Association Between Leukocyte Telomere Length and Plasma Homocysteine in a Singapore Chinese Population

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Abstract

Rationale: Leukocyte telomere length (LTL) and plasma homocysteine (HCY) have been independently associated with cardiovascular disease (CVD) morbidity and mortality. However, few studies have investigated the association between LTL and HCY levels.

Objective: This study investigated the association of LTL with CVD risk factors, including HCY, in an overt CVD-free Singapore Chinese population comprised of middle aged and elderly, the age group at risk of developing CVD.

Approach: The association of plasma HCY and other CVD biomarkers with LTL were assessed in 100 samples drawn from the Singapore Chinese Health Study (SCHS). SCHS, a population-based cohort, recruited Chinese individuals, aged 45–74 years, between 1993 and 1998. Questionnaire data were collected via face-to-face interviews. Known CVD biomarkers were measured from the blood collected at the time of recruitment, and LTL was measured using the conventional Southern blot method.

Results: After adjustment for age, gender, smoking status, education, and dialect, LTL was found to be inversely associated with plasma HCY levels (p for trend = 0.014). Serum urate showed a weak association (p for trend = 0.056). Other CVD risk factors and nutrients, namely total cholesterol, low-density lipoprotein (LDL), triglycerides and creatinine, high-density lipoprotein (HDL), folate, and vitamin B6 showed the expected trend with LTL, but did not reach statistical significance.

Conclusion: LTL displayed an inverse association with plasma HCY. This LTL–HCY inverse association in subjects lacking obvious cardiovascular events suggests that telomere length may be an intermediary in the biological mechanism by which elevated HCY leads to CVD.

Introduction

TELOMERES ARE TTAGGG REPEAT REGIONS at the ends of human chromosomes, protecting them from end-to-end fusions and thus maintaining chromosomal stability.¹ Telomere length (TL) decreases with every cell division. Hence it is the primary predictor of cellular senescence and cell cycle arrest *in vitro*^{2,3} and is considered as a cellular marker for biological aging *in vivo*.^{4,5} Over the past few

decades, TL has been implicated as a pathognomonic indicator in several aging-related diseases whose hallmarks are increased oxidative stress and inflammation. These diseases include cardiovascular diseases (CVD), diabetes, and Alzheimer's disease.^{6–8} Experimental evidence suggests that cellular senescence due to increased inflammation and oxidative stress may increase the replication rate of cells and damage the G-rich telomeric DNA, thus cumulatively accelerating telomere loss, leading to these aging-related

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diseases.^{9,10} Particularly in CVD, which is the leading cause of death worldwide, short leukocyte telomeres have been associated with stroke, heart failure, atherosclerosis, and myocardial infarction.^{10–12} Apart from CVD events, leukocyte telomere length (LTL) associates independently with CVD risk factors such as low density lipoproteins (LDL), male gender, and insulin resistance, suggesting TL's role in the development of CVD.^{12,13} However, the utility of LTL as a potential marker for cardiovascular aging needs further validation due to inconsistent association of LTL with certain risk factors, such as body mass index (BMI) and hypertension.^{12,14,15} These disparities make it necessary to investigate the association of LTL and CVD in multiple populations.

Homocysteine (HCY) is an amino acid intermediate product of the methionine pathway. Epidemiological evidence has identified HCY as an independent risk marker for age-associated diseases of vascular etiology, such as CVD and Alzheimer's disease.^{16–19} Elevated plasma HCY levels have been associated with the severity of atherosclerotic diseases, carotid artery stenosis, stroke, and stroke-related mortality.^{20,21} Mechanistically, experimental increase in HCY levels by oral administration of L-methionine in humans was shown to cause endothelial dysfunction via increased oxidative stress.²² Consistently, studies in murine models revealed an enhancement of vascular inflammation and accelerated atherosclerosis when HCY levels were elevated.²³ However, limited knowledge about the pathophysiological link of HCY to CVD warrants further exploration.

Interestingly, Richards et al. first reported an inverse association between plasma HCY levels and LTL, suggesting a possible link between the two.²⁴ A similar observation was reported in elderly South Australian men. However, no conclusive association was found in younger populations or in both young and old women.²⁵ Also, no association was found between HCY and LTL in individuals without evident CVD.¹⁵ In view of conflicting findings reported on HCY and LTL, studies in healthy populations are required to investigate any possible mechanistic association between HCY and LTL in the pathogenesis of CVD. To date, there is a paucity of data associating LTL with CVD risk factors in Asian populations. The aim of this study is to explore the association of known biomarkers of CVD risk with LTL in a relatively healthy population-based cohort.

Materials and Methods

Study population

The Singapore Chinese Health Study (SCHS) is a prospective population-based cohort designed to investigate the risk factors for cancer and other chronic diseases and diet. The details of the study design have been described previously.²⁶ In brief, 63,257 Chinese men and women who were permanent residents or citizens of Singapore living in government housing estates (86% Singaporeans resided in these buildings at the time of recruitment) were recruited between April, 1993, and December, 1998. The participants, aged 45–74 years at the time of recruitment, were restricted to two major dialect groups in Singapore, the Hokkiens and the Cantonese, who originated from Fujian and Guangdong provinces in southern China, respectively. Written informed consent was obtained and the study was approved by the Institutional Review Board at the National University of Singapore.

Baseline assessment at recruitment was conducted through a face-to-face interview by a trained interviewer using a structured questionnaire, which included questions on demographics, medical history, cigarette smoking, alcohol consumption, physical activity, and detailed menstrual and reproductive history (women only). The habitual diet of the study participants over the past year was recorded using a 165-item semi-quantitative food frequency questionnaire, which incorporated common and distinct food items in Singapore. The dietary intake of each nutrient was derived based on the Singapore Food Composition Database developed specially for this cohort.²⁶

In April, 1994, 1 year after the initiation of cohort subject recruitment, we began to collect blood and single-void urine specimens from a random 3% sample of study enrollees. Details of the biospecimen collection, processing, and storage procedures have been described.²⁷ All blood specimens were processed and separated into their various components (plasma, serum, red cells, buffy coat) prior to storage at -80°C . The biospecimens used in this study were 100 samples randomly selected from the first 483 subjects (214 men and 269 women) for whom plasma HCY was quantified in a prior study.

Laboratory measurement

Plasma total HCY, B vitamins, and urate were quantified in previous studies.^{28,29} Briefly, plasma total HCY concentrations were measured using high-performance liquid chromatography (HPLC) with electrochemical detection as reported by Hughes and Ong.²⁹ Plasma folate and vitamin B12 concentrations were analyzed with the Quantaphase II B-12/folate radioimmunoassay kit (Bio-Rad Laboratories Inc, Hercules, CA).²⁹ Plasma vitamin B6 concentrations were determined with a radioenzymatic assay (Buhlmann Laboratories AG, Allschwil, Switzerland) on the basis of a method originally described by Shin et al.³⁰ Serum urate was measured using the direct enzymic assay where serum urate was oxidized by uricase to allantoin and hydrogen peroxide, and the intensity of the resultant red chromogen was measured at 545 nm.³¹

Serum creatinine was measured using the Enzymatic Creatinine_2 method.³² Glycated hemoglobin (HbA1c) was measured using the Bio-Rad Variant II, a fully automated HbA1c analyzer that uses the principles of ion-exchange HPLC, and detection was performed at 415 nm and 690 nm.^{33,34} Total cholesterol, high-density lipoprotein (HDL), and triglycerides (TGs) were measured on the Bayer Advia 1650 Autoanalyzer using standard reagents.^{35,36} Total cholesterol was measured using a method based on an enzymatic assay using cholesterol ester hydrolase and cholesterol oxidase conversion followed by a colorimetric Trinder end point reaction. HDL-cholesterol (HDL-C) plasma concentrations were measured using the direct HDL-C method in serum and plasma without prior separation.³⁷ The Friedewald formula was used to calculate the plasma concentrations of LDL-cholesterol (LDL-C).³⁸ TGs were measured based on the Fossati three-step enzymatic reaction with a Trinder end point.

Telomere length measurement

Genomic DNA was isolated from peripheral blood leukocytes (buffy coat) using the QIAamp DNA Mini Kit as per the manufacturer's instructions and stored at -80°C . TL was measured by Terminal Restriction Fragment (TRF)

analysis using TRF diagnostic kits (TeloTTAGG Telomere Length Assay, Roche Diagnostics; cat. no. 12209136001). The assay was performed while blinded for participant characteristics. In brief, 1 μ g of DNA was digested using Hinf I/Rsa I at 37°C for 2 hr and resolved on 0.8% agarose gel. DNA was transferred onto a nylon membrane (Hybond, N⁺, Amersham, UK) overnight and hybridized at 42°C with a digoxigenin-labeled telomeric probe. The probe was detected using digoxigenin luminescence, and the signal was recorded on X-ray film. Images were digitized, and the mean TL was calculated by comparison to the 1-kb plus DNA ladder provided in the kit. One sample greater than 12,000 base pairs (bp) and nine samples less than 2000 bp were excluded from the analysis. Thus, the remaining 90 samples were included in this study because TL < 2000 bp is normally found in individuals above 80 years,³⁹ and this exclusion criteria was also based on the range reported in Chinese populations (generally longest < 12,000 bp).⁴⁰

Statistical analysis

The LTL values followed a normal distribution across the study population and arithmetic means of LTL were presented. The analysis of co-variance (ANCOVA) method was used to examine the difference of LTL across different groups of factors of interest. The relationship between LTL and other biomarkers was assessed by tertile categories while adjusting for age, gender, dialect (Hokkien, Cantonese), education (none, primary, secondary, and above), and smoking status (ever, never). Additional analysis was con-

ducted for HCY with further adjustment for serum urate because urate was a potential confounding factor. All linear trend tests were based on the ordinal values of the categories defined by tertiles.⁴¹

All statistical analysis was conducted using SAS (v. 9.2, SAS Institute, Inc., Cary, NC). All reported *p* values were two-sided; *p* < 0.05 was considered statistically significant.

Results

Distribution of selected demographic characteristics of study participants is described in Table 1. The mean age was 59.4 years (range, 47–77 years) and 57.8% were women. The LTL values had a mean of 5330 bp and ranged from 2600 bp to 8200 bp. The mean HCY concentration was 10.11 μ mol/L (range 4.37 μ mol/L to 20.27 μ mol/L). The participants did not have any history of CVD (self-reported) except one who had self-reported physician-diagnosed coronary heart disease at baseline. Exclusion of this participant did not change the results materially. Although LTL did not significantly associate with age, gender, and smoking status, expected direction was observed. LTL decreased with increasing age (Table 2), which was in agreement with previously published data.^{5,42} Participants aged 65 years and above had shorter LTL (5254 bp) as compared to participants aged 50–65 years (5350 bp) and participants aged below 50 years (5373 bp). Moreover, males had shorter LTL than females (males, 5279 bp; females, 5360 bp), which corroborated with reported findings.¹² LTL of subjects that were either current smokers or had smoked before had 95 bp

TABLE 1. SELECTED GENERAL CHARACTERISTICS OF STUDY SUBJECTS BY LEUKOCYTE TELOMERE LENGTH VALUES

Variable n (%) or mean $\pm$ SD	Leukocyte telomere length, bp			Total (n=90)
	1 st tertile (n=48)	2 nd tertile (n=13)	3 rd tertile (n=29)	
Age, years	60.1 $\pm$ 8.3	57.7 $\pm$ 7.6	59.2 $\pm$ 6.8	59.4 $\pm$ 7.7
Women	26 (54.2)	10 (76.9)	16 (55.2)	52 (57.8)
Dialect				
Cantonese	28 (54.2)	5 (38.5)	17 (58.6)	48 (53.3)
Hokkien	22 (45.8)	8 (61.5)	12 (41.4)	42 (46.7)
Education				
None	13 (27.1)	6 (46.2)	11 (37.9)	30 (33.3)
Primary	22 (45.8)	7 (53.8)	16 (55.2)	45 (50.0)
Secondary and above	13 (27.1)	0 (0)	2 (6.9)	15 (16.7)
Smoking				
Never	33 (68.8)	10 (76.9)	20 (69.0)	63.0 (70.0)
Ever	15 (31.2)	3 (23.1)	9 (31.0)	27.0 (30.0)
LTL, bp	4602 $\pm$ 502	5477 $\pm$ 344	6455 $\pm$ 505	5330 $\pm$ 960
HCY, μ mol/L	10.48 $\pm$ 3.03	9.79 $\pm$ 2.74	9.65 $\pm$ 2.87	10.11 $\pm$ 2.94
Serum urate, μ mol/L	340.15 $\pm$ 74.62	340.31 $\pm$ 97.41	309.52 $\pm$ 78.96	330.30 $\pm$ 79.93
Total cholesterol, mmol/L	5.78 $\pm$ 1.01	6.46 $\pm$ 0.94	5.42 $\pm$ 1.06	5.76 $\pm$ 1.06
LDL, mmol/L	3.55 $\pm$ 0.92	4.29 $\pm$ 0.88	3.19 $\pm$ 1.04	3.54 $\pm$ 1.01
HDL, mmol/L	1.38 $\pm$ 0.38	1.33 $\pm$ 0.21	1.41 $\pm$ 0.36	1.38 $\pm$ 0.35
Triglycerides, mmol/L	1.91 $\pm$ 1.07	1.89 $\pm$ 1.01	1.82 $\pm$ 0.96	1.88 $\pm$ 1.02
HbA1c, %	6.05 $\pm$ 0.84	6.15 $\pm$ 1.24	6.33 $\pm$ 1.84	6.16 $\pm$ 1.29
Creatinine, μ mol/L	65.13 $\pm$ 12.16	59.62 $\pm$ 16.49	61.31 $\pm$ 15.77	63.10 $\pm$ 14.07
Folate, nmol/L	15.01 $\pm$ 7.33	18.49 $\pm$ 6.85	15.52 $\pm$ 5.63	15.68 $\pm$ 6.79
Vitamin B6, nmol/L	42.63 $\pm$ 27.25	36.91 $\pm$ 12.92	39.07 $\pm$ 22.12	40.66 $\pm$ 23.95
Vitamin B12, pmol/L	346.78 $\pm$ 179.06	418.86 $\pm$ 214.90	346.98 $\pm$ 103.56	357.25 $\pm$ 164.87

SD, standard deviation; LTL, leukocyte telomere length; HCY, homocysteine; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

TABLE 2. MEAN LEUKOCYTE TELOMERE LENGTH (BP) BY CATEGORIES OF SELECTED DEMOGRAPHIC FACTORS

Variable	n	Mean $\pm$ standard deviation	p value
Age, years			
< 50	11	5372 $\pm$ 926	0.904
50 to < 65	53	5350 $\pm$ 939	
65 +	26	5253 $\pm$ 1063	
Gender			
Male	38	5279 $\pm$ 1089	0.698
Female	52	5360 $\pm$ 873	
Smoking			
Never	63	5354 $\pm$ 835	0.672
Ever	27	5259 $\pm$ 1233	
Education			
None	30	5370 $\pm$ 995	0.343
Primary	45	5407 $\pm$ 1046	
Secondary and above	15	4993 $\pm$ 534	
Dialect			
Cantonese	48	5331 $\pm$ 1016	0.953
Hokkien	42	5319 $\pm$ 915	

shorter LTL than those that had never smoked (5354 bp). There was no significant difference in LTL between participants in the different educational groups (no education [5370 bp], primary education [5407 bp]), and secondary education [4993 bp] or different dialect groups, Cantonese (5331 bp) and Hokkien (5319 bp).

Plasma HCY concentration was inversely associated with LTL (Pearson correlation coefficient, $r = -0.227$, $p = 0.031$). After adjusting for gender, dialect, age, education, and smoking status (Table 3), the mean LTL of subjects in the highest tertile of plasma HCY was 693 bp shorter than subjects in the lowest tertile (p for trend = 0.014). Even after controlling for the effect of serum urate (Table 4), another confounding variable, the significant association between LTL and HCY was still maintained (p for trend = 0.040). Similarly, the mean LTL of subjects in the highest tertile of serum urate was 532 bp shorter than subjects in the lowest tertile (p for trend = 0.056), which was at borderline significance. LTL was the longest corresponding to the highest tertiles of HDL and folate, whereas LTL was the shortest corresponding to the highest tertiles for LDL, TGs, and creatinine. Although the associations of LTL with these biomarkers were in the expected directions, they did not reach statistical significance.

We further analyzed the association of LTL and HCY in men and women separately. In men, the means (95% confidence interval [CI]) of LTL from the lowest to the highest tertiles of plasma HCY were 5697 bp (5009–6385), 5596 bp (4844–6347), and 4894 bp (4386–5402), respectively. LTL values associated at borderline significance with HCY in men (p of trend = 0.066). In women, the corresponding values were 5505 bp (5129–5885) for tertile 1, 5390 bp (5042–5738) for tertile 2, and 4953 bp (4394–5512) for tertile 3. There was no evidence of interaction by gender (p for interaction = 0.9), implying that the relationship between LTL and HCY did not vary by gender. Serum urate exhibited a significant inverse association with LTL in women (p of trend = 0.035) but not in men (p of trend = 0.838).

Discussion

In the present study, we aimed to associate known risk factors for CVD with LTL in an Asian population-based cohort study consisting of middle-aged and elderly recruits. A decrease in LTL was observed with increasing age that is consistent with published epidemiological studies.^{5,42} A strong concomitant finding from many telomere studies is that females have longer LTL than males.¹² A similar observation was also reported in the present study. Interestingly, this is the first study in the Singapore Chinese population that demonstrated significant inverse relationship (p value = 0.014) between HCY and LTL. Separate analysis of this correlation in males and females also demonstrated that individuals with higher HCY levels have shorter LTL. The other CVD risk factors, namely LDL, HDL, TGs, cholesterol, and creatinine, also exhibited the expected relation with LTL, although the association did not reach statistical significance.

LTL values shorter than 2000 bp and longer than 12,000 bp were considered as outliers. These samples with extreme LTL values were excluded from the analysis, as studies from literature suggests that the average LTL less than 2000 bp is found in individuals aged more than 80 years old.³⁹ In addition, a study in a Chinese population with similar age range of participants as our study reported that the LTL was generally less than 12,000 bp.⁴⁰

The association between HCY and telomeres was first explored by Bekaert et al. in 2007 as part of a study in a Belgian cohort aimed at investigating the link between CVD risk markers and LTL.¹⁵ However, an association between LTL and CVD risk markers, including cholesterol, TGs, blood pressure, and plasma HCY, was not found. The authors attributed this lack of an association to relative youth of the cohort and the narrow age-range (35–55 years) of the recruits. In our study of a comparatively older cohort with an age range of 47–77 years, LTL did not significantly associate with the conventional CVD risk factors such as cholesterol and LDL. Nonetheless, the inverse association with serum urate did reach a borderline significance (p for trend = 0.056). In addition, LDL, serum urate, TGs, total cholesterol, HDL, and creatinine exhibited a difference in LTL of –313 bp, –532 bp, –91 bp, –154 bp, 314 bp, and –432 bp, respectively, between the highest and the lowest tertiles, where “–” indicated a decrease in LTL. The lack of statistical significance in these results was probably attributable to insufficient power due to relatively small sample size.

Consistent with our finding, Richards et al. reported that LTL negatively correlated with plasma HCY concentrations in a population-based study cohort of twins from the United Kingdom.²⁴ In these subjects with a mean age of 49 years (standard deviation [SD] = 12.5), LTL was relatively longer in individuals with the lowest HCY and the highest folate tertile levels and shorter in those with the highest tertile level for HCY and the lowest for folate. Bull et al. measured TL in an Australian population consisting of a younger cohort (18–32 years) and an older cohort (65–83 years).²⁵ TL associated inversely with HCY levels in older men, but not in older females and the younger cohort. The authors attributed this to the small sample size or the narrow range of HCY concentrations in older females and relative youth of the younger cohort. Our study, the first in Singapore

TABLE 3. THE MEAN OF LEUKOCYTE TELOMERE LENGTH (BP) BY TERTILE CATEGORIES OF BIOMARKERS

Biomarker*	n	Mean** (95% CI)	p for trend
HCY ($\mu\text{mol/L}$)			0.014
T1: 7.43 (6.62–8.21)	30	5583 (5231–5935)	
T2: 9.89 (9.16–10.37)	32	5451 (5106–5795)	
T3: 12.95 (12.14–14.99)	28	4890 (4514–5265)	
Serum urate ($\mu\text{mol/L}$)			0.056
T1: 251.50 (231–259)	30	5628 (5247–6009)	
T2: 321.00 (299–350)	31	5276 (4928–5625)	
T3: 406.00 (394–442)	29	5096 (4738–5454)	
Total cholesterol (mmol/L)			0.543
T1: 4.56 (4.37–4.98)	30	5482 (5123–5841)	
T2: 5.69 (5.49–5.95)	32	5147 (4800–5493)	
T3: 6.92 (6.53–7.35)	28	5328 (4962–5693)	
LDL (mmol/L)			0.230
T1: 2.58 (2.09–2.81)	30	5503 (5139–5866)	
T2: 3.53 (3.39–3.75)	30	5267 (4909–5625)	
T3: 4.58 (4.2–4.91)	29	5190 (4830–5551)	
HDL (mmol/L)			0.246
T1: 1.06 (0.98–1.13)	30	5256 (4904–5607)	
T2: 1.34 (1.24–1.40)	31	5135 (4775–5494)	
T3: 1.73 (1.59–1.90)	29	5570 (5202–5937)	
Triglycerides (mmol/L)			0.723
T1: 0.92 (0.83–1.18)	32	5352 (4995–5708)	
T2: 1.65 (1.47–1.78)	29	5330 (4957–5703)	
T3: 3.00 (2.44–3.69)	29	5261 (4898–5625)	
HbA1c (%)			0.541
T1: 5.50 (5.30–5.60)	35	5258 (4918–5598)	
T2: 5.90 (5.80–6.10)	34	5293 (4957–5628)	
T3: 7.10 (6.40–8.40)	21	5441 (5007–5875)	
Creatinine ($\mu\text{mol/L}$)			0.209
T1: 50 (45–53)	30	5587 (5148–6026)	
T2: 61.5 (59–66.5)	32	5246 (4898–5592)	
T3: 78.5 (71–87)	28	5155 (4733–5576)	
Folate (nmol/L)			0.271
T1: 08.73 (07.65–10.75)	30	5227 (4851–5603)	
T2: 14.81 (13.54–15.93)	31	5214 (4854–5574)	
T3: 21.89 (19.44–26.09)	29	5510 (5148–5871)	
Vitamin B6 (nmol/L)			0.777
T1: 18.40 (13.45–22.40)	30	5115 (4733–5496)	
T2: 37.40 (32.15–41.20)	31	5578 (5231–5924)	
T3: 62.10 (55.50–75.40)	29	5223 (4860–5586)	
Vitamin B12 (pmol/L)			0.758
T1: 241.88 (209.06–270.75)	30	5274 (4905–5643)	
T2: 334.69 (311.09–354.52)	31	5460 (5112–5808)	
T3: 438.32 (402.83–552.98)	29	5188 (4802–5575)	

Adjusted for age, gender, smoking status, education, and dialect.

*Values for each biomarker are median levels by tertile. Tertile 1 (T1), tertile 2 (T2), and tertile 3 (T3) refer to tertile values in an increasing order, *i.e.*, T1 < T2 < T3.

Chinese, used a Southern blot-based method for LTL measurement in individuals belonging to an age range that is at risk of developing CVD. Our finding also demonstrated significant negative association between LTL and plasma HCY after adjustment for age, gender, smoking status, education, and dialect. We did not observe any statistically significant association with folate, but the difference in mean LTL between the highest and the lowest tertile level was 283 bp.

Despite the strong epidemiologic evidence linking LTL to CVD risk, the underlying mechanism remains largely unknown. The triad of inflammation, oxidative stress, and

cellular aging has become the holy grail of the cellular basis for CVD development *in vivo*.¹⁰ Given the role of telomeres as a mitotic clock for biological aging *in vitro* and *in vivo*, it has secured an important position as a measurable outcome of the interaction between the three. The association of LTL with inflammation is evidenced by its correlation to inflammatory markers like the C-reactive protein, tumor necrosis factor- α (TNF α), and interleukin-6 (IL-6).^{43,44}

Oxidative stress-induced telomere shortening has been noted in human cultured fibroblasts,⁴⁵ which is further underscored by the association of LTL with dietary anti-oxidants

TABLE 4. MEAN LEUKOCYTE TELOMERE LENGTH (LTL IN BP) BY TERTILE CATEGORIES OF HOMOCYSTEINE AND URATE WITH THEIR MUTUAL ADJUSTMENT

Biomarker	n	Mean LTL ^a (95% CI)	p for trend
Homocysteine (μmol/L)			0.040
T1: 7.43 (6.62–8.21)	30	5539 (5185–5893)	
T2: 9.89 (9.16–10.37)	32	5487 (5141–5832)	
T3: 12.95 (12.14–14.99)	28	4946 (4566–5326)	
Serum urate (μmol/L)			0.172
T1: 251.50 (231–259)	30	5532 (5147–5917)	
T2: 321.00 (299–350)	31	5317 (4973–5660)	
T3: 406.00 (394–442)	29	5147 (4792–5501)	

^aAdjusted for age, gender, smoking status (never vs. former, vs. current), education, dialect, and serum urate for homocysteine, and serum homocysteine for urate.

like vitamins C and E *in vivo*.⁴⁶ Links between telomere dysfunction and vascular senescence were demonstrated in human aortic endothelial cells.⁴⁷ Moreover, telomerase RNA component knockout mice develop cardiomyopathy, heart failure, and cardiomyocyte apoptosis.⁴⁸ In humans, short telomeres were related to atherosclerosis in vascular biopsy tissues and senescent endothelial cells from atherosclerotic plaques. Despite the link between TL and the components of the triad individually, the mechanism by which they all come together in development and progression of CVD remains inconclusive.

Our epidemiological study here may further support the hypothesis that LTL may be involved in mediating the effects of HCY on CVD risk and CVD development. HCY can influence telomeric DNA directly because of its involvement in pathways affecting DNA methylation and nucleotide synthesis.¹⁶ Demethylation of the human telomerase reverse transcriptase gene was found to be associated with shorter LTL in atherosclerosis patients with elevated HCY.⁴⁹ A high level of HCY reflects reduced methylation potential, and in mice models this resulted in increased oxidative stress and compromised endothelial function and thrombogenicity.¹⁶ HCY-induced oxidative stress could probably be another mechanism by which it may affect LTL. Thus, LTL could be the bridge between oxidative stress, inflammation, HCY, and CVD, which warrants further mechanistic investigation. In prospective studies, HCY was found to be associated with CVD events like stroke and mortality. In addition, CVD and CVD risk factors, including HCY and LTL, are independently associated with increased risk of cognitive impairment and Alzheimer's disease; the risk is higher in individuals with subclinical CVD.^{18,19} Like CVD, Alzheimer's disease also has a vascular etiology and is characterized by increased oxidative stress and inflammation. Reduction in HCY upon vitamin B12, vitamin B6, and folate supplementation was associated with a decrease in the rate of brain atrophy in cognitive and clinical decline in mild cognitive impairment patients.⁵⁰ Such nutritional interventions might act as possible alternatives to reduce the risk of CVD in individuals with high HCY levels.

In summary, our finding emphasizes the association between LTL and HCY in CVD risk development because this association was observed in a population-based study with

no obvious CVD events. The age group comprising middle-aged and elderly subjects in the age range of 47–77 is generally considered at risk for CVD. Observation of this association in a healthy population of a similar age range may provide insights into the role of LTL in CVD development. Use of TRF length analysis enables us to correlate LTL in an Asian population and other populations with greater confidence due to low variation in the technique as compared to other methods like quantitative polymerase chain reaction. However, the power of this study is a potential limitation because the direction of association of other CVD risk factors with LTL was observed as expected but lacked statistical significance.

It is reasonable to postulate that in a population without any overt CVD LTL correlates with a relatively novel marker, *i.e.*, HCY, which induces elevated oxidative stress leading to inflammation. Importantly, TL registers the cumulative brunt of both inflammation and oxidative stress during a lifetime, highlighting its major advantage over the other biomarkers currently available that provide only point estimates. In this scenario, LTL can be used to monitor the ramifications of these processes in addition to other biomarkers, thus adding value to LTL as an early complementary biomarker for aging-related diseases like CVD. Furthermore, dietary modulation with respect to folate and vitamin B12 intake can influence HCY levels, suggesting potential intervention strategies for optimal telomere maintenance and CVD prevention.

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Author Disclosure Statement

No competing financial interests exist.

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