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Short communication

Early and late childhood telomere length predict subclinical atherosclerosis at age 14 yrs. – The CardioCAPS study

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ABSTRACT

Introduction: Carotid Intima Media Thickness (CIMT) is a marker of subclinical atherosclerosis, associated with cardiovascular risk in adults. Telomere length (TL) is a marker of cellular ageing. We sought to determine whether telomere length in early childhood and/or at 14-years is associated with CIMT in adolescence, in a community-based cohort study.
Methods: 118 children had TL measured at mean age 3.6-years and 165 children had TL and CIMT, measured at 14-years, from the community-based Childhood Asthma Prevention Study.
Results: TL in early childhood was significantly inversely associated with CIMT at 14 years, $p = 0.04$. TL in teenage life was also significantly inversely associated with CIMT at 14 years, $p = 0.03$. This latter association was no longer significant, however, after adjusting for early life TL.
Conclusion: TL measured in early childhood and adolescence is significantly associated with CIMT at 14-years, suggesting that telomere length is a biological marker or even early determinant of late cardiovascular risk.

1. Introduction

Telomere length (TL) is a measure of cell replication and telomere attrition results in cellular senescence [1]. TL is thought to be a key marker of biological ageing and is associated with cardiovascular disease and subclinical atherosclerosis in adults [2,3]. TL varies at birth and rates of shortening vary during life. We have previously reported, from the community-based CardioCAPS cohort, that TL in early childhood (age 3-years) predicts CIMT in mid-childhood (age 8-years) [4]. CIMT is a marker of subclinical atherosclerosis known to be associated with future cardiovascular outcomes [5–7]. We hypothesized that TL in early childhood would be a better predictor than TL in adolescence or TL attrition rate, for prediction of CIMT at 14-years.

2. Methods

2.1. Study population

Participants were from the Childhood Asthma Prevention Study (CAPS), a prospective birth cohort study in the effect of Omega-3 supplementation and dust mite avoidance in asthma prevention in 616 healthy children, who were born in Sydney from 1997 to 1999 as previously described [8]. At 8-years 405 children, and at 14-years, 191 children agreed to return for assessment of anthropometry, non-fasting lipid profiles and CIMT. At 14-years 165 of these 191 participants had TL measured; 118 of these had TL measured in early childhood. Steroid use in this asthma cohort was modest with 2.5% use at 14-years and 11% use in early life. Ethics approvals and informed consent were obtained.

2.2. Leukocyte telomere length

Leukocyte TL was measured between 18-months and 5-years of age (mean 3.6-years), as previously described [4]. Briefly, leukocyte genomic DNA was extracted from blood samples by a salting-out method [9] and quantified by UV–Vis spectrophotometry (NanoDrop 2000, NanoDrop technologies, USA). Mean telomere length for each sample was measured using Quantitative PCR in quadruplicates, 5 replicates of leukocyte DNA from an adult female (2.5_serial dilution) was used as a reference sample on each plate. Leukocyte TL was again measured at 14-years of age, by the more contemporary method of genomic DNA isolation (ReliaPrep Blood gDNA Miniprep System, Promega Madison, Wisconsin, USA). Control samples were from two sourced cell lines, Human monocytic THP-1 and T-Cell 1301 and 5 replicates of leukocyte DNA from an adult female (2.5_serial dilution). Results were expressed as Telomere to Single copy gene (T/S) ratio, a unitless

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measure corresponding to the relative TL of DNA. The TL used in this analysis was a mean of quadriplicate T/S ratios per participant [10].

2.3. CIMT

CIMT was measured at 14-years of age as previously described [4]. The CIMT was measured 0-10 mm proximal to the carotid bulb, (GE Vivid 9), by a single blinded observer, using automated edge detection software [11].

2.4. Statistical analysis

The analysis was performed in participants with data for TL and CIMT. Chi squared and *t*-tests were used to compare the analyzed with the remaining cohort participants. Multivariable linear regression models were used to test the association between TL and CIMT. TL was modelled as a continuous variable and using quartiles. The distribution of TL was skewed, and thus was transformed for analysis (Ln [telomere length]/Ln2), reflecting a change in CIMT per doubling of telomere length. TL quartiles were calculated after the TL variable was transformed. The TL quartiles, measured in early childhood, were adjusted for age at DNA collection using the residuals method. Linear regression models were adjusted for sex and age. Models adjusting for early life risk factors also included omega-3 supplementation group, maternal and paternal education, and smoking in pregnancy, this was based on univariate association $p < 0.1$. The independent effect of TL in early childhood and TL at 14-years was tested by including both variables in a linear regression model. Statistical analyses were undertaken in IBM SPSS version 24 (IBM Corp.USA).

3. Results

There were no significant differences between the 118 subjects analyzed for early childhood TL and the remaining CAPS cohort, for early life markers. Those with 14-year TL ($n = 165$) were more likely to have had older and tertiary educated parents, compared to the remaining cohort (data not shown). Mean CIMT at 14-years of age was 646 μm(SD = 71). Median(IQR) Telomere length in early childhood was 1.72(1.46, 2.14) and at 14-years of age 1.02 (0.88, 1.18) (Table 1).

Table 1
Characteristics of subjects with telomere measurements performed from DNA collected during early life and at 14 years of age.

	Telomere measurement			
	Early life		14 years	
	N	n(%) or Mean (SD)	N	n(%) or Mean (SD)
Parental, birth, infant				
Sex, Female %	118	55(47%)	165	73(44%)
Maternal age, years	118	29.3(5.05)	165	29.3(4.98)
Paternal age, years	118	31.5(5.76)	164	31.1(5.56)
Maternal tertiary education %	118	60(51%)	165	91(55%)
Paternal tertiary education %	117	58(49%)	164	83(51%)
Maternal smoking during pregnancy %	118	22(19%)	165	33(20%)
Gestation, weeks	118	39.6(1.24)	165	39.7(1.26)
Birth weight, grams	118	3470(529)	165	3460(495)
Breastfeeding ≥6 months %	118	50(42%)	165	74(45%)
Omega-3 supplementation group Active %	118	61(52%)	165	83(50%)
Weight gain from 0 to 18 months, kg	117	8.02(1.12)	164	8.04(1.17)
BMI *Z*-score 3 years	114	0.28(1.02)	161	0.32(0.12)
Telomere data				
Age DNA collection, years	118	3.62(1.06)	165	13.90(0.16)
Telomere length[a], T/S ratio (median (IQR))	118	1.72(1.46, 2.14)	165	1.02(0.88, 1.18)
Cardiovascular measures at 14 years				
Maximum carotid IMT, μm	118	649(74)	165	646(71)
BMI z-score	118	0.60(1.008)	165	0.53(1.085)
nonHDL, mmol/L	118	3.01(0.70)	165	3.01(0.69)
HDL, mmol/L	118	1.33(0.24)	165	1.30(0.24)
SBP, mmHg	115	114(9.87)	161	114(10.31)
Age, years	118	14(0.21)	165	14(0.21)

BMI, body mass index; IMT, intima-media thickness; HDL, high density lipoprotein; SBP, systolic blood pressure.
Early life: refers to subjects who had telomere measurements performed from DNA taken between the ages of 1.5–5 years and DNA collected at 14 years.
[a] Telomere length expressed as median (IQR = interquartile range) of T/S ratio.

3.1. Leukocyte telomere length in early childhood and CIMT at 14 years

Shorter TL in early childhood was significantly associated with increased CIMT at 14-years of age (beta = 28 μm per halving of TL, (95% CI 1, 55), $p = 0.04$). CIMT quartiles were also significantly associated with TL ($p = 0.002$) (Table 2). Compared to the longest TL quartile, CIMT in those in the shortest quartile was on average 54 μm thicker (95%CI 19, 89). This association remained significant after adjustment for early life factors ($p = 0.006$).

3.2. Telomere length in adolescence and CIMT at 14-years

Shorter TL at 14-years was also significantly associated with thicker CIMT at 14-years (beta = 40 μm per halving of TL, (95%CI 4, 76), $p = 0.03$) (Table 2). This association remained significant after adjusting for early life factors ($p = 0.03$). TL measured in quartiles was also associated with CIMT at 14-years after adjusting for early life risk factors ($p = 0.013$). This association was no longer significant, however, after adjusting for early life TL (beta = 31 μm per halving of TL, (95%CI -77, 16), $p = 0.2$).

3.3. Attrition of telomere length and CIMT at 14-years

The annualized reduction in TL from early life to 14-years (mean = 0.080 T/S per year, SD = 0.059) was not significantly associated with CIMT at 14-years ($p = 0.9$) (Table 2).

4. Discussion

Many studies have shown TL to be associated with cardiovascular outcomes and subclinical atherosclerosis in adults [1,3,12,13]. There is variation in TL at birth [14] and although longitudinal studies have been undertaken in adulthood, there is little information on the changes and effects of TL during the first decades of life. In this study, we have demonstrated a significant association of shorter TL measured at 3.6-years and at 14-years with subclinical atherosclerosis in adolescence. This is in keeping with a previous finding of TL at 3.6-years being independently associated with CIMT in mid-childhood [4]. TL shortens with oxidative stress and inflammation, which are also associated with cardiovascular disease in adulthood [2,15]. The attrition of TL in adults has also been shown to be associated with thickening of CIMT >75th centile in adults [16]. In our study, however, we did not find an association between TL attrition from 3.6 to14-years and CIMT. Our novel finding that TL length in early life is associated with CIMT in adolescence as opposed to attrition of TL suggests the path of biological ageing may be largely set in early life. This may be reflective of heritability of TL, as previous studies have shown genetic variants associated with shorter TL are associated with coronary artery disease in adult life [17] or perhaps the increased rate of shortening in the first few years of life compared to adulthood [18], which may be adversely affected by environmental influences during this time of more rapid turnover.

In our community-based low risk cohort, established risk factors for cardiovascular disease were not associated with CIMT at 14-years. This is in contrast to longitudinal studies in adulthood which have shown traditional cardiovascular risk factors contributed far more to the variation in CIMT than early life socioeconomic factors [19]. The short exposure and the small relative difference between healthy adolescents in our cohort may have reduced the effect size, in this instance.

Limitations of this study include the small cohort at 14-years, limited by the expected marked loss to follow up during adolescence. However, this smaller cohort was generally representative of the larger birth cohort. Strengths include the longitudinal study design, serial measures of TL and assessment of a community-based cohort. Although telomere

Table 2
Association between telomere length and carotid IMT at 14 years.

	Carotid IMT(μm) at 14 years					
	Adjusted for age, sex			Adjusted for early life factors[e]		
	Beta coefficient	95%CI	P-value[f]	Beta coefficient	95%CI	P-value[f]
Early childhood (N = 118)						
Telomere length[a]	−28	−55, −1	0.04	−25	−52, 1	0.06
Telomere length Quartiles[b]						
1st Quartile (shortest)	54	19, 89	0.002	50	15, 84	0.006
2nd Quartile	3	−40, 34		3	−32, 39	
3rd Quartile	−5	−39, 29		−6	−39, 28	
4th Quartile (longest)	0 (referent)			0 (referent)		
14 years (N = 165)						
Telomere Length[c]	−40	−76, −4	0.03	−41	−77, −4	0.029
Telomere length Quartiles[d]						
1st Quartile (shortest)	33	3, 62	0.015	33	3, 63	0.013
2nd Quartile	42	12,71		43	13, 72	
3rd Quartile	7	−23, 36		6	−23,36	
4th Quartile (longest)	0 (referent)			0 (referent)		
Telomere attrition (N = 118)						
Telomere attrition per year	13	−212, 239	0.91	22	−202, 247	0.85

[a] Telomere length transformed to [Ln(telomere length)/Ln(2)] and adjusted for age at DNA collection.
[b] Telomere length quartiles, after transformation to [Ln(telomere length)/Ln(2)] and adjusted for age at DNA collection.
[c] Telomere length transformed to [Ln(telomere length)/Ln(2)].
[d] Telomere length quartiles, after transformation to [Ln(telomere length)/Ln(2)].
[e] Early life risk factors model adjusts for sex, age at 14y, CAPS diet intervention group, and maternal and paternal education (early childhood models) or smoking in pregnancy (14 years).
[f] P-value from a linear regression model.

length was measured at both time points by PCR, the DNA extraction method used at 14-years was a column-based purification method rather than a salting-out method. These methods are both reliable for DNA extraction, however our data concerning the non-significant relationship between telomere attrition rate and CIMT should be interpreted with caution. We cannot make a causal link based on this relationship nor can we be certain that TL is associated with atherosclerosis in adolescence, independent of unmeasured environmental influences.

5. Conclusion

Shorter TL in early childhood and at 14-years of age is significantly associated with thicker CIMT at 14-years. The relationship of TL at 14-years is not independent of early childhood TL, indicating that TL in early childhood may be a more important biological marker or determinant of cardiovascular risk.

Declarations of interest

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Author contributions

All authors made a significant contribution to the study design, data acquisition, analysis or interpretation. All authors critically reviewed and contributed to the writing of the manuscript and all authors gave final approval and agree to be accountable for integrity and accuracy of this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2018.12.065.

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