Short Telomeres Are Associated With Increased Carotid Atherosclerosis in Hypertensive Subjects

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Abstract—Recent studies have shown that individuals with shorter telomeres present a higher prevalence of arterial lesions and higher risk of cardiovascular disease mortality. As a group, patients with high blood pressure are at an increased risk for cardiovascular diseases. However, some hypertensive patients are more prone than others to atherosclerotic lesions. The main objective of this study was to examine the relationship between telomere length, as expressed in white blood cells, and carotid artery atherosclerotic plaques in hypertensive males. Data from 163 treated hypertensive men who were volunteers for a free medical examination were analyzed. Extracranial carotid plaques were assessed with B-mode ultrasound. Telomere length was measured from DNA samples extracted from white blood cells. The results of this study show that telomere length was shorter in hypertensive men with carotid artery plaques versus hypertensive men without plaques (8.17 ± 0.07 kb versus 8.46 ± 0.07 kb; $P<0.01$). Multivariate analysis showed that in addition to age, telomere length was a significant predictor of the presence of carotid artery plaques. The findings from this study suggest that in the presence of chronic hypertension, which is a major risk factor for atherosclerotic lesions, shorter telomere length in white blood cells is associated with an increased predilection to carotid artery atherosclerosis. (*Hypertension*. 2004;43:182-185.)

Key Words: atherosclerosis ■ hypertension, essential ■ aging ■ carotid arteries

It is well established that hypertensive subjects are at higher risk for atherosclerosis and an accelerated cardiovascular aging. However, not all hypertensive patients ultimately manifest cardiovascular complications. The reasons for this are unknown but may reflect environmental and genetic factors such as oxidative stress, inflammation, and other molecular and cellular mechanisms that are involved in aging.[1,2]

Telomere length in white blood cells (WBCs) may register the cumulative burden of oxidative stress and inflammation in the circulation during an individual's lifetime.[3] It is reasonable to suggest, therefore, that telomere length may explain, in addition to chronological age, interindividual variation in the predilection for cardiovascular disease associated with aging and hypertension. Support for this proposition has been provided by studies showing that WBC telomere length is inversely correlated with pulse pressure, an index of aortic stiffness that not only increases with age but also is associated with an increased predisposition to cardiovascular diseases.[4–6] In fact, telomere length is shorter in patients with atherosclerotic coronary heart disease than in their age-matched peers.[6] Moreover, a recent study found that among individuals older than 60 years, those with shorter telomeres in WBCs had a 3.18-fold higher mortality rate from heart disease than their peers.[7] This finding was based on cause of death from death certificates, which are notoriously inaccurate in ascribing cause of death.[8] Therefore, the mortality rates caused by cardiovascular disease in subjects with relatively shorter telomere length in WBCs may be even higher than the ones reported in the study. Collectively, these data suggest that people with shorter telomeres present a higher prevalence of arterial lesions and a higher risk of cardiovascular disease mortality. Accordingly, the goal of this study was to examine the relationship between WBC telomere length and carotid artery atherosclerotic plaques in men with chronic hypertension, a major risk factor for the development of atherosclerotic plaques.

Methods

The French national health care system offers working and retired persons a free medical examination every 5 years. The Centre d'Investigations Préventives et Cliniques (the IPC Center) is one of the largest medical facilities in France that provides this service. The IPC Center has performed approximately 20 000 examinations annually since 1970 for people living in the Paris area.

In this report, we analyzed data derived from 163 consecutive men with chronic treated essential hypertension for whom telomere length

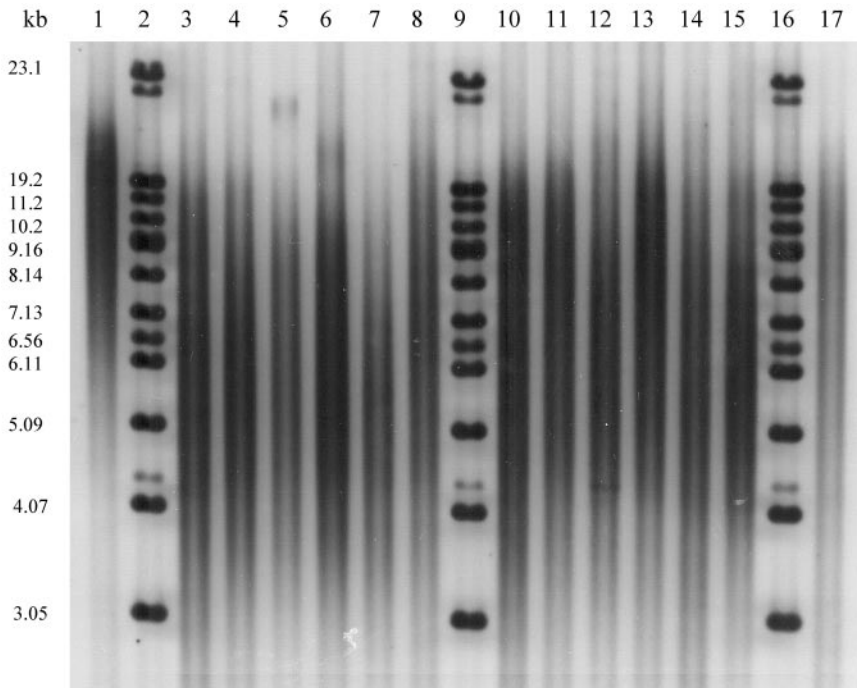DOI: 10.1161/01.HYP.0000113081.42868.f4

Figure 1. Representative autoradiogram showing the TRF from reference DNA samples (lanes 1, 17) and WBC from 12 patients (lanes 3 to 8, 10 to 15). Lanes 2, 9, and 16 are molecular weight reference ladders (mol wt (in kb) of markers are indicated); mean TRF were calculated with respect to the closest mol wt ladder. The mean TRF length was calculated as $TRF=\Sigma OD_i/\Sigma(OD_i/MW_i)$, where OD_i is optical density at a given position in the lane and MW_i is molecular weight at that position (see Reference 4). The mean TRF length for samples measured in duplicate gels was used for data analysis. TRF lengths (in kb) in the illustrated autoradiogram were: 1=10.26; 3=7.00; 4=6.80; 5=6.99; 6=7.34; 7=6.20; 8=7.85; 10=7.52; 11=7.96; 12=7.68; 13=8.63; 14=7.46; 15= .43; 17=8.25.

in WBCs and carotid artery parameters were determined. All hypertensive subjects had their hypertension diagnosed at least 6 months previously and were undergoing antihypertensive treatment. These men were a part of a cohort that had been followed-up at the IPC Center to assess age-dependent changes in cardiovascular parameters. The details of this cohort have been presented in previous publications from our group.[9,10]

The study protocol was approved by an ethics committee (Comité d'Ethique du Centre Hospitalier Universitaire de Cochin) and written informed consent was obtained from all study participants.

Medical History, Blood Pressure Measurements, and Standard Biological Procedures

All participants were administered a standardized questionnaire that provided information about demographic background, occupation, medical history, drug use (namely antihypertensive and hypolipemic drugs), and personal habits such as cigarette smoking. Subjects were classified as non-smokers, ex-smokers, or current smokers. Among the 163 hypertensive patients, 55 received a hypolipemic treatment, 86 received antihypertensive monotherapy, 77 received a combination therapy, 73 received an angiotensin-converting enzyme inhibitor, 77 received a diuretic, 60 received a beta blocker, and 47 received a calcium channel blocker. Blood pressure was measured after a 10-minute rest in the supine position and was measured 3 times in the right arm using a manual mercury sphygmomanometer. A blood sample was collected for DNA analyses, total serum cholesterol, HDL-cholesterol, LDL-cholesterol, and fasting plasma glucose.

Ultrasonography

Ultrasound examinations were performed by 2 trained ultrasonographers using the Aloka SSD-650, with a transducer frequency of 7.5 MHz. Acquisition, processing, and storage of B-mode images were computer-assisted with the new version of a software previously described (M'ATHS, Metris, France).[9]

The protocol involved scanning of the common carotid arteries, the carotid bifurcations, and the origin (first 2 cm) of the internal carotid arteries. At the time of the examination, the near and far walls of these arterial segments were scanned longitudinally and transver-

sally to assess the presence of plaques. The presence of plaques was defined as localized echo-structures encroaching into the vessel lumen for which the distance between the media-adventitia interface and the internal side of the lesion was $\geq$1 mm. For intima-media thickness and lumen diameter measurements, near and far walls of the right and the left common carotid arteries 2 to 3 cm proximal to bifurcation were imaged. In patients with carotid artery plaques, intima-media thickness measurements were realized in plaque-free segments of the common carotid arteries. Details of the methodology used have been previously described.[9]

Measurements of the Terminal Restriction Fragments Length

Terminal restriction fragments (TRF) lengths were measured as previously described.[4,5] Briefly, DNA samples were digested overnight with restriction enzymes Hinf I (10 U) and Rsa I (10 U) (Boehringer Mannheim). Eighteen DNA samples ($\approx$5 μg each) and 4 DNA ladders (1 kb DNA ladder plus γ DNA/Hind III fragments; GIBCO) were resolved on a 0.5% agarose gel (20 cm$\times$20 cm) at 50V (GNA-200 Pharmacia Biotech). After 16 hours, the DNA was depurinated for 30 minutes in 0.25 N HCl, denatured 30 minutes in 0.5 mol/L NaOH/1.5 mol/L NaCl, and neutralized for 30 minutes in 0.5 mol/L Tris, pH 8/1.5 mol/L NaCl. The DNA was transferred for 1 hour to a positively charged nylon membrane (Boehringer Mannheim) using a vacuum blotter (Appligene, Oncor). The membranes were hybridized at 65°C with the telomeric probe (digoxigenin 3'-end labeled 5'-(CCTAAA)$_3$) overnight in 5$\times$SSC 0.1% Sarkosyl, 0.02% sodium dodecyl sulfate (SDS), and 1% blocking reagent (Boehringer Mannheim). The membranes were washed 3 times at room temperature in 2$\times$SSC 0.1% SDS, each for 15 minutes and once in 2$\times$SSC for 15 minutes. The digoxigenin-labeled probe was detected by the digoxigenin luminescent detection procedure (Boehringer Mannheim) and exposed on x-ray film. Each DNA sample was measured in duplicate. For an illustration and further details, see Figure 1.[4]

Statistical Analysis

Mean values of TRF length, blood pressure, heart rate, and anthropometric and biological parameters were compared in subjects with

TABLE 1. Mean Values of the Main Anthropometric, Hemodynamic, and Biological Parameters in Hypertensive Men With or Without Carotid Plaques

Carotid Plaques	Without	With
N	90	73
Age (y)	60.1±0.9	63.6±1.0*
SBP (mm Hg)	147.5±1.8	149.5±2.0
DBP (mm Hg)	90.6±1.0	88.8±1.1
PP (mm Hg)	56.8±1.5	60.7±1.7
HR (bpm)	65.8±1.0	66.9±1.1
Height (cm)	171±1	171±1
Weight (kg)	83.1±1.6	84.5±2.2
Smokers (%)	20	19
Diuretics (%)	38	37
Beta blockers (%)	33	41
ACEI (%)	46	45
CCB (%)	30	29
Other antihypertensives (%)	14	14
Combined therapy (%)	44	49
Hypolipemic drugs (%)	30	38
Total cholesterol (g/L)	2.22±0.04	2.25±0.05
HDL cholesterol (g/L)	0.56±0.02	0.55±0.02
Glycemia (g/L)	1.03±0.03	1.04±0.02
Triglycerides (g/L)	1.28±0.07	1.17±0.06
Carotid IMT (mm)	0.74±0.01	0.82±0.01***
Carotid diameter (mm)	6.02±0.08	6.17±0.08
Carotid R/H	4.15±0.07	3.85±0.07**
TRF length (kb)	8.46±0.07	8.17±0.07**

Carotid parameters are the mean values of left and right side. ACEI indicates angiotensin converting enzyme inhibitors; CCB, calcium channel blockers; TRF, terminal restriction fragments.

*$P<0.05$, **$P<0.01$, ***$P<0.001$ with vs without plaques; these differences persist after adjustment for age.

and without carotid plaques by multivariate analysis including age. The risk for carotid artery plaques was assessed using a logistic regression model (odds ratios) that included variables that were correlated with the presence of plaques in the univariate analysis.

The criterion for significance is $P<0.05$. Statistical analyses were performed using the SAS statistical software package.

Results

Table 1 summarizes the population characteristics. Among hypertensive subjects, those with carotid plaques were older ($P<0.001$) and had increased intima-media thickness (measured in a plaque-free carotid segment) ($P<0.0001$). This difference persisted after adjustment for age. The age-adjusted TRF length was shorter in hypertensive subjects with carotid artery plaques versus hypertensive subjects without plaques ($8.21±0.08$ kb versus $8.43±0.07$ kb; $P=0.03$) (Figure 2). This difference persisted ($P<0.05$) after adjustment for blood pressure levels (systolic blood pressure, diastolic blood pressure, mean arterial pressure, pulse pressure), total cholesterol levels, presence of hypolipemic treatment, type of antihypertensive treatment, and cigarette smok-
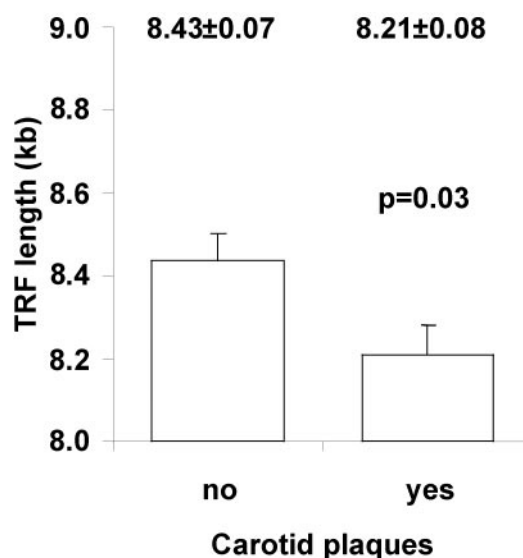

Figure 2. Age-adjusted TRF length according to the presence or absence of carotid plaques in hypertensive men.

ing. TRF length was negatively correlated with age ($r=0.25$; $P<0.01$).

Table 2 presents the odds ratios for the presence of carotid artery plaques, evaluated with a multiple logistic regression model. Age ($P<0.05$) and TRF length ($P<0.033$) were significant determinants of the presence of carotid artery plaques.

To evaluate the possible impact of hypolipemic treatment in the association between TRF length and carotid artery plaques, we conducted the same analyses after excluding the 55 patients who were receiving such a treatment. Among the 108 remaining patients, TRF length was $8.50±0.08$ kb in those without carotid plaques (n=63) versus $8.13±0.09$ kb in those who had plaques (n=45) ($P=0.0026$). The difference was statistically significant after adjustment for age ($8.47±0.08$ kb versus $8.17±0.09$ kb, respectively; $P=0.017$).

Discussion

One of the enigmatic questions about the pathophysiology of essential hypertension is, why are some hypertensive patients more prone than others to developing atherosclerotic lesions? Our study shows that hypertensive men with shorter telomeres in WBC have an increased risk for carotid artery atherosclerotic plaques. This relationship persisted after adjustment for age. An increase in TRF length was associated

TABLE 2. Odds Ratios (OR) and 95% Confidence Intervals (CI) for Carotid Plaques Associated With a Decrease of 0.1 kb in TRF Length and Age 1 Year

Carotid Plaques in Hypertensive Patients	OR (95% CI)	P
Model 1		
TRF length only	1.06 (1.02–1.12)	0.010
Model 2		
Age	1.04 (1.00–1.08)	0.047
TRF length	1.05 (1.00–1.11)	0.033

with a decrease in atherosclerotic plaques of the carotid artery. Among the other parameters studied, only age was a significant determinant of carotid plaques. Total cholesterol, HDL cholesterol, and LDL cholesterol did not differ between patients with or without plaques.

Several large clinical studies have shown an association between total cholesterol and the presence of carotid artery plaques.[11] It is possible that the lack of such an association in the present study is because of the relatively small number of patients. In fact, patients with plaques were treated with hypolipemic drugs more frequently (38% versus 30%), but this difference was not statistically significant. Interestingly, the association between telomere length and carotid artery plaques persisted even after eliminating patients who were receiving a hypolipemic treatment.

In the present study, the mean difference in the age-adjusted TRF length between patients with and without carotid plaques was 220 bp. Based on previously published data and the results of the present study, WBC telomere attrition rates are approximately 25 to 38 bp per year.[5,6,12] Therefore, the observed difference between patients with or without carotid plaques corresponds to a higher biological age of approximately 7 years in hypertensive patients with plaques.

Inflammation and reactive oxygen species are central to the pathobiology of age-related cardiovascular disorders, including atherosclerosis and arterial stiffness.[1,2,13] Telomere length registers the turnover rate of cells, including WBC, a rate that may be augmented by chronic inflammation and an increase in the cumulative oxidative burden.[14]

Telomere length is highly heritable.[3,4] At a given age, it reflects telomere length at birth and telomere attrition rate thereafter. Age-dependent telomere attrition results from replication[15] and telomere repeat loss, which may depend on oxidative stress.[16] Therefore, telomere length registers, in part, the cumulative burden of oxidative stress and inflammation during the individual's lifetime. Indeed, studies in cultured cells have shown that telomere erosion per replication is inversely related to antioxidant capacity.[16,17] In a recent study, Minamino et al[18] evaluated the effects of telomere dynamics on phenotypes of cultured coronary artery endothelial cells. Replicative senescence, associated with telomere erosion, was expressed by an increase in ICAM-1 and a decrease in eNOS activity, both of which are implicated in atherogenesis. By contrast, in the same study, it was found that the ectopic expression of telomerase, which retards telomere erosion, protects against these changes. Thus, a complex biology marks the relationship between telomere length, oxidative stress, and antioxidant capacity. The presence of fewer atherosclerotic plaques in patients with longer telomeres may hence reflect less cumulative oxidative stress or an innate increased antioxidant capacity of these subjects.

In conclusion, our findings suggest that in the presence of chronic hypertension, shorter telomere length in WBC is associated with an increased predilection to carotid artery atherosclerosis.

Perspectives

Telomere length may be a bioindicator of cardiovascular risks and biological aging of the vasculature in humans. More studies are needed to understand the determinants of telomere length and attrition rates in humans.

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