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DNA methylation-based telomere length is more strongly associated with long-term all-cause mortality than quantitative polymerase chain reaction-based telomere length among middle-aged and older hypertensive adults

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Abstract

Background Telomere length (TL) has been linked to mortality risk across various populations. However, its predictive value for mortality risk specifically in hypertensive adults remains unclear.

Methods This cohort study utilized data from the 1999–2000 and 2001–2002 cycles of the National Health and Nutrition Examination Survey (NHANES). TL was assessed using DNA methylation (DNAmTL) and quantitative polymerase chain reaction (qPCRTL). Cox proportional hazards models were employed to examine the relationship between TL and mortality risk.

Results This study included 1601 participants, with 988 deaths occurring during a median follow-up of 184 months, including 279 from cardiovascular disease (CVD). Deceased participants exhibited significantly lower levels of DNAmTL (6.45 ± 0.30 vs. 6.70 ± 0.28, $P < 0.001$) and qPCRTL (0.89 ± 0.22 vs. 0.99 ± 0.24, $P < 0.001$) compared to survivors. After full adjustment, each 1-kb decrement in DNAmTL and qPCRTL was associated with a 52% and 38% reduction in all-cause mortality risk, respectively. Participants in the highest TL quartile (Q4) for DNAmTL and qPCRTL had a 36% and 25% reduced risk of all-cause mortality than those in the lowest quartile (Q1), respectively. Receiver operating characteristic (ROC) curves demonstrated that DNAmTL had superior predictive value compared to qPCRTL (area under curve [AUC] 0.73 vs. 0.63, $P < 0.001$).

Conclusion TL is inversely associated with all-cause mortality risk in middle-aged and older hypertensive adults, with DNAmTL showing greater predictive accuracy for long-term mortality than qPCRTL.

Keywords Telomere length, Mortality, Hypertension, DNA methylation, Aging

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Introduction

Telomeres are specialized structures located at the ends of chromosomes, consisting of repetitive TTAGGG sequences and associated proteins [1, 2]. These structures play a crucial role in preserving genomic integrity by preventing the fusion of chromosome ends. As cells divide, telomeres gradually shorten, which serves as a marker of cellular aging [3]. Due to this, telomeres are increasingly recognized as significant biomarkers for aging and related diseases such as cardiovascular disease (CVD), cancer, and diabetes [4, 5].

Hypertension, characterized by consistently elevated levels of blood pressure (BP), is a well-known risk factor for increased mortality. It also contributes to the development of other serious conditions, including stroke, heart failure (HF), and coronary heart disease (CHD) [6, 7]. Previous studies have confirmed a significant association between shorter TL and increased risk of hypertension [8].

Over the past few decades, several methods for measuring TL have been developed, including Southern blot telomere restriction fragment (TRF) analysis [9], fluorescence in situ hybridization (flow FISH) [10], and quantitative polymerase chain reaction (qPCR) [11]. While Southern blot and flow FISH are considered gold standards, they require large quantities of high-quality DNA, specialized skills, and are costly. Currently, qPCR is the most widely used method due to its efficiency and minimal DNA requirements. Recently, a novel method using the methylation patterns of cytosine residues in cytosine-phosphate-guanine dinucleotides (CpGs) to estimate TL (DNAmTL) has shown a stronger association with age and mortality compared to qPCR-based TL measurements (qPCRTL) [12].

Fyhrquist et al. [13] observed that among hypertensive adults with left ventricular hypertrophy (LVH), short telomeres measured by Southern blot showed only a weak correlation with age and an insignificant association with mortality, despite a significant link to cardiovascular disease risk. In this study, we aimed to: (1) investigate the relationship between TL, as estimated by qPCR and DNAm, and long-term mortality among middle-aged and older adults with hypertension; (2) compare the predictive value of DNAmTL and qPCRTL for long-term mortality.

Methods

Study design and population

This cohort study utilized data from the 1999–2000 and 2001–2002 cycles of the National Health and Nutrition Examination Survey (NHANES). NHANES is a nationwide program that employs a multistage, complex sampling design to assess the health and nutritional status of adults and children in the U.S.A. The study protocols were approved by the Research Ethics Review Board of the National Center for Health Statistics (NCHS), and all participants provided written informed consent. Participants were excluded if they had missing data for DNAmTL or qPCRTL, without hypertension, or did not have follow-up information. Hypertension was defined as either a self-reported physician diagnosis, the use of antihypertensive medications, or systolic BP (SBP) $\geq$ 140 mmHg or diastolic blood pressure (DBP) $\geq$ 90 mmHg [14, 15]. CVD was identified based on self-reported diagnoses of HF, CHD, angina pectoris, myocardial infarction, or stroke [16, 17].

DNAmTL measurement

DNA methylation was analyzed in Dr. Yongmei Liu's laboratory at Duke University. Bisulfite conversion was performed on 500 ng of DNA using the Zymo EZ DNA Methylation Kit, following the manufacturer's instructions. The converted DNA was then processed using PCR conditions optimized for Illumina's Infinium Methylation assay. Methylation profiles were generated using the Illumina Infinium MethylationEPIC BeadChip. The bisulfite-treated DNA was hybridized to the BeadChip, and post-hybridization, the samples underwent denaturation, amplification, fragmentation, precipitation, and resuspension, followed by another round of hybridization. The BeadChip was then washed, labeled for primer extension, and scanned using the Illumina iScan system. DNAmTL was calculated using an elastic net regression model based on the DNA methylation data [12].

qPCRTL measurement

qPCRTL measurements were conducted in Dr. Elizabeth Blackburn's laboratory at the University of California, San Francisco. Telomere length was normalized to a standard reference DNA and expressed as the T/S ratio. Each sample was analyzed in triplicate on separate days, resulting in six data points per sample. Assay plates were processed in batches, with control wells included on each plate. Assays with eight or more invalid control wells were excluded from analysis, affecting less than 1% of the runs. Inter-run variability was controlled using calibration values from control DNA, with exclusions made for runs where control DNA values deviated by more than 2.5 standard deviations from the mean, impacting less than 6% of runs. Outliers, constituting less than 2% of samples, were also excluded. The mean and standard deviation of the T/S ratio were calculated, with an inter-assay coefficient of variation of 6.5%.

Mortality ascertainment

Mortality data were obtained by linking NHANES records to the National Death Index (NDI) through December 31, 2019. Cause of death was classified using the International Statistical Classification of Diseases, 10th Revision (ICD-10).

Statistical analysis

Sampling weights were applied to account for the NHANES complex sampling design. Participants were divided into quartiles based on their DNAmTL and qPCRTL levels. Continuous variables were presented as mean ± standard deviation (SD) or median (interquartile range) and were analyzed using weighted *t*-tests or Wilcoxon rank-sum tests, depending on their distribution. Categorical variables were reported as frequencies (percentages) and analyzed using weighted Chi-square tests. Pearson correlation was used to examine the relationships between DNAmTL, qPCRTL, and age.

Kaplan–Meier curves were used to compare mortality rates across DNAmTL and qPCRTL quartiles. The association between TL and mortality risk was assessed using three weighted Cox proportional hazards models: Model I adjusted for age and gender; Model II further adjusted for smoking status, alcohol consumption, educational attainment, poverty income ratio (PIR), and body mass index (BMI); and Model III additionally adjusted for CVD, diabetes, total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), uric acid (UA), and estimated glomerular filtration rate (eGFR). Restricted cubic spline (RCS) curves were employed to assess potential nonlinear dose–response relationships between DNAmTL, qPCRTL, and mortality risk. The predictive performance of DNAmTL and qPCRTL for mortality was evaluated using receiver operating characteristic (ROC) curves, with the area under the curve (AUC) tested by the DeLong test. Subgroup and sensitivity analyses were performed to test the robustness of the associations across various populations. Statistical analyses were conducted using R software (version 4.0.3), with significance set at a two-sided P value < 0.05.

Results

Baseline characteristics of participants

A total of 1601 participants were enrolled in this study, with a mean age of 68.02 ± 9.85 years, and 761 (47.53%) were male (Fig. 1). Over a median follow-up period of 184 months, 988 participants died, including 279 due to CVD. The baseline characteristics are presented in Table 1. Compared to survivors, those who died were generally older, more likely to be male, predominantly white, and had higher smoking rates. They also had lower levels of educational attainment, reduced levels of TC, and eGFR, but higher levels of UA. Furthermore, there was a higher prevalence of diabetes and CVD among decedents. TL, as assessed by DNAmTL (6.45 ± 0.30 vs. 6.70 ± 0.28, $P < 0.001$) and qPCRTL (0.89 ± 0.22 vs. 0.99 ± 0.24, $P < 0.001$), was significantly shorter in those who died compared to those survivors (Supplementary file 1: Fig. S1). Additionally, DNAmTL exhibited a stronger correlation with age than qPCRTL ($r = -0.59$ vs. $r = -0.27$) (Supplementary file 1: Fig. S2).

Association between TL and mortality in hypertensive adults

Weighted Cox regression models, detailed in Table 2, identified a significant association between shorter TL and an increased risk of all-cause mortality. Specifically,

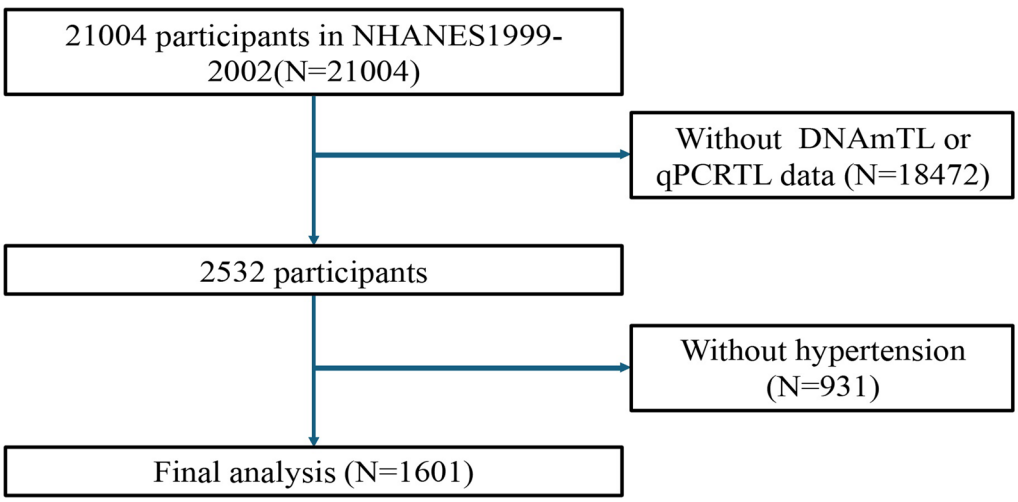

Figure 1 Flow-chart of the participants selection

Table 1 Baseline characteristics of participants

Variables	Overall (*N* = 1601)	Died (*N* = 988)	Alive (*N* = 613)	*P* value
Age (years)	68.02 ± 9.85	71.87 ± 9.21	61.80 ± 7.34	< 0.001
Male, *n* (%)	761 (47.53)	512 (51.82)	249 (40.62)	< 0.001
BMI, kg/m^2	29.28 ± 5.92	28.86 ± 6.06	29.91 ± 5.64	< 0.001
Race//ethnicity, *n* (%)				< 0.001
Mexican American	424 (26.48)	249 (25.20)	175 (28.55)	
Hispanic	96 (6.00)	47 (4.76)	49 (7.99)	
White	627 (39.16)	426 (43.12)	201 (32.79)	
Black	410 (25.61)	249 (25.20)	161 (26.26)	
Others	44 (2.75)	17 (1.72)	27 (4.40)	
Education levels, *n* (%)				< 0.001
Less than high school	779 (48.66)	527 (53.34)	252 (41.11)	
High school or equivalent	338 (21.11)	207 (20.95)	131 (21.37)	
Above high school	484 (30.23)	254 (25.71)	230 (37.52)	
PIR	2.46 ± 1.55	2.22 ± 1.44	2.84 ± 1.64	
Smoking status, *n* (%)				0.005
Never	765 (47.78)	443 (44.84)	322 (52.53)	
Current	212 (13.24)	146 (14.78)	66 (10.77)	
Former	624 (38.98)	399 (40.38)	225 (36.70)	
Alcohol consumption, n (%)	903 (56.40)	546 (55.26)	357 (58.24)	0.243
Diabetes, (%)	451 (28.17)	311 (31.48)	140 (22.84)	< 0.001
CVD, *n* (%)	395 (24.67)	309 (31.28)	86 (14.03)	< 0.001
TC, mmol/L	5.31 ± 1.06	5.27 ± 1.08	5.38 ± 1.03	0.039
HDL, mmol/L	1.34 ± 0.40	1.33 ± 0.41	1.35 ± 0.38	0.371
TG, mmol/L	1.48 (1.06, 2.16)	1.47 (1.05, 2.22)	1.50 (1.07, 2.08)	0.875
eGFR, ml/min/1.73 m^2	81.68 ± 22.57	76.45 ± 23.67	90.12 ± 17.69	< 0.001
UA, umol/L	344.44 ± 96.12	352.17 ± 99.53	331.97 ± 89.02	< 0.001

CVD cardiovascular disease, *BMI* body mass index, *PIR* poverty income ratio, *TC* total cholesterol, *HDL* high-density lipoprotein, *TG* triglyceride, *eGFR* estimated glomerular filtration rate, *PIR* poverty income ratio, *UA* uric acid

each 1-kilobase (kb) decrement in DNAmTL and qPCRTL corresponded to a 52% (hazard ratio (HR) 0.48, 95% confidence interval (CI) 0.32–0.74, $P = 0.001$) and 38% (HR 0.62, 95% CI 0.39–0.99, $P = 0.047$) reduction in all-cause mortality risk, respectively. Participants in the highest quartile (Q4) of TL, as measured by DNAmTL and qPCRTL, experienced a 36% (HR 0.64, 95% CI 0.45–0.91, $P = 0.013$) and 25% (HR 0.75, 95% CI 0.58–0.97, $P = 0.031$) reduction in the risk of all-cause mortality than those in the lowest quartile (Q1), respectively. Kaplan–Meier curves indicated a significantly higher mortality rate with increasing TL for both DNAmTL and qPCRTL (Fig. 2). However, no significant association was found between TL and CVD mortality (Supplementary file 1: Table S1). Restricted cubic spline (RCS) curves suggested a linear dose–response relationship between DNAmTL (*P* for nonlinearity: 0.422) and qPCRTL (*P* for nonlinearity: 0.841) with all-cause mortality risk among hypertensive adults (Fig. 3). Receiver operating characteristic (ROC) curve analysis revealed that DNAmTL had a superior predictive value for long-term all-cause mortality compared to qPCRTL (AUC 0.73 vs. 0.63, $P < 0.001$) (Fig. 4).

Subgroup and sensitive analysis

Subgroup analyses were performed to assess the robustness of the association between TL and all-cause mortality, stratified by age, gender, smoking status, alcohol consumption, obesity (BMI ≥ 30 kg/m^2), and the presence of CVD and diabetes. The effect of TL on mortality was consistent across these subgroups (Supplementary file 1: Table S2, all *P* for interaction > 0.05). Sensitivity analyses, which excluded participants who died within the first two years of follow-up, corroborated the consistent association between both DNAmTL and qPCRTL with all-cause mortality among hypertensive adults (Supplementary file 1: Table S3).

Table 2 Association between the TL and all-cause mortality in patients with hypertension

	Model I	Model II	Model III
DNAmTL			
Continuous			
Per 1 kb increment	0.40 (0.29–0.55) < 0.001	0.47 (0.31–0.70) < 0.001	0.48 (0.32–0.74) 0.001
Categorical			
Q1	Reference	Reference	Reference
Q2	0.77 (0.63–0.96) 0.018	0.79 (0.62–1.00) 0.051	0.75 (0.58–0.97) 0.028
Q3	0.72 (0.56–0.93) 0.012	0.83 (0.62–1.11) 0.213	0.81 (0.61–1.08) 0.157
Q4	0.53 (0.38–0.73) < 0.001	0.66 (0.46–0.94) 0.021	0.64 (0.45–0.91) 0.013
P for trend	< 0.001	0.032	0.022
qPCRTL			
Continuous			
Per 1 kb increment	0.58 (0.38–0.90) 0.016	0.60 (0.37–0.95) 0.031	0.62 (0.39–0.99) 0.047
Categorical			
Q1	Reference	Reference	Reference
Q2	1.00 (0.80–1.25) 0.990	0.93 (0.73–1.17) 0.523	0.94 (0.74–1.19) 0.586
Q3	0.82 (0.64–1.04) 0.108	0.82 (0.63–1.06) 0.133	0.80 (0.61–1.05) 0.109
Q4	0.75 (0.59–0.95) 0.018	0.74 (0.58–0.96) 0.024	0.75 (0.58–0.97) 0.031
P for trend	0.006	0.015	0.016

Data were shown as HR, 95% CI and *P* value

Model I was adjusted for age and gender, Model II was adjusted for age, gender, race, education levels, smoking status, alcohol consumption, BMI and PIR, Model IIII was further adjusted for age, gender, race, education levels, smoking status, alcohol consumption, BMI, PIR, TC, TG, HDL, eGFR, UA, CVD and diabetes

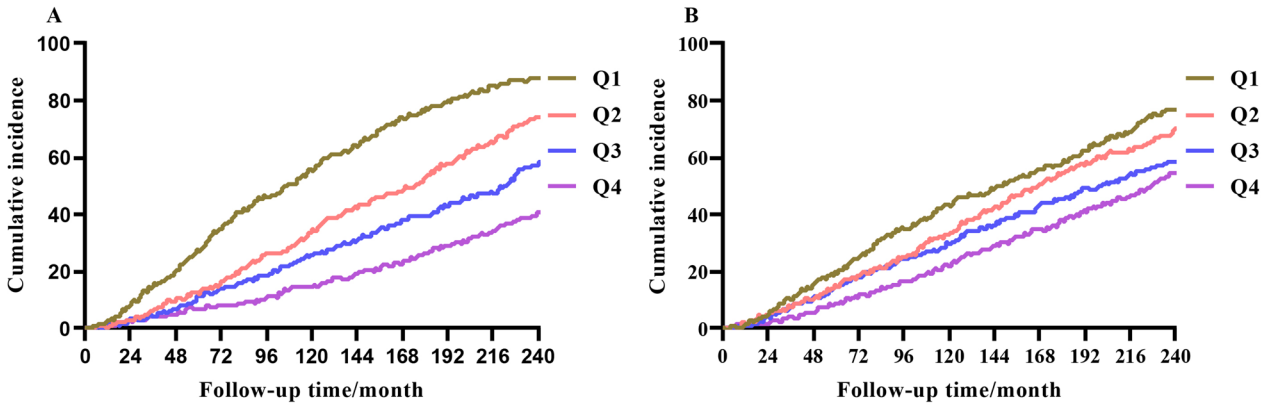

Figure 2 Cumulative incidence of death among groups of DNAmTL (**A**) and qPCRTL (**B**)

Discussion

In this study, we investigated the association between TL and mortality among middle-aged and older adults with hypertension. Our main findings include: (1) TLs, measured by both DNAm and qPCR, were significantly shorter in died participants compared to survivors. (2) Shorter TLs were associated with an increased risk of all-cause mortality. (3) DNAmTL exhibited a stronger association with age and superior predictive value for all-cause mortality compared to qPCRTL.

TL and all-cause mortality

Numerous studies, with few exceptions [18], have established a significant inverse association between TL and all-cause mortality across diverse populations [19, 20]. Rode et al. [21] used data from two large Danish cohorts, encompassing over 64,000 individuals, and found that those with the shortest TLs had a 1.4-fold increased risk of all-cause mortality compared to those in the longest decile. A meta-analysis of 25 studies further indicated that each SD decrease in leukocyte TL was associated

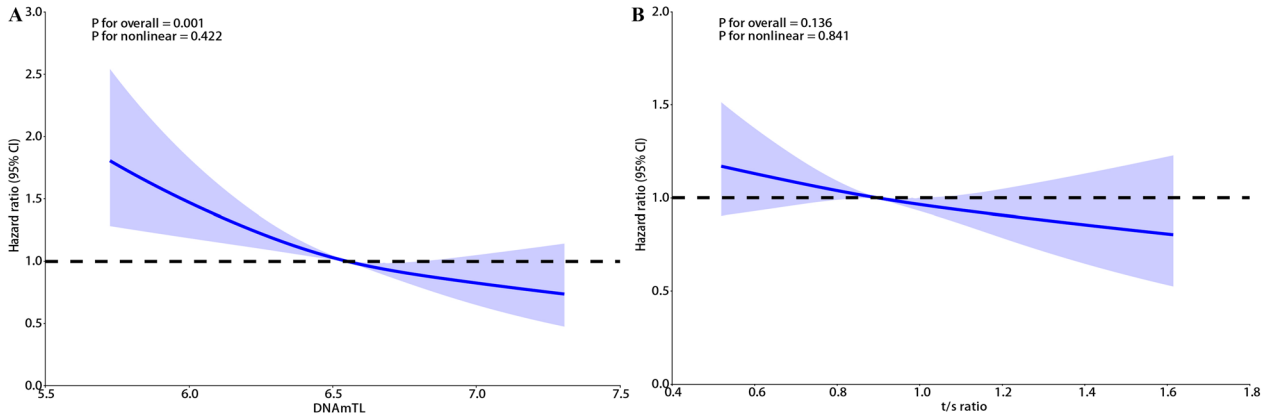

Figure 3 Nonlinear dose–response association between DNAmTL (**A**) and qPCRTL **B** with all-cause moratality

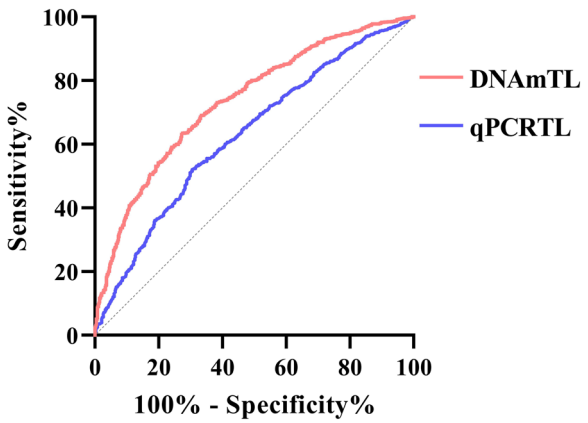

Figure 4 ROC curve analysis for the predictive value of DNAmTL and qPCRTL to all-cause moratality

with a 13% increase in all-cause mortality risk, with individuals in the lowest TL quartile having a 44% higher hazard compared to those in the highest quartile [22]. Additionally, Parving et al. [23] found that patients with type-1 diabetes in the third tertile of TL had an 87% increased risk of all-cause mortality relative to those in the first tertile.

However, it is noteworthy that these studies primarily used traditional methods for measuring TL of Southern blotting. Recently, a novel method based on DNA methylation has been shown to correlate more strongly with age and time-to death compared to qPCRTL [12]. A recent cohort study demonstrated a negative association between DNAmTL and all-cause mortality in patients with HIV infection [24]. Fyhrquist et al. demonstrated an insignificant association between leukocyte TL measured by Southern blot and all-cause mortality in hypertensive patients with LVH. Our study revealed a significant negative association between TL—whether measured by DNAm or qPCR—and long-term all-cause mortality in middle-aged and older hypertensive adults. Furthermore, DNAmTL was found to have a stronger correlation with age and superior predictive value for all-cause mortality compared to qPCRTL. The discrepancy between our findings and the previous study might be due to different methods of TL assessment. We used qPCR and DMAm methods, whereas the earlier study used Southern blotting. Additionally, our study had a longer follow-up period and higher mortality rate (61.71% vs. 3.78%), which could have contributed to the more significant findings.

In this study, subgroup analysis stratified by age revealed a significant association between DNAmTL and long-term all-cause mortality in both age groups (≥ 65 and < 65 years). This finding aligned with our previous study, which investigated the association between DNAmTL and all-cause mortality in patients with CVD [25]. However, in this study, a significant association between qPCRTL and all-cause mortality was only observed in adults aged 65 years and older, not in those younger than 65 years. This is consistent with prior studies suggesting that qPCRTL may be less sensitive in younger populations. For instance, Li et al. [26] reported that shorter qPCRTL was significantly linked to all-cause mortality in elderly patients with diabetes, but not in younger individuals. Similarly, a recent study indicated that shorter qPCRTL was associated with an increased risk of all-cause mortality only in older adults (> 65 years) with alcohol-associated liver disease [27]. Our findings, combined with these previous studies, suggest that the predictive value of qPCRTL for mortality risk becomes more pronounced with advancing age, possibly due to the cumulative effects of aging on telomere dynamics. This may explain why the association was observed only in those aged ≥ 65 years in our study.

TL and CVD mortality

The association between TL and CVD mortality remains inconclusive. For instance, a cohort study of 4926 patients with moderate chronic kidney disease (CKD) found that each 0.1 unit decrease in relative TL was associated with a 20% increased risk of CVD mortality [28]. Similarly, a large-scale study involving 472,432 participants from the UK Biobank reported that reduced TL was linked to increased CVD mortality. Conversely, other studies have reported mixed results. Chen et al. found that while shorter TLs were significantly associated with increased all-cause mortality, this association did not extend to CVD mortality among individuals with diabetes [26]. Yeap et al. found no association between TL and CVD mortality in a community-based study of men [29]. Similarly, an earlier study involving elderly hypertensive adults with LVH indicated no significant association between TL, measured by Southern blotting, and CVD mortality [13]. In our study, we found no significant association between qPCRTL and CVD mortality among middle-aged and older hypertensive adults, aligning with several previous reports. Additionally, for the first time, we confirmed that DNAmTL also does not correlate with CVD mortality in this population.

TL, hypertension and mortality

Previous research has shown that TL is inversely correlated with SBP in both healthy and hypertensive adults [30]. Elevated SBP has also been associated with increased telomerase activity and reduced TL in peripheral blood leukocytes [31]. Telomerase, functioning as a reverse transcriptase, generates telomeric repeats using a template provided by TERC. For example, Diego et al. demonstrated that elevated plasma endothelin-1 (ET-1) led to hypertension in mice with dysfunctional telomerase activity, a result of ECE-1 overexpression [32]. Additionally, TL was notably shorter in female hypertensive patients with coronary plaques compared to those without plaques, and multivariate analysis identified TL as a significant predictor of coronary artery plaque presence, linking TL to coronary atherosclerosis in hypertensive individuals [33]. Our study is the first to demonstrate that TL, assessed through qPCR or DNA methylation, is significantly associated with an increased risk of all-cause mortality in hypertensive adults. However, no significant association was found between TL and CVD mortality. This lack of association could be due to several factors: (1) The hypertensive nature of the study population may influence the relationship between TL and cardiovascular outcomes, with comorbid conditions potentially obscuring the specific impact of TL on CVD mortality. (2) CVD mortality encompasses a variety of conditions, such as CHD, HF, and stroke, each of which may differently impact the relationship with TL.

This study has several limitations that should be acknowledged. Firstly, due to its observational design, we cannot draw causal inferences between TL and mortality. Secondly, the study cohort was limited to American participants, and further research is needed to evaluate these associations in more diverse populations. Additionally, in this study, TL, as estimated by DNA methylation, may not directly reflect absolute TL. However, as an innovative method for measuring TL, DNAmTL offers greater insight into the epigenetic modifications associated with telomere biology. Moreover, it may capture broader aspects of telomere maintenance mechanisms, going beyond the mere measurement of length itself. Lastly, while we adjusted for potential confounding factors, there may still be unaccounted variables, such as environmental factors, that could influence the results.

Conclusions

TLs, either measured by DNAm or qPCR, were negatively associated with all-cause mortality risk in middle-aged and older hypertensive patients. DNAmTL was superior in predicting long-term all-cause mortality than qPCRTL.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13148-025-01833-z.

Supplementary Material 1

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Author contributions

WQ. designed the study and wrote the original manuscript. GY., DLR., and SJ performed the data analysis. DH collected the data. YX., TB., and ZX. reviewed the manuscript.

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Data availability

The datasets used and analyzed in this study are available from the corresponding author for reasonable use.

Declarations

Ethics approval and consent to participate

The study protocol of NHANES was approved by the National Center for Health Statistics of the Center for Disease Control and Prevention Institutional Review Board. Informed consent was obtained from all participants included in the study.

Conflict of interest
The authors declare no competing interests.

Consent for publication
Not applicable.

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