Telomeric G-Tail Length and Hospitalization for Cardiovascular Events in Hemodialysis Patients

Shuma Hirashio,* Ayumu Nakashima,* Shigeaio Doi,* Kuniko Anno,† Eiko Aoki,‡ Akira Shimamoto,§ Noriaki Yorioka,‡ Nobuoki Kohno,∗ Takao Masaki,* and Hidetoshi Tahara†

Abstract

**Background and objectives** Telomeric G-tails play a pivotal role in maintaining the intramolecular loop structure of telomeres. Previous *in vitro* studies have suggested that the erosion of telomeric G-tails triggers cellular senescence, leading to organ dysfunction and atherosclerosis. The authors recently established a method to measure telomeric G-tail length using a hybridization protection assay. Using this method, this study investigated whether telomeric G-tail length could be used as a novel predictor for future cardiovascular events in hemodialysis patients.

**Design, setting, participants, & measurements** A prospective observational study was performed involving a cohort of 203 Japanese hemodialysis patients to examine the lengths of telomeric G-tails and total telomeres and subsequent cardiovascular events during a median follow-up period of 48 months. The lengths of telomeric G-tails and total telomeres were also measured in 203 participants who did not have CKD and who were age- and sex-matched to hemodialysis patients.

**Results** The lengths of telomeric G-tails and total telomeres were significantly shorter in hemodialysis patients than in control subjects. Telomeric G-tails, but not total telomeres, were independently and negatively associated with clinical history of cardiovascular disease. During follow-up, 80 cardiovascular events occurred. Total telomere length did not predict cardiovascular events. However, the length of telomeric G-tails was associated with new-onset cardiovascular events (hazard ratio per log luminescence signals, 0.12; 95% confidence interval, 0.12 to 0.50) that persisted after adjustment for age, sex, diabetes mellitus, clinical history of cardiovascular disease, inflammation, use of vitamin D, and serum levels of phosphate and intact parathyroid hormone.

**Conclusions** Longer telomeric G-tail length is associated with a lower risk of future cardiovascular events in hemodialysis patients.


Introduction

Human telomeres contain 10–15 kilobase pairs of the repeating telomeric DNA sequence, 5'-TTAGGG-3', followed by 75–300 bases of a G-rich single-stranded 3' overhang, the so-called G-tail (1). The length of telomeric G-tails plays a pivotal role in maintaining the intramolecular loop structure of the telomere, a structure that is also known as the “t loop” (2). The collapse of the t-loop structure leads to telomere dysfunction and is accompanied by end-to-end fusion of chromosomes. Consequently, this structure is considered to be essential for the maintenance of chromosome terminal structure and function and its assembly into stable protein-telomeric DNA complexes (3). In fact, previous reports have shown that telomeric G-tail erosion, rather than total telomere length, serves as a trigger of cellular senescence and eventually leads to loss of cellular viability *in vitro* (4). We reported that telomeric G-tails in human umbilical vein endothelial cells gradually shortened with cell division and that introduction of the human telomerase reverse transcription (*TERT*) gene rejuvenated cellular functions (5).

Clinical studies have shown that total telomere shortening is associated with increasing chronological age (6,7), progression of arteriosclerosis (8,9), and CKD (10–12). Patients with CKD have a markedly increased risk for cardiovascular disease (CVD) (13) and total telomere shortening is associated with progression of CVD (14). We thus examined whether telomeric G-tail length could be a sensitive marker for CVD in hemodialysis patients. Until recently, no reliable method for the measurement of telomeric G-tail length in clinical samples has been available. However, we recently established a technique to measure telomeric G-tail length using a hybridization protection assay (HPA) (15). In this study, our new method enabled us to measure a large number of clinical samples accurately and with high sensitivity.

Materials and Methods

Participants

We enrolled 203 outpatients receiving maintenance hemodialysis at Hiroshima University Hospital (Hiroshima, Japan), Hakuai Clinic (Kure, Japan), Onomichi Clinic (Onomichi, Japan), and Motomiya Clinic (Motomiya, Japan).
(Onomichi, Japan), East Clinic (Hiroshima, Japan), and Chuonaika Clinic (Kure, Japan). These patients received di-
alysis three times a week. Patients with a history of malignant
tumor, currently receiving treatment for a malignant tumor,
currently receiving steroid therapy, or who were infected at
the time of blood sampling were excluded from this study. The
clinical history of CVD was also checked by review of medical
records at the time of initiating this study. The clinical history
of CVD was defined as a previous history of angina pectoris,
myocardial infarction, treatment of percutaneous coronary
intervention, aortic disease (acute aortic dissection, thoracic
aortic aneurysm, or abdominal aortic aneurysm), cerebral
hemorrhage, cerebral infarction, or arteriosclerosis obliterans
(lower limb amputation, extremity gangrene, or treatment of
extremity percutaneous transluminal angioplasty). Afterward,
patients were prospectively followed for assessment of a new-
onset cardiovascular event in relation to telomeric G-tail length
or total telomere length. A new-onset cardiovascular event
was defined as angina pectoris, myocardial infarction, treat-
ment of percutaneous coronary intervention, aortic disease
(acute aortic dissection, thoracic aortic aneurysm, or abdomi-
nal aortic aneurysm), cerebral hemorrhage, cerebral infarction,
or arteriosclerosis obliterans (lower limb amputation, extremity
gangrene, or treatment of extremity percutaneous transluminal
angioplasty during the observational period). These new-onset cardiovascular events were also checked
using medical records and, in selected cases, diagnosis by
specialists. To assemble control participants for this study,
blood samples were obtained from a total of 493 volunteers,
417 of whom were enrolled as participants without CKD
based on completion of a medical questionnaire. From that
group, 203 samples were assessed for telomeric G-tail and total
telomeres as control participants. We selected control partic-
ipants who were matched with hemodialysis patients for age
and sex. The Ethics Committee of our hospitals approved the
study protocol (Analysis of Telomere Instability Mechanism in
Dialysis Therapy, approval number Hi-129, registered April 2,
2008) and written informed consent was obtained from each
patient. This study was conducted in accordance with the
Declaration of Helsinki.

Procedures

After PBMCs were isolated from each whole blood sample,
DNA was extracted by the modified phenol-chloroform
method within 24 hours. Telomeric G-tails and total telo-
meres were measured by HPA (16). The research staff who
measured telomeric G-tails and total telomeres did not know
the details regarding clinical data or outcome in this study.

Briefly, the extracted DNA was adjusted to 100 μg/ml
using a Nanodrop 2000 spectrophotometer (Thermo Fisher
Scientific K.K., Yokohama, Japan), and was subsequently dis-
persed into three wells of a 96-well plate. The sample plates
were set in a JANUS automated workstation (PerkinElmer
Japan Co Ltd, Yokohama, Japan) and the HPA reaction was
performed. First, a diluted telomere HPA probe labeled with
acridinium ester (AE; Fujirebio Inc, Tokyo, Japan) was added
to each well, and incubated at 60°C for 20 minutes (hybrid-
ization step). Hydrolyzation buffer was then added to each
well, and samples were incubated at 60°C for 10 minutes
(hydrolyzation step). The plate was then set on an EnVision
multilabel reader (PerkinElmer Japan Co Ltd) to measure the
luminescence of AE. This assay additionally served as a pos-
tive control for each HPA using a synthetic 35-mer single-
stranded DNA composed of a repeating 5'-TTAGGG)n-3'
sequence. We measured telomeric G-tail length using 1 μg
of purified nonadenatured genomic DNA, and total telomere
length using 0.2 μg of heat-denatured genomic DNA. Unlike
conventional methods such as Southern blotting, telomeric
G-tails, and total telomeres are represented as luminescence
signals (in relative light units [rlu]) in this technique. We used
control genomic DNA isolated from the Henrietta Lacks
(HeLa) cancer cell line and control telomere oligonucleotides
to normalize the luminescence. To confirm the reliability
of HPA, two independent experiments were performed in trip-
licate. We determined that the average coefficient of variations
(CVs) were 4.1% and 4.3% for the telomeric G-tail and total
telomeres, respectively.

Laboratory Analyses

Blood samples were collected from hemodialysis patients
before the first session of dialysis in a given week. Study
parameters were derived from the periodic blood test data
collected at the facilities for maintenance dialysis for individual
patients. We averaged three consecutive readings during the
2-month period, measuring five parameters: hemoglobin and
serum levels of albumin, calcium, phosphate, and C-reactive
protein. The adjusted calcium level was calculated by Payne's
equation (17). Serum intact parathyroid hormone (PTH) level
was measured by electrochemiluminescence immunoassay
with the use of the ECLusys reagent PTH (Roche Diagnostics
K.K., Tokyo, Japan). Serum β2 microglobulin levels are shown
as the average of two consecutive readings during the 3
months before sampling. Kt/V was calculated with the follow-
ing single-pool equation: (Kt/V sp=−Ln(Ce/Cs−0.008×td)+(4.35×Ce/Cs)×ΔBW/BW), where Ce is prehemodialysis
urea nitrogen, Cs is posthemodialysis urea nitrogen, ΔBW
is weight loss during dialysis, BW is body weight after
a session of dialysis, and td is dialysis time (18).

Statistical Analyses

All variables were expressed as the mean±SD or the median
and interquartile range (25th–75th percentiles), unless other-
wise indicated. Comparisons between two groups were as-
sessed with the Wilcoxon signed-rank test or chi-squared
test. Spearman’s rank correlation analysis was used to de-
termine possible associations between the lengths of telo-
meric G-tails or total telomeres with selected parameters.
Multivariate regression analyses were used to assess in-
dependent predictors of telomeric G-tails and total telomeres,
whereas logistic regression approaches were used to assess
determinants of existing CVD. Analyses of subsequent car-
diovascular events were made with the Cox proportional
hazard model. The univariate and multivariate Cox regres-
sion analyses are presented as hazard ratios (HRs) and 95%
confidence intervals (95% CIs). Statistical significance was set
at a level of P<0.05. All analyses were carried out with SPSS
software (version 21.0; IBM, Armonk, NY).

Results

The clinical characteristics of hemodialysis patients are
shown in Table 1. In the patient group, the mean age was
62.7±10.1 years and dialysis duration was 79 (31–138)
months. Diabetes mellitus was present in 86 patients (42.4%), and 88 patients (43.3%) had a history of CVD. Classification of the clinical history of CVD is summarized in Supplemental Table 1. These data were similar to those of general Japanese hemodialysis patients (19). Control subjects included 104 men (51.7%) and the mean age was 62.6±6.2 years. Age and sex did not differ between hemodialysis patients and control participants.

The luminescence signals showed that telomeric G-tails were significantly shorter in length in hemodialysis patients than in control participants (Table 1). Similarly, total telomeres were also significantly shorter in hemodialysis patients than in control participants (Table 1). We previously found that there was a positive correlation between the lengths of telomeric G-tails and total telomeres in vitro (5). In this study, we examined the correlation between telomeric G-tail length and total telomere length in control participants and hemodialysis patients. As expected, a strong correlation was observed between telomeric G-tail length and total telomere length in control participants and hemodialysis patients (ρ=0.51, P<0.001) and hemodialysis patients (ρ=0.48, P<0.001).

In univariate analysis (Table 1), there was a positive correlation between telomeric G-tail length and serum phosphate level and a negative correlation between telomeric G-tail length and clinical history of CVD. In addition, total telomere lengths were positively associated with vitamin D use and serum phosphate level, whereas they were negatively associated with age and clinical history of CVD. Next, we performed a multivariate regression analysis of factors associated with the lengths of telomeric G-tails and total telomeres. Table 2 shows the results of multivariate regression analysis of factors predicting telomeric G-tail and total telomere lengths. Specifically, a clinical history of CVD and serum phosphate levels were independently associated with telomeric G-tail length, whereas age and serum phosphate level were independently associated with total telomere length. We also performed an analysis that excluded hemodialysis patients with a history of CVD at baseline (Supplemental Table 2). In this analysis, only serum phosphate levels were independently associated with telomeric G-tail.

Patients with a clinical history of CVD had shorter telomeric G-tail lengths than those without a clinical history of CVD (21,669 rlu [19,162–23,910] for patients with CVD versus 23,078 rlu [20,182–25,972] for patients without CVD; P=0.01). In a logistic regression model, the association remained and was independent of age, sex, diabetes mellitus, serum phosphate level, and inflammation (odds ratios [OR], 0.12 per log rlu; 95% CI, 0.01 to 0.90; P=0.04) (see Telomeric G-tail in Table 3). Although patients with a clinical history of CVD had shorter total telomere lengths than those without a clinical history of CVD (218,939 rlu [201,131–242,396] for patients with CVD versus 228,917 rlu [210,971–265,989] for patients without CVD; P=0.01), the association disappeared after adjustments for age, sex, diabetes mellitus, serum phosphate level, and inflammation (see Total telomere in Table 3). See Total telomere, per log rlu in table 3 shows the control of these models (without telomeric G-tail lengths and total telomere lengths).

Cardiovascular events were assessed after a median follow-up period of 48 months (range, 3–57; interquartile

<table>
<thead>
<tr>
<th>Table 1. General characteristics of hemodialysis patients and healthy controls, and univariate associations with telomeric G-tail and total telomere lengths in patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Telomeric G-tail, rlu</td>
</tr>
<tr>
<td>Total telomere, rlu</td>
</tr>
<tr>
<td>Age, yr</td>
</tr>
<tr>
<td>Men</td>
</tr>
<tr>
<td>Dialysis duration, mo</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>History of CVD</td>
</tr>
<tr>
<td>Vitamin D use</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
</tr>
<tr>
<td>Albumin, g/dl</td>
</tr>
<tr>
<td>Adjusted calcium, mg/dl</td>
</tr>
<tr>
<td>Phosphate, mg/dl</td>
</tr>
<tr>
<td>Intact PTH, pg/ml</td>
</tr>
<tr>
<td>β2 microglobulin, mg/L</td>
</tr>
<tr>
<td>C-reactive protein, mg/dl</td>
</tr>
<tr>
<td>Kt/V</td>
</tr>
</tbody>
</table>

Data are means±SD or median (interquartile range) for continuous variables and n (%) for categorical variables. Univariate correlations were assessed by Spearman’s rank correlation analysis. rlu, relative light unit; CVD, cardiovascular disease; PTH, parathyroid hormone.

<sup>a</sup>Significantly different (P<0.05) from healthy individuals as assessed by Wilcoxon signed-rank test or chi-squared test.

<sup>b</sup>P<0.01.

<sup>c</sup>P<0.05.
range, 23–55). During the follow-up period, 80 patients (39.4%) were hospitalized with a new-onset cardiovascular event. Causes of new-onset cardiovascular events are summarized in Supplemental Table 1. Cox proportional hazard crude analyses showed that patients with shorter telomeric G-tail lengths had an increase of new-onset cardiovascular events (HR, 0.12 per log rlu; 95% CI, 0.03 to 0.50; \( P = 0.004 \)). This difference persisted after adjustment for age, sex, diabetes mellitus, history of CVD, inflammation, vitamin D use, and serum levels of phosphate and intact PTH (see Telo-meric G-tail in Table 4). By contrast, total telomere length was not associated with future cardiovascular events (see Total telomeres in Table 4). After multivariate adjustment, HRs were not statistically significant.

### Discussion

In this study, we report for the first time that telomeric G-tail lengths were shortened in hemodialysis patients. The reduction of telomeric G-tail length was associated with future cardiovascular events. The association was independent of age, sex, diabetes mellitus, baseline CVD, vitamin D use, inflammation, and serum levels of phosphate and intact PTH. Our findings indicate that telomeric G-tail length but not total telomere length could be a novel predictor for future cardiovascular events in hemodialysis patients.

Oxidative stress and chronic inflammation are well known risk factors of CVD and play major roles in total telomere shortening (9,20). A previous study demonstrated that patients with CVD had shortening of total telomere length

### Table 2. Multivariate regression model predicting telomeric G-tail and total telomere length in 203 hemodialysis patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>( \beta )</th>
<th>( P ) Value</th>
<th>Adjusted ( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Telomeric G-tail length</strong></td>
<td></td>
<td></td>
<td>0.08</td>
</tr>
<tr>
<td>Age, per 1 yr</td>
<td>−0.03</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>−0.10</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus, presence</td>
<td>0.03</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>Clinical history of CVD, presence</td>
<td>−0.16</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Phosphate, per 1 mg/dl</td>
<td>0.16</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Inflammation, presence</td>
<td>0.05</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td><strong>Total telomere length</strong></td>
<td></td>
<td></td>
<td>0.15</td>
</tr>
<tr>
<td>Age, per 1 yr</td>
<td>−0.25</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>−0.08</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus, presence</td>
<td>0.02</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>Clinical history of CVD, presence</td>
<td>−0.05</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Phosphate, per 1 mg/dl</td>
<td>0.23</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Inflammation, presence</td>
<td>0.02</td>
<td>0.73</td>
<td></td>
</tr>
</tbody>
</table>

\( \beta \) shows standard regression coefficient. Inflammation was defined as C-reactive protein \( > 0.5 \) mg/dl.

### Table 3. ORs and 95% CI for factors predicting the clinical history of CVD in 203 hemodialysis patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Odds Ratio (95% CI)</th>
<th>( P ) Value</th>
<th>Adjusted ( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Telomeric G-tail</strong></td>
<td></td>
<td></td>
<td>0.16</td>
</tr>
<tr>
<td>Age, per 1 yr</td>
<td>1.10 (1.06 to 1.14)</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>1.14 (0.59 to 2.20)</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus, presence</td>
<td>1.80 (0.93 to 3.52)</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Phosphate, per 1 mg/dl</td>
<td>1.06 (0.83 to 1.35)</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>Inflammation, presence</td>
<td>0.76 (0.31 to 1.86)</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td>Telomeric G-tail, per log rlu</td>
<td>0.12 (0.01 to 0.90)</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td><strong>Total telomere</strong></td>
<td></td>
<td></td>
<td>0.15</td>
</tr>
<tr>
<td>Age, per 1 yr</td>
<td>1.10 (1.06 to 1.14)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>1.18 (0.62 to 2.25)</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus, presence</td>
<td>1.67 (0.87 to 3.20)</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Phosphate, per 1 mg/dl</td>
<td>1.03 (0.81 to 1.31)</td>
<td>0.81</td>
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</tr>
<tr>
<td>Inflammation, presence</td>
<td>0.89 (0.38 to 2.09)</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td><strong>Total telomere, per log rlu</strong></td>
<td>0.47 (0.05 to 3.98)</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>Without telomeric G-tail and total telomere</td>
<td>1.10 (1.06 to 1.14)</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>1.18 (0.62 to 2.23)</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus, presence</td>
<td>1.71 (0.90 to 3.26)</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Phosphate, per 1 mg/dl</td>
<td>0.98 (0.78 to 1.24)</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Inflammation, presence</td>
<td>0.84 (0.36 to 1.94)</td>
<td>0.68</td>
<td></td>
</tr>
</tbody>
</table>

Inflammation was defined as C-reactive protein \( > 0.5 \) mg/dl. OR, odds ratio; 95% CI, 95% confidence interval.
compared with healthy participants (21). Notably, previous reports have found that total telomere shortening per se causes cellular senescence in vascular endothelial cells (22) and eventually leads to atherosclerotic plaques (23). It raises the possibility that shortening of total telomere length directly involves pathologic conditions of CVD through cellular senescence.

However, there have been no studies that showed a correlation between total telomere length and the incidence of CVD in hemodialysis subjects. This study revealed that telomeric G-tail length (but not total telomere length) independently predicted new-onset cardiovascular events in hemodialysis patients. Telomere length is well correlated with G-tail length under normal conditions; however, we found a link between G-tail and CVD, but not total telomere length. This result is similar to our previous report of in vitro experiments using a G-quadruplex inhibitor that induced telomere dysfunction by t-loop destruction (24). Therefore, we postulate that G-tail reduction in patients with CKD is due to oxidative stress exposure. A possible explanation is that oxidative stress has a predilection for a guanine-rich single-stranded 3’ overhangs of G-tails (25). Although shelterin components play an important role in t-loop formation and protection (26), past studies did not establish a link between those proteins and CKD. These results suggest that telomeric G-tail length may be a sensitive marker for CVD in patients with CKD.

Our data show that both telomeric G-tail length and total telomere length in hemodialysis patients were shorter than those in control participants. Telomeric G-tail and total telomere shortening in aging healthy individuals is mainly caused by cell division, whereas other factors such as oxidative stress and inflammation might contribute to telomeric G-tail shortening in hemodialysis patients. In fact, it was reported that total telomere length is shorter in CKD before starting RRT (27). It is therefore likely that kidney insufficiency, rather than hemodialysis therapy, contributes to shortened telomeric DNA.

In addition to the clinical history of CVD, increased phosphate levels showed a significant correlation with both telomeric G-tail length and total telomere length, suggesting that high phosphate reduced telomere shortening. However, previous studies suggested that dysfunctional phosphate metabolism in hemodialysis patients increased CVD events and mortality (28). This discrepancy might be because patients with high phosphate levels are also highly active (29). In fact, shortened telomeres are reportedly associated with low levels of physical activity (30).

We previously developed the HPA to measure telomeric G-tail length in many clinical samples of PBMCs (15). Telomeric G-tail length and total telomere length measurement by HPA does not include subtelomeric sequences because the AE-labeled probes are quite specific. Actual telomeric G-tail length and total telomere length can be compared with conventional methods using a radioisotope assay (15,31). By contrast, conventional methods are semiquantitative because telomeric G-tail length is measured by densitometry of the smear pattern of an autoradiogram. In this article, we used a modified G-tail telomere HPA, and CV values were better than the original assay. Therefore, the method used in this study is a reliable technique for measurement of telomeric G-tail and telomere lengths.

The strengths of our observational study include its prospective design, the large number of clinical samples from hemodialysis patients, the long duration of follow-up, measurement by an accurate method, and the ability to adjust for various important risk factors. By contrast, some limitations of our study merit comment. Although PBMCs provide an easily accessible source for DNA analysis, it is not clear whether telomeric G-tail length in PBMCs reflects that in tissues. Although the methods used for the assessments of telomeric G-tail and total telomere lengths in this study are reliable, the average CVs of those measurements were relatively wide. Because our cohort comprises Japanese hemodialysis patients, our results may not necessarily be generalizable to other populations.

This is the first study to report simultaneous measurements of telomeric G-tail length and total telomere length in hemodialysis patients. This study shows that telomeric G-tail length is shortened in hemodialysis patients. Telomeric G-tail length of hemodialysis patients is also associated with CVD history and the likelihood of future hospitalization for a cardiovascular event.

### Table 4. Hazard ratios for telomeric G-tail length and total telomere length with cardiovascular events in patients

<table>
<thead>
<tr>
<th>Model</th>
<th>Covariate</th>
<th>Cardiovascular Events</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td><strong>Telomeric G-tail</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Crude (per log rlu)</td>
<td>0.12 (0.03 to 0.50)</td>
</tr>
<tr>
<td>2</td>
<td>1 plus age, sex, and diabetes mellitus</td>
<td>0.16 (0.04 to 0.65)</td>
</tr>
<tr>
<td>3</td>
<td>2 plus baseline CVD and inflammation</td>
<td>0.18 (0.04 to 0.78)</td>
</tr>
<tr>
<td>4</td>
<td>3 plus vitamin D use, phosphate, and intact PTH</td>
<td>0.14 (0.03 to 0.63)</td>
</tr>
<tr>
<td><strong>Total telomeres</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Crude (per log rlu)</td>
<td>0.39 (0.08 to 1.31)</td>
</tr>
<tr>
<td>2</td>
<td>1 plus age, sex, and diabetes mellitus</td>
<td>0.50 (0.11 to 2.14)</td>
</tr>
<tr>
<td>3</td>
<td>2 plus baseline CVD and inflammation</td>
<td>0.48 (0.11 to 2.11)</td>
</tr>
<tr>
<td>4</td>
<td>3 plus vitamin D use, phosphate, and intact PTH</td>
<td>0.25 (0.05 to 1.28)</td>
</tr>
</tbody>
</table>

Indicated are crude HRs or with various degrees of adjustment (models 2–4) for cardiovascular events according to telomeric G-tail and total telomere length. Inflammation was defined as C-reactive protein $>$0.5 mg/dl. HR, hazard ratio.
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Disclosures
H.T is a founder and the board director of MiRTeL Inc, and owns stock of MiRTeL Inc.

References