LEUKOCYTES TELOMERE LENGTH SHORTENING IN FAMILIAL HYPERCHOLESTEROLEMIA

Baragetti A^{1,2}, Pellegatta F^{2,3}, Cicolari S^{1,2}, Norata GD^{1,2}, Grigore L^{2,3}, Zampoleri V^{1,2}, Catapano AL^{1,2,3}

1: Dept of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy
2: SISA Center for the Study of Atherosclerosis, Bassini Hospital, Cinisello Balsamo, Milan, Italy
3: IRCCS, Multimedica Hospital, Sesto San Giovanni, Milan, Italy

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INTRODUCTION AND AIM

Telomeres are DNA-protein complexes which protect chromosome ends from degradation and fusion, thus regulating cellular lifespan and fate. At each cell cycle, a specialized reverse transcriptase, the telomerase, adds new telomeric sequences at the end of chromosomes in order to preserve the telomere length (TL). This system is more active in cells with a high-turnover (germ and stem cells), while in somatic cells, telomere length (TL) is reduced over time and reflects cellular senescence.

In humans, TL assessed in DNA from blood leukocytes (LTL) inversely associates with the onset and progression of several cardiometabolic diseases, including diabetes, metabolic syndrome, but also chronic kidney disease and coronary heart disease (CHD). While TL shortening has been proposed as a valid tool to track premature vascular aging associated with common cardiovascular risk factors, including smoking, hypertension, conflicting data have been reported for hypercholesterolemia from large epidemiological studies.

However, since Familial Hypercholesterolemia (FH) is characterized by elevated LDL-C and massive premature atherosclerosis and CHD risk, the **driving hypothesis** of the study is:

To investigate whether lifelong exposure to genetically elevated LDL-C is causal for atherosclerosis and CHD risk because it results on premature LTL shortening.

The long range goal of this investigation is not only to focus on LTL as a novel molecular and cellular aspect to study but also to strengthen the need of a more and more early and aggressive lipid lowering intervention to contain the CHD risk burden.

HYPERCHOLESTEROLEMIA RESULTS IN SHORTER LEUKOCYTES TELOMERE LENGTH (LTL)

A total of **206 hypercholesterolemic patients (HC)** followed at the Lipid Clinic of the Center for the Study of Atherosclerosis, E. Bassini Hospital (Milan, Italy) were recruited for this study. All patients underwent clinical and biochemical characterization for clinical diagnosis of FH. This population included **135 HeFH patients** (defined on the basis of genetic evaluation, by NGS on FH-related genetic loci) and **71 non-FH hypercholesterolemic patients (non-FH HC)**. Clinical history and anthropometric parameters were collected for each patient, their lipid profile was determined and compared to that of **320 normolipidemic subjects (controls)** from the general population of the PLIC Study, as indicated in **Table 1** below.

	Controls (n=320)	Total HC (n=206)	Non-FH HC (n=71)	HeFH (n=135)	P †
Age (years)	49±13	41±18	48±15	37±18 ^{§*}	<0.001
Women, n (%)	184 (57.7)	109 (52.9)	41 (57.7)	68 (50.37)	0.345
BMI (Kg/m ²) ⁽¹⁾	25.72±0.24	25.72±0.31	25.24±0.52	25.28±0.39	0.272
Waist circumference (cm) ⁽¹⁾	88.05±0.62	89.58±0.94	89.70±1.48	89.53±1.22	0.118
Anti-hypertensives, n (%)	70 (21.8)	25 (12.1)	13 (18.3)	12 (8.8) ^{§*}	0.009
Corneal arcus, n (%)	-	9 (4.3)	4 (5.6)	5 (3.7)	
Tendon xanthomata, n (%)	-	20 (9.7)	3 (4.2)	17 (12.6)	
Achilles' tendon echo (mm)	-	5.23±1.82	4.38±0.29	5.57±0.17	
Fasting glucose (mg/dL) ⁽³⁾	88.82±0.66	92.27±0.93	93.76±1.57*	91.43±1.17*	0.004
Diabetes, n (%)	4 (1.2)	2 (1.0)	0 (0)	2 (1.5)	0.873
TC (mg/dL) ⁽⁴⁾	212.06±2.90	316.89±3.88	291.26±6.00*	333.27±4.86 ^{§*}	<0.001
HDL-C (mg/dL)	61.14±1.39	59.94±1.82	60.43±2.92	59.60±2.33	0.661
TG (mg/dL) ⁽⁴⁾	99.60±3.76	123.12±4.93	124.29±7.82*	122.35±6.38*	<0.001
LDL-C (mg/dL) ⁽⁴⁾	133.52±2.75	232.27±3.74*	203.76±5.86*	250.06±4.62 ^{§*}	<0.001
Non-HDL-C (mg/dL) ⁽⁴⁾	156.97±2.95	259.69±3.99	230.21±6.18*	278.46±4.93 ^{§*}	<0.001
Remnant cholesterol (mg/dL) ⁽⁴⁾	20.26±0.90	26.40±1.19	27.44±1.92*	25.76±1.52*	<0.001
ApoB (mg/dL) ⁽⁴⁾	108.25±1.74	172.19±3.45	149.94±6.01*	182.40±4.07 ^{§*}	<0.001
ApoA-I (mg/dL) ⁽⁴⁾	147.59±1.50	154.86±2.90	160.50±5.16 [°]	152.28±3.48	0.017
Statin treatment (n) (%)	39 (12.2)	60 (29.1)	17 (23.9)	43 (31.8)	<0.001
Aspirin (n)	17 (5.3)	19 (9.2)	9 (12.6)	10 (7.4)	0.046
Previous CV events, n (%)	15 (4.6)	26 (12.6)*	11 (15.5)*	15 (11.1)*	<0.001

†P derived from ANCOVA multivariable analysis between controls, non-FH HC and HeFH (P in red when <0.05). (1)adjusted by age and gender; (2)adjusted by age, gender; (3)adjusted by age, gender and anti-hypertensive treatments; (4)adjusted by age, gender and statin treatments. *P<0.05 controls vs others; §P<0.05 HeFH vs non-FH HC; °P<0.05 controls vs non-FH HC. ||Achille's tendon available for 41 non-FH HC and 110 HeFH (P derived from non parameter's U-Mann Whitney).

Plasma LDL-C levels were significantly higher in HC subjects compared with controls (232.27±3.74 mg/dL and 133.52±2.75 mg/dL, respectively, p<0.001) as was the prevalence of cardiovascular events (12.6% vs 4.6%, p<0.001). In spite of these differences, LTL was similar in hypercholesterolemic patients and controls (1.39±0.05 mg/dL vs 1.51±0.04 mg/dL, p= 0.091) (**Figure 1A**), suggesting that elevated LDL-C levels 'per se' do not seem to correlate with markers of premature cellular senescence.

Despite consistent difference in LDL-C between HeFH patients and non-FH HC subjects (**Table 1**), LTL values were similar in the two groups (1.34±0.06 vs 1.44±0.07, p= 0.281) (**Figure 1B**). By contrast, only, HeFH subjects, presenting LDL-C levels almost doubled compared to controls (**Table 1**), showed significantly shorter LTL compared to controls (1.34±0.06 vs 1.52±0.04, p= 0.045) (**Figure 1B**). Of note HeFH were significantly younger than controls (37±18y vs 49±13y, p<0.001) (**Table 1**), suggesting that **the lifelong exposure to genetically elevated LDL-C levels might be responsible for earlier shortening of telomeres compared with healthy normocholesterolemic subjects.**

PREMATURE LTL SHORTENING IN FAMILIAL HYPERCHOLESTEROLEMIA

In light of previous data, to then verify whether it is the lifelong exposure to LDL-C rather the LDL-C *per se* to result into shorter LTL, we firstly explored LTL stratifying by age. When comparing LTL in younger subjects (<35 years-old), thus reducing the years of exposure to elevated LDL-C, LTL was still significantly shorter in HeFH compared to controls (1.32±0.11 vs 1.64±0.11, p=0.050) but not non-FH HC (1.32±0.11 vs 1.52±0.15, p=0.305) (**Figure 2A**), suggesting that, in FH, already at early age, telomeres are shorter. It is worth noting that when we considered elder subjects (>50y), similar LTL values were observed in HeFH and the other two groups (**Figure 2A**).

To verify whether the latter observation could mark the effect of statin treatment, we further focused our analysis in elder FH subjects naive to statin therapy and observed that LTL values were significantly shorter compared with controls (0.99±0.25 vs 1.62±0.06, p=0.022) but not with non-FH HC (0.99±0.25 vs 1.20±0.16, p=0.447) (**Figure 2B**). This observation reconciles with that of **Figure 1C**, highlighting the hypothesis that shorter LTL is associated with the lifelong burden of LDL-C in genetically determined hypercholesterolemia.

Finally, to evaluate the potential role of lifelong exposure to elevated LDL-C unrelated to other factors, we compared two subgroups of HeFH (N=90) and non-FH HC (N=30) subjects matched for age (mean 35 years-old) and LDL-C levels. LTL was significantly lower in HeFH (1.33±0.05 vs 1.55±0.08, p=0.019) (**Figure 2C**), a finding confirmed also when only statin-naïve subjects were evaluated (**Figure 2D**). These differences were lost when matching older patients (58-60 years-old), confirming the relevance of the time-exposure to elevated LDL-C (not shown).

LDL-C LOWERING AND LTL SHORTENING

To finally hypothesize the effect of statins, as LDL-C lowering drugs, on LTL shortening rate over lifetime in non-FH HC and in HeFH, we derived correlation coefficients from the cross-sectional relation between age and LTL in our cohorts (assumed as a linear function) (**Figure 3**).

Of note, while both in non-FH HC and in HeFH statins would reduce the LTL change slope over time, this would be more prominent in HeFH free from statin treatments. Moreover, treating HeFH with statins would be benefit to reach similar LTL to that of non-FH HC in advanced decades.

Advanced longitudinal analyses are warranted, tracking LTL shortening in vivo since early stages of life, to derive the function of the relation between biological age and LTL in familial hypercholesterolemia and in elevated CVD risk.

CONCLUSIONS

Our data show that genetically determined familial hypercholesterolemia and the consequent lifelong exposure to elevated LDL-C levels result in shorter LTL, thus marking early cellular senescence and perhaps contributing to premature cardiovascular morbidity in FH patients.

Longitudinal studies and experimental models are warranted to confirm these hypotheses. Clinically, these data strongly support that shorter LTL calls for more and more early and aggressive lipid lowering treatments in familial hypercholesterolemia.