the NCSS 9 statistical software package (NCSS, Kaysville, UT).

Results

Cardiovascular Characteristics

Between the baseline and follow-up examinations, participants showed an increase in BMI ($P=0.03$), carotid IMT ($P=0.02$), and heart rate ($P<0.0001$), a drop in mean DBP ($P=0.008$) but no change in mean SBP (Table). Gr yes/yes was older than Gr no/no ($P<0.001$) and showed higher baseline carotid artery IMT ($P<0.001$), BMI ($P<0.001$), SBP ($P<0.001$), and DBP ($P<0.01$). Gr no/yes showed intermediate values (trend test) for carotid IMT ($P<0.0001$), SBP ($P<0.0001$), DBP ($P<0.002$), and BMI ($P<0.001$). No differences in smoking and in heart rate were observed across the 3 CAP groups.

Relationship Between LTL Dynamics and CAP at Baseline and Follow-Up Examinations

For the entire cohort, mean LTL was 6.46±0.56 kb at baseline and 6.23±0.53 kb at follow-up ($P<0.001$). The average rate of LTL attrition during the 9.5-year follow-up period was 24.2±16.0 base pairs (bp)/y (Table). Among the 154 participants, 149 displayed LTL shortening and 5 (3%) displayed LTL lengthening.

There were no differences in LTL attrition across the 3 CAP groups ($P<0.24$; Table). This finding held after adjusting for age, sex, and baseline LTL: Gr yes/yes, 23.3±2.0 bp/y; Gr no/yes, 26.5±2.0 bp/y; Gr no/no, 22.5±2.3 bp/y ($P=0.79$; Figure 1B).

Across the 3 CAP groups, LTL was shorter at both baseline and follow-up examinations in Gr yes/yes than Gr no/yes, which, in turn, was shorter than Gr no/no (ie, LTL, Gr yes/yes<LTL, Gr no/yes<LTL, Gr no/no; $P=0.0004$ for baseline LTL; and $P=0.0009$ for follow-up LTL; Table). This finding held for baseline examination after adjusting for age and sex, that is, LTL for Gr yes/yes=6.30±0.08 kb, as compared with the 2 other groups: LTL, Gr no/yes=6.52±0.07 kb and LTL, Gr no/no=6.51±0.07 kb (Figure 1A; trend ANOVA, $P=0.03$ and ANOVA Gr yes/yes versus 2 other groups $P=0.02$).

After adjustments (for baseline LTL, age, and sex), LTL attrition during the follow-up period was not associated with SBP, DBP, heart rate, carotid IMT, BMI, or smoking.

Table. Characteristics at Baseline and Changes During the 9.5-Year Follow-Up Period in the Entire Population and in the 3 Subgroups According to the Presence of Carotid Atherosclerotic Plaques at Baseline/Follow-Up Visits

Parameter	Whole Population	Presence of CAPs (Baseline/Follow-Up)			
		No/No	No/Yes	Yes/Yes	*P* Value (Trend ANOVA)
No. of subjects	154	56	56	42	
Sex (women %)	31%	32%	32%	26%	0.77
Age at BL, y	58.4±9.6	53.3±9.4	60.5±8.8*	62.5±8.0*	<0.0001
FU duration, y	9.5±0.5	9.5±0.5	9.7±0.5	9.3±0.5	0.017
Smoking at BL, %	47%	46%	50%	46%	0.89
BMI at BL, kg/m^2	25.4 (23.8 to 28.3)	24.2 (22.5 to 26.8)	25.7 (24.8 to 28.0)†	28.1 (24.6 to 31.5)*	0.0002
ΔBMI, kg/m^2	0.30 (−0.62 to 1.22)§	0.74 (−0.39 to 1.54)	0.00 (−0.68 to 0.90)	0.28 (−1.01 to 1.33)	0.12
SBP at BL, mm Hg	141±19	130±16	145±18*	151±17*	<0.0001
ΔSBP, mm Hg	2.0±17.1	4.7±14.1	1.7±17.9	−1.0±19.3	0.27
DBP at BL, mm Hg	87±10	84±10	89±9†	90±9‡	0.002
ΔDBP, mm Hg	−2.1±9.8§	0.9±9.7	−3.6±9.8†	−4.1±9.0†	0.02
HR at BL, bpm	66±9	66±8	66±10	67±8	0.92
ΔHR, bpm	3.6±9.2§	2.6±7.7	3.2±10.6	5.5±9.1	0.28
Carotid IMT at BL, mm	0.71 (0.66 to 0.81)	0.68 (0.60 to 0.74)	0.72 (0.67 to 0.83)‡	0.80 (0.70 to 0.85)*	<0.0001
Δ carotid IMT, mm	0.01 (−0.04 to 0.08)§	0.02 (−0.02 to 0.06)	0.01 (−0.06 to 0.11)	0.01 (−0.02 to 0.13)	0.98
No. of regions with CAPs at BL	0 (0 to 1)	0	0	1 (1 to 2)	…
Δ no. of regions with CAPs	1 (0 to 2)§	0	2 (1 to 2)*	1 (1 to 2)*	<0.0001
LTL at BL, kb	6.46±0.56	6.64±0.60	6.46±0.51	6.20±0.46*	0.0004
LTL at FU, kb	6.23±0.53	6.39±0.57	6.21±0.50	6.02±0.42*	0.0009
LTL attrition, bp/y	24.2±16.0§	26.1±19.4	26.0±14.9	19.5±10.7	0.08

Values are mean±SD, median (interquartile range), or percentages (%). No/No indicates absence of CAP in both baseline and follow-up examinations; No/Yes, presence of CAP only at the follow-up examination; and Yes/Yes, presence of CAP in both baseline and follow-up examinations. Smoking at BL indicates current and past smoking at baseline (BL); and Δ, changes between baseline and follow-up visits. BMI indicates body mass index; bp, base pair; CAP, carotid atherosclerotic plaque; DBP, diastolic blood pressure; FU, follow-up; HR, heart rate; IMT, intima-media thickness; kb, kilobase pairs; LTL, leukocyte telomere length; and SBP, systolic blood pressure.

*P<0.001; †P<0.05; ‡P<0.01; Tukey–Kramer or Kruskal–Wallis post hoc test and χ^2 vs Gr No/No.

§Follow-up–baseline significantly different from 0.

Relationship Between LTL Dynamics and Number of CAPs

LTL at baseline was inversely correlated with the number of regions with CAPs at baseline examination (adjusted for age and sex, trend ANOVA, P=0.005; Figure 2A).

LTL attrition rates during the follow-up period were not correlated with the number of plaques at baseline: Gr 0, 25.0±1.4 bp/y; Gr 1, 22.3±3.2 bp/y; Gr≥2, 22.3±3.5 bp/y (P=0.46; Figure 2B). LTL at baseline and LTL attrition rates during the follow-up period were not correlated with the number of plaques at the follow-up examination (data not shown).

Age Effect on LTL–CAP Correlation

We further analyzed the association of CAP with LTL as a function of participant's age when CAPs were detected. LTL was negatively associated with age (P<0.001), and the presence of CAPs (P<0.02) with an interaction (age × presence CAPs; P=0.03) indicating that the younger the participant's age of the detection of the plaque, the stronger is the association between LTL and CAPs.

Discussion

The key finding of this longitudinal study is that compared with participants with no CAPs at baseline and follow-up examinations, LTL attrition was not higher in participants with CAPs at baseline or in those who developed CAPs during the follow-up period. This finding challenges the convention that the association of short LTL with ACVD principally reflects a heightened LTL attrition because of increased burdens of inflammation and oxidative stress.[15,16] Although some studies showed that in adults, cardiovascular risks associated with increased inflammation and oxidative stress are also associate with short LTL,[6,28] the influence of such factors on LTL might be small compared with those that define LTL before adulthood.

Previous studies observed that short LTL was principally associated with severe forms of carotid artery atherosclerosis[8,11] or with presence of CAPs in young individuals.[9] In 1 study, the CAPs–short LTL association was limited to young women.[5] These findings are largely compatible with our findings; they infer that individuals with early-onset

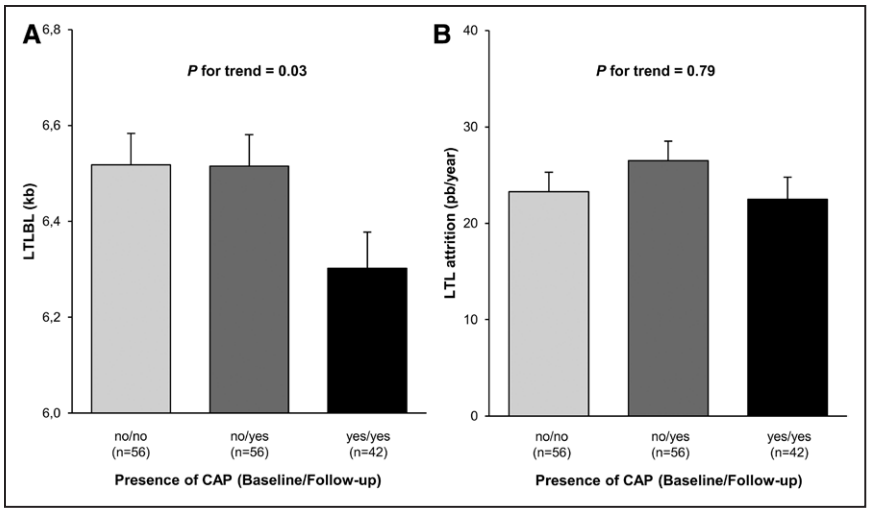

Figure 1. Baseline leukocyte telomere length (LTL; **A**) and leukocyte telomere attrition during the follow-up period (**B**) vs the presence of carotid atherosclerotic plaques during the baseline (BL)/follow-up visits. Adjusted BL LTL (kb) vs the presence of carotid atherosclerotic plaque (CAP) at BL and follow-up (**A**); adjusted LTL attrition (bp/y) vs the presence of CAP at BL and follow-up (**B**). Values are mean±SEM. BL LTL is adjusted to age and sex; LTL attrition is adjusted to age, sex, and BL value of LTL. no/no indicates absence of CAP in both BL and follow-up examinations; no/yes, presence of CAP only at the follow-up examination; and yes/yes, presence of CAP in both BL and follow-up examinations.

atherosclerosis and those with severe forms of atherosclerosis display comparatively short LTL.[29]

In practice, having carotid artery atherosclerosis is a question of when and how severe because most individuals develop some degree of atherosclerosis if they live long enough. For instance, at least some buildup of CAPs is found in (1) 48% of men and 36% of women <45 years; (2) 71% of men and 54% of women between the ages of 50 and 54 years; and (3) 94% of men and 93% of women ≥80 years.[30] Thus, having shorter LTL might not apply to all individuals with carotid artery atherosclerosis. Our results go along with this hypothesis suggesting that short LTL largely denotes early onset and more severe manifestation of carotid artery atherosclerosis.

Our findings do not support the view that a higher pace of LTL attrition during adulthood explains the association of short LTL with ACVD and that LTL is a biomarker of the aging of the vasculature. They do support the competing view that ascribes an active role of telomere length, as expressed in LTL, in the development of ACVD. This view is based on the following series of observations: (1) LTL is highly heritable (≈65%),[31–33] a finding already observed at birth[34]; (2) alleles associated with short LTL are over-represented in individuals with clinical manifestation of ACVD,[35–37] thus largely excluding reverse causality, that is, that ACVD accelerates LTL attrition; (3) the wide LTL variation is already observed across newborns (SD of ≈0.7 kb),[34] suggesting that having short or long LTL is primarily determined before adulthood; and (4) individuals who enter adult life with short or long LTL typically display long or short LTL for the rest of their adult life course.[38,39] Individually, each of these observations hardly infers causality, but collectively they suggest an active role of telomeres in ACVD.

Study Strengths and Limitations

The key strengths of study are the relatively long follow-up duration that is critical for obtaining valid data in longitudinal studies [40] and the precise LTL measurements by Southern blots.[27,41] The modest sample size can be considered as a limitation of the study. However, given the longitudinal nature of the study, each participant's LTL at follow-up examination is scaled to his/her LTL at baseline examination. Thus, there may not be a need for a large sample size to answer the critical question: Is LTL attrition in individuals with CAPs from the outset different that of participants who developed CAPs during the 9.5 years of follow-up and that of participants with no evidence of CAPS (no/no). Using a relative long follow-up period, our approach circumvents the high interindividual variation in LTL across participants. However, the temporal

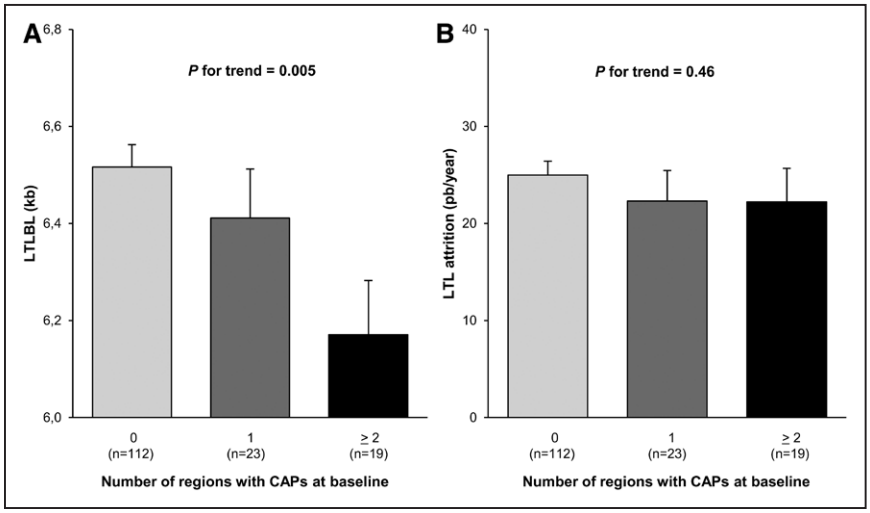

Figure 2. Baseline (BL) leukocyte telomere length (LTL; **A**) and leukocyte telomere attrition during the follow-up period (**B**) vs the number of regions with carotid atherosclerotic plaques at BL. Values are mean±SEM. BL LTL is adjusted to age and sex; LTL attrition is adjusted to age, sex, and BL value of LTL.

relationship between CAPs and LTL across the population warrants replication in large-scale studies.

Conclusions and Perspectives

This study shows that carotid atherosclerosis was not associated with increased LTL attrition during a 9.5-year follow-up period, yet carotid atherosclerosis was associated with short LTL, principally in younger participants. Based on these findings, the concept of precedence, that is, LTL is primarily determined before adulthood,[22,38,39] the high LTL heritability,[31–33] and recent genetic studies,[35–37] we infer that telomere length might be actively involved in the development of ACVD. In the final analysis, atherosclerosis and other aging-related degenerative diseases may arise from a progressive increase in the imbalance between injury and repair.[42,43] Perhaps, the vascular repair potential of individuals with ACVD is compromised because of their shorter telomeres. Given that LTL is a complex genetic trait, elucidating more LTL gene variants and learning their potential role in the development of ACVD will take us a long way toward understanding the role of telomere biology in ACVD and human aging in general.

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Disclosures

None.

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Novelty and Significance

What Is New?

- Leukocyte telomere length (LTL) attrition rate during a relatively long follow-up period in a population of adults was not associated with the presence and magnitude of carotid atherosclerotic plaques.
- Short LTL predicted development of carotid atherosclerosis, as expressed by increased number of anatomic regions with carotid atherosclerotic plaques.
- The association between short LTL and carotid atherosclerotic plaques applies principally to individuals with an early or more severe form of carotid atherosclerosis.

What Is Relevant?

- Shorter LTL observed in individuals with carotid atherosclerosis is not because of a higher LTL attrition as compared with controls.
- Short LTL ostensively precedes the development of carotid atherosclerosis and predicts its early onset and progression.

Summary

This longitudinal study shows no increased LTL attrition in subjects with atherosclerotic plaques, suggesting that short LTL precedes the manifestations of atherosclerosis.

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Short Telomeres, but Not Telomere Attrition Rates, Are Associated With Carotid Atherosclerosis

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