Results from the German Chronic Kidney Disease (GCKD) study support association of relative telomere length with mortality in a large cohort of patients with moderate chronic kidney disease

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Telomere length is known to be inversely associated with aging and has been proposed as a marker for aging-related diseases. Telomere attrition can be accelerated by oxidative stress and inflammation, both commonly present in patients with chronic kidney disease. Here, we investigated whether relative telomere length is associated with mortality in a large cohort of patients with chronic kidney disease stage G3 and A1-3 or G1-2 with overt proteinuria (A3) at enrollment. Relative telomere length was quantified in peripheral blood by a quantitative PCR method in 4,955 patients from the GCKD study, an ongoing prospective observational cohort. Complete four-year follow-up was available from 4,926 patients in whom we recorded 354 deaths. Relative telomere length was a strong and independent predictor of all-cause mortality. Each decrease of 0.1 relative telomere length unit was highly associated with a 14% increased risk of death (hazard ratio1.14 [95% confidence interval 1.06-1.22]) in a model adjusted for age, sex, baseline eGFR, urine albumin/creatinine ratio, diabetes mellitus, prevalent cardiovascular disease, LDL-cholesterol, HDL-cholesterol, smoking, body mass index, systolic and diastolic blood pressure, C-reactive protein and serum albumin. This translated to a 75% higher risk for those in the lowest compared to the highest quartile of relative telomere length. The association was mainly driven by 117 cardiovascular deaths (1.20 [1.05-1.35]) as well as 67 deaths due to infections (1.27 [1.07-1.50]). Thus, our findings support an association of shorter telomere length with all-cause mortality, cardiovascular mortality and death due to infections in patients with moderate chronic kidney disease.

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Telomeres are non-coding, repetitive nucleotide sequences (TTAGGG) ranging from 5 to 15 kilobase pairs in length that are located at the end of eukaryotic chromosomes.[1] Their functions include protection of the DNA and maintenance of chromosomal integrity. Telomeres shorten at each cycle of cell division due to the incapacity of DNA polymerase to replicate the very ends of linear chromosomes.[2] Approximately 50–200 base pairs are lost during each cell division, and when a critical telomere length is reached, cells undergo replicative senescence or apoptosis. Consequently, telomere length (TL) has been proposed as a marker of biological age,[3] and its predictive role in aging-related disease has been investigated in many epidemiologic studies.[4–6]

Telomere attrition, accelerated by oxidative stress and inflammation, leads to cell senescence, which compromises regeneration and functionality of vital organs, including the kidneys.[7] In particular, it has been shown that chronic inflammation leads to lymphocyte telomere attrition, cell senescence, and finally impairment of the immune response.[8] This T-cell dysfunction can contribute to increased susceptibility to kidney infections and injury.[7]

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Table 1 | Characteristics of all patients available (n = 4926) for analysis grouped by relative telomere length quartiles

RTL quartiles	Total	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
RTL range	**0.4–2.31**	**0.40–0.82**	**0.82–0.92**	**0.92–1.05**	**1.05–2.31**	
RTL mean ± SD	**0.95 ± 0.19**	**0.73 ± 0.07**	**0.87 ± 0.03**	**0.98 ± 0.04**	**1.20 ± 0.14**	
RTL median [25th, 75th percentile]	**0.92 [0.82, 1.05]**	**0.75 [0.69, 0.78]**	**0.87 [0.84, 0.90]**	**0.98 [0.95, 1.01]**	**1.16 [1.10, 1.26]**	***P* value**
N	4926	1232	1232	1232	1230	
Age, yr	60.2 ± 11.9	64.8 ± 8.7	62.4 ± 10.1	59.5 ± 11.7	54.1 ± 13.6	8.9e-110
	63 [53, 70]	68 [61, 71]	65 [57, 70]	63 [52, 69]	56 [45, 65]	
Sex (female)	1 959 (39.8)	384 (31.2)	445 (36.1)	540 (43.8)	590 (48)	6.2e-19
Body mass index, kg/m^2	29.8 ± 6.0	30.4 ± 6.0	29.9 ± 5.8	29.9 ± 5.8	29.0 ± 6.2	3.5e-10
	28.9 [25.7, 33.2]	29.5 [26.2, 34.1]	29.1 [26.1, 33.2]	29.0 [25.9, 33.1]	27.8 [24.5, 32.4]	
Current smoker	785 (16)	170 (13.9)	175 (14.2)	208 (16.9)	232 (18.9)	1.3e-3
Diabetes mellitus	1768 (35.9)	530 (43)	474 (38.5)	405 (32.9)	359 (29.2)	6.9e-13
Prevalent CVD	1261 (25.6)	408 (33.1)	346 (28.1)	302 (24.5)	305 (16.7)	4.7e-20
eGFR (CKD-EPI formula), ml/min per 1.73 m^2	49.5 ± 18.2	46.4 ± 15.8	47.6 ± 17.2	50.1 ± 18.4	53.8 ± 20.4	6.0e-22
	46 [37, 58]	44 [35, 54]	45 [36, 55]	47 [38, 58]	50 [40, 62]	
UACR, mg/g	430 ± 969	413 ± 1052	350 ± 784	440 ± 912	519 ± 1090	1.6e-04
	51 [9, 383]	140 [9, 304]	44 [9, 294]	58 [10, 441]	65 [10, 522]	
Serum albumin, mg/l	38.3 ± 4.3	37.9 ± 4.2	38.4 ± 3.9	38.5 ± 4.1	38.5 ± 4.8	4.3e-04
	38.7 [36.2, 40.8]	38.3 [36.0, 40.5]	38.7 [36.2, 40.7]	38.9 [36.4, 41]	39 [36.2, 41.1]	
Systolic blood pressure, mm Hg	139.5 ± 20.2	141.1 ± 20.0	139.7 ± 20.2	140.1 ± 20.9	137.2 ± 19.8	2.1e-06
	138 [126, 152]	140 [128, 154]	139 [126, 152]	138 [125, 152]	135 [124, 149]	
Diastolic blood pressure, mm Hg	79.2 ± 11.7	77.4 ± 11.5	78.6 ± 11.7	79.7 ± 11.7	81.1 ± 11.6	3.5e-14
	79 [71, 87]	77 [70, 85]	78 [71, 86]	79 [72, 87]	81 [73, 88]	
High-sensitivity C-reactive protein, mg/l	4.77 ± 8.48	5.25 ± 8.04	4.80 ± 7.82	4.73 ± 8.51	4.31 ± 9.41	2.0e-07
	2.27 [1.02, 5.01]	2.53 [1.22, 5.57]	2.34 [1.06, 4.97]	2.27 [1.01, 5.10]	1.91 [0.85, 4.4]	
HDL-cholesterol, mg/dl	51.8 ± 18	50.2 ± 17.2	51.1 ± 17.8	52.4 ± 18.3	53.5 ± 18.4	1.5e-05
	48.3 [39.2, 61.3]	46.9 [38.2, 58.9]	47.7 [39.0, 60.3]	49.2 [39.8, 61.4]	50.1 [40.1, 64.1]	
LDL-cholesterol, mg/dl	118.2 ± 43.5	113.1 ± 41.1	115.8 ± 40.4	120.7 ± 42.5	123.3 ± 48.8	5.6e-08
	113.6 [89.1, 142.7]	108.8 [84.9, 138.9]	112.2 [87.3, 140.5]	117.9 [90.7, 146.3]	117.0 [92.3, 147.2]	

CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RTL, relative telomere length; UACR, urine albumin–creatinine ratio.
Data are given as mean ± SD, with median [25th, 75th percentile], or n (%), unless otherwise indicated.

Chronic kidney disease (CKD) is a complex disease, and its heritability has been estimated to be 30%–70%.[9–12] In past years, genome-wide association studies have identified many genetic loci associated with kidney function and CKD.[13–17] However, index single nucleotide polymorphisms at the identified loci explain only a minor part of the heritability, and additional genetic contributors might be missing. To date, only a few small studies have investigated the association between TL and kidney disease. Some studies found that short TL correlates with impaired kidney function in the general population,[18,19] as well as in heart failure patients.[20] We recently described significantly shorter relative TL (RTL) in patients with moderately severe CKD who have prevalent cardiovascular disease (CVD),[21] as well as an association with duration[22] and progression of CKD.[23] Patients who have reached kidney failure treated by hemodialysis are described as having reduced TL in comparison with healthy controls,[24–27] and reduced TL is inversely associated with mortality.[28] Only a few investigations have been conducted in non-dialysis-dependent kidney patients.[21–23,29,30] To our knowledge, the current study is the first prospective study that investigates the association between leukocyte RTL and causes of mortality in a large non-dialysis-dependent CKD cohort.

RESULTS

Baseline characteristics of the study population

RTL was quantified in peripheral blood by a quantitative polymerase chain reaction method in 4955 patients from the German Chronic Kidney Disease study. Complete 4-year follow-up was available from 4926 patients. Baseline characteristics of these 4926 patients according to quartiles of the RTL are provided in Table 1. RTL ranged from a minimum of 0.40 to a maximum of 2.31 (Supplementary Figure S1), with a mean ± SD of 0.95 ± 0.19 and a median of 0.92 (1st quartile = 0.82; 3rd quartile = 1.05). RTL was negatively correlated with age (r = –0.36, $P < 0.001$) and positively correlated with estimated glomerular filtration rate (eGFR; r = 0.17, $P < 0.001$) and urine albumin–creatinine ratio (r = 0.05, $P < 0.001$). When we adjusted RTL for age and sex, we no longer observed a significant correlation with eGFR and urine albumin–creatinine ratio.

Prospective follow-up and mortality

A total of 354 deaths occurred during a median follow-up period of 4 years (1483 days). The causes of death were CVD including myocardial infarction, coronary heart disease, sudden cardiac death, congestive heart failure, pulmonary embolism, cardiac valve disease and ischemic stroke (117 patients, 33.1%), infections (67 patients, 18.9%), non-ischemic cerebrovascular causes (9 patients, 2.5%), peripheral vascular disease (7 patients, 2.0%), kidney failure (8 patients, 2.3%), various other causes (103 patients, 29.1%) and unknown causes (43 patients, 12.1%).

Cumulative incidence plots show that incidence of all-cause mortality (Figure 1a) increases with shorter RTL, with

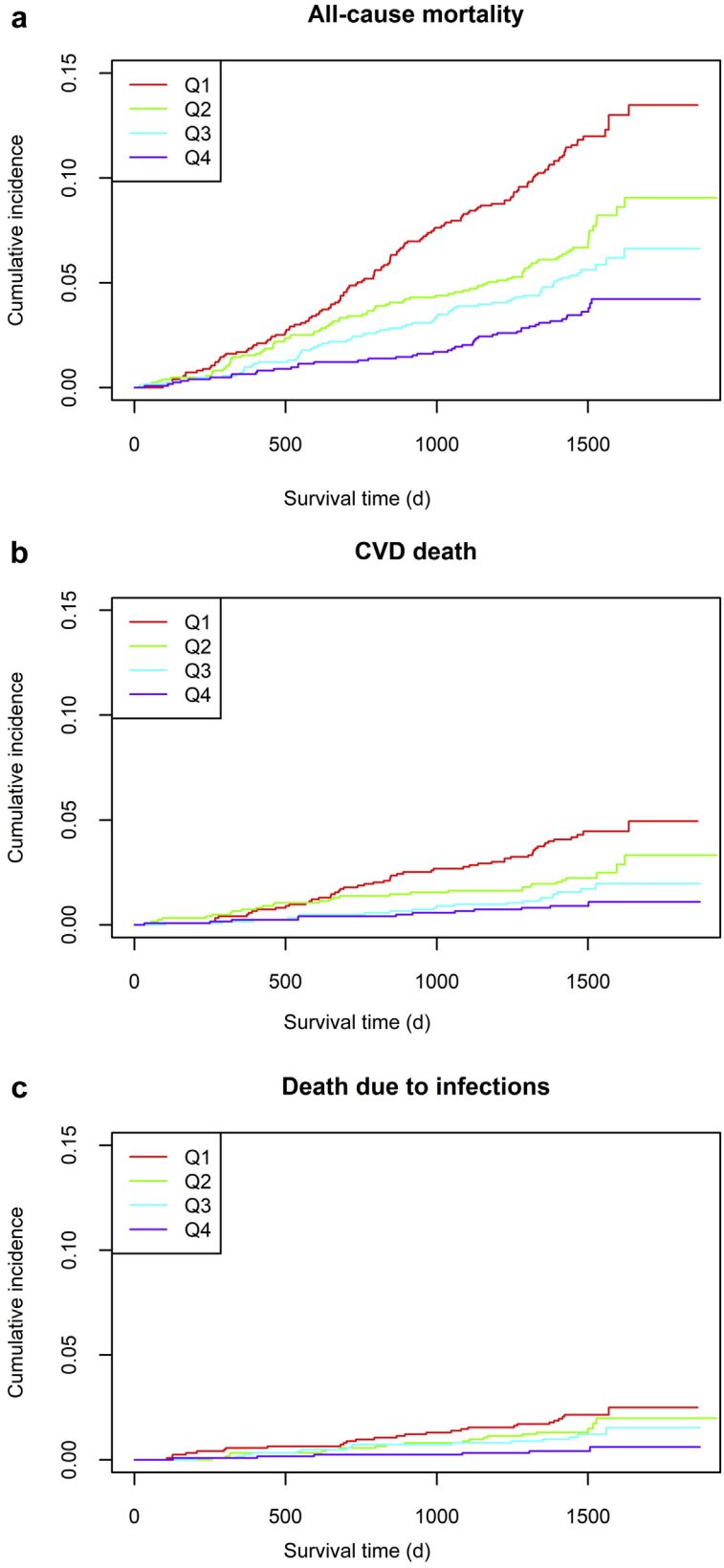

Figure 1 | Cumulative incidence function for (a) all-cause mortality, (b) cardiovascular disease (CVD) mortality, and (c) death due to infections, by quartiles (Q) of relative telomere length (RTL). Q1 is the quartile including patients with the shortest RTL.

Table 2 | Results of Cox model on all-cause mortality, death due to cardiovascular disease (cause-specific hazard ratios [HRs] are given), and death due to infections (cause-specific HRs are given) for each decrease in 0.1 units of relative telomere length (RTL) as well as the first quartile of RTL versus quartiles 2 to 4 (combined as reference category)

	For each decrease of 0.1 RTL	
Adjustment model[a,b]	HR [95% CI]	*P* value
All-cause mortality		
Model 1 (354 events)	1.16 [1.08–1.24]	1.7e-05
Model 2 (343 events)	1.16 [1.08–1.24]	4.4e-05
Model 3 (333 events)	1.14 [1.06–1.22]	3.5e-04
Cardiovascular death		
Model 1 (117 events)	1.22 [1.08–1.38]	0.0014
Model 2 (116 events)	1.21 [1.07–1.36]	0.0026
Model 3 (113 events)	1.20 [1.05–1.35]	0.0052
Death due to infections		
Model 1 (67 events)	1.26 [1.07–1.48]	0.005
Model 2 (65 events)	1.28 [1.08–1.51]	0.0024
Model 3 (63 events)	1.27 [1.07–1.50]	0.0051

CI, confidence interval.
[a]Model 1: adjusted for age and sex; model 2: adjusted for age, sex, estimated glomerular filtration rate, urine albumin–creatinine ratio, diabetes mellitus, prevalent cardiovascular disease; model 3: adjustment as in model 2 plus low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, smoking, body mass index, systolic blood pressure, diastolic blood pressure, C-reactive protein, and serum albumin.
[b]Due to missing values, not all models include the same number of events.

the highest incidence with lowest RTL quartile. In the cumulative incidence function curves of cardiovascular (Figure 1b) and infection mortality (Figure 1c), the difference between quartiles was less pronounced, but the order of the quartiles was the same.

Results of Cox regression models applying different adjustments are provided in Table 2 and showed a significant association between shorter RTL and the risk of all-cause mortality. Evaluated continuously, each decrease of 0.1 RTL units was associated with a 16% increased risk of death in a model adjusted for age and sex (hazard ratio [HR], 1.16; 95% confidence interval [CI], 1.08–1.24; $P = 1.7e-05$). The association remained significant after an extended adjustment for eGFR, urine albumin–creatinine ratio, diabetes mellitus, and prevalent cardiovascular disease (model 2: HR, 1.16; 95% CI, 1.08–1.24) as well the additional CVD risk factors low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, smoking, body mass index, systolic blood pressure, diastolic blood pressure, C-reactive protein, and serum albumin at baseline (model 3: HR, 1.14; 95% CI, 1.06–1.22; $P = 3.5e-04$). Nonlinear P spline analyses are given in Figure 2 and revealed an almost linear association of RTL with all-cause mortality. Patients with the shortest RTL (1st quartile) had a 75% higher risk for all-cause mortality compared to those in the quartile with the longest RTL (Supplementary Table S1, fully adjusted model: HR, 1.75; 95% CI, 1.22–2.50; $P = 0.0024$).

Next, we analyzed what is driving the association of RTL with all-cause mortality (Table 2). We evaluated the 2 most frequent specific causes of death and observed that each decrease of 0.1 RTL units was associated with a 20% increased risk of CVD death in the fully adjusted model (HR, 1.20; 95% CI, 1.05–1.35; $P = 0.0052$). Reduced RTL was also significantly inversely associated with death due to infections. Each 0.1 unit decrease of RTL was associated with a 1.27-fold higher risk for death due to infections (HR, 1.27; 95% CI, 1.07–1.50; $P = 0.0051$). Looking at the estimates for the various quartiles in Supplementary Table S1 revealed for death due to infections that the estimates for each of the quartiles 1, 2, and 3 were similarly elevated compared to that for quartile 4. The analysis with other causes of death as well as unknown causes of death was obviously too heterogeneous and did not reveal any association with RTL (data not shown).

The graph of the scaled Schoenfeld residuals and test on proportional hazard assumptions did not suggest any time-varying effects for RTL on any of the investigated outcomes. The subdistribution HRs for both cardiovascular and infection death, reported in Supplementary Table S2, are only slightly attenuated compared to the cause-specific HRs.

We also evaluated whether the effect of RTL on the 3 different outcomes differed between men and women and for patients with and without diabetes mellitus, but we did not detect a significant interaction for these variables, or for age (all *P* values of interaction >0.1 in the fully adjusted models).

Given that we recently observed a U-shaped association between duration of CKD and RTL,[22] we performed a sensitivity analysis additionally adjusting for the duration of CKD at baseline defined as less than 6 months, between 6 month and 5 years, and more than 5 years. This additional adjustment resulted in only marginal changes of the HRs obtained for all 3 endpoints (Supplementary Table S3).

DISCUSSION

The results of this study showed a significant association of RTL with all-cause mortality in a non-dialysis-dependent CKD cohort. Shorter RTL was associated with higher risk of mortality independently from kidney function and traditional CVD risk factors. This association was driven by death due to CVD as well as death due to infections.

Association with all-cause mortality

Prior studies,[5,6,31–35] with few exceptions,[36,37] have demonstrated a negative association between RTL and all-cause mortality in the general population. The largest study so far (n = 64,637) was performed by Rode *et al.*, with an adjusted HR for mortality of 1.40 for the decile with the shortest versus the decile with the longest RTL.[35] In accordance with these results, our study showed with each decrease of 0.1 RTL units a 14% higher risk for all-cause mortality, which translates to a 75% higher risk for those in the lowest compared to the highest quartile of RTL. To our knowledge, only Carrero *et al.*[28] have investigated the relationship between RTL and mortality risk in CKD patients. They studied 175 patients with end-stage kidney disease treated by hemodialysis, of whom 70 died during a median of 31 months of observation. The authors observed that TL independently predicted

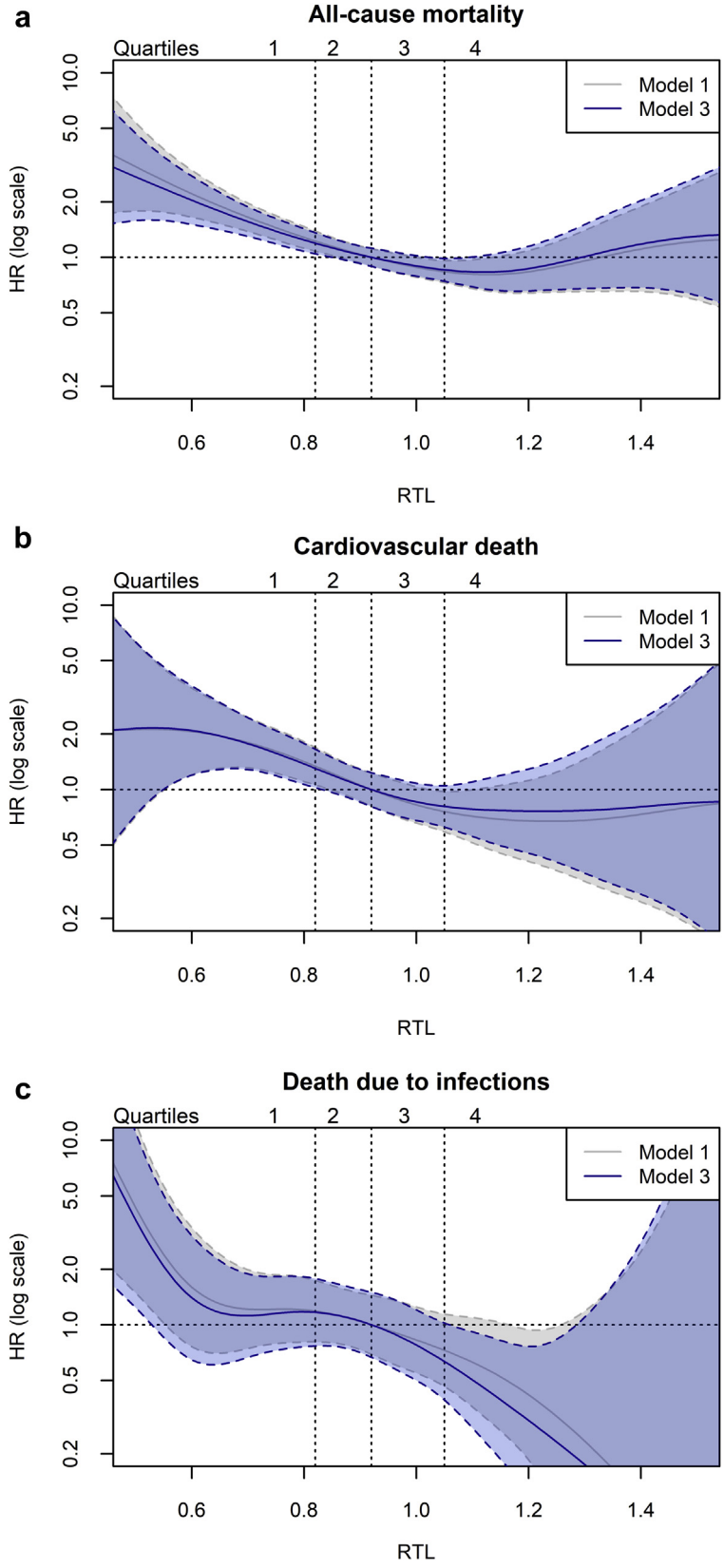

Figure 2 | Adjusted nonlinear splines (and 95% confidence bands) for the association between increasing relative telomere length (RTL) and hazard ratio (HR) of (a) all-cause mortality, (b) cardiovascular death, and (c) death due to infections. HR is given as log-scale on the y-axes. Gray line: adjustment model 1 (adjusted for age and sex). Blue line: adjustment model 3 (adjusted for age, sex, estimated [continued]

patient survival after additional adjustment for age, sex, and inflammation. The current study extends these observations to the much larger group of individuals with CKD who do not require dialysis.

Association with CVD mortality

No studies have investigated the association of RTL with CVD mortality in CKD patients so far, although this is the major cause of death in these patients. Depending on the investigated ethnicity and on the data adjustment models, some, but not all, studies in the general population reported an association between low RTL and CVD outcomes.[33,38–41] Strong support for a causal association came from a Mendelian randomization study in which genetic variants associated with shorter RTL were found to be associated with ischemic heart disease.[40] In the present study, we identified a significant association of RTL with cardiovascular deaths, with a 20% higher risk with each decrease of RTL by 0.1 units, or a 75% higher risk for those patients in the quartile with the shortest TL compared to the quartile with the longest TLs. This finding is in line with our earlier report of an association with prevalent cardiovascular events in this patient population: each decrease of RTL by 0.1 units was significantly associated with a 6% higher odds for prevalent CVD in a model adjusting for age, sex, current smoking, hypertension, diabetes status, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, C-reactive protein, eGFR, and body mass index.[21] The prospective follow-up in these patients in the present investigation revealed that especially the lowest quartile of RTL was associated with a markedly increased risk, whereas the other 3 quartiles showed very similar estimates (Figure 1b).

Association with death due to infections

Although experimental evidence supports a role of cell senescence and short TL in impaired immune response, epidemiologic studies are sparse, especially in CKD patients. Helby *et al.* conducted the largest (n = 75,309) prospective population-based study investigating RTL and the risk of hospitalization for infectious disease and the risk of infection-related death. During 7 years of follow up, they observed a higher risk of any infections in the quartile with the shortest compared with the quartile with the longest RTL.[42] Previous studies with smaller sample sizes reported conflicting results.[32,43,44] Our findings in CKD patients describe for the first time the association between short RTL and higher risk for death due to infections in this high-risk population.

Potential mechanism

The biological mechanism underlying the relationship between RTL and mortality is still unclear. The association identified by our study does not elucidate whether RTL shortening is causally related to cardiovascular disease and infections. However, a genome-wide association study followed by a genetic risk score analysis combining lead variants at 7 genetic loci showed an association of the alleles associated with shorter RTL with increased risk of coronary artery disease. This finding provides possible support for a potential causal role of RTL in CVD.[45,46] Furthermore, cellular senescence induced by telomere attrition could be a trigger of atherosclerosis as well arteriosclerosis. The accumulation of senescent cells in the vessel contributes to atherosclerotic plaque formation and media calcifications resulting in increased arterial stiffness as a dominant feature of uremic arterial disease.[47] Most of the studies have measured RTL in the DNA from peripheral leukocytes. However, a close correlation has been shown between leukocyte and aortic wall tissue TL.[48] Therefore, cellular senescence could affect endothelial cells leading to dysfunction in the vascular wall and promoting the adhesion of immune cells, a primary event in atherosclerosis. Furthermore, short telomeres activate p53 and autophagy in cardiac progenitor cells, destabilizing the balance of quiescence and proliferation toward differentiation and senescence, leading to an exhaustion of cardiac progenitor cells.[49] Telomere dysfunction has been shown to induce a profound p53-dependent repression of the master regulators of mitochondrial biogenesis and function, which leads to bioenergetic compromise due to impaired oxidative phosphorylation and adenosine triphosphate generation.[50,51]

There are also several links between RTL and infections. The loss of telomeres has been observed during T cell differentiation,[52] in chronic viral infections,[53] and with age.[54] Furthermore, short leukocyte TL has been reported as a risk factor in various immune-related diseases[55] and diabetes.[56] Leukocyte shorter TL causes cell senescence that is followed by a reduction of immune cell proliferative capacity. TL may also be involved in age-related declines in immune function related to an insufficient response to vaccines and acute infections.[57–59] A potential role of TL in infections is also supported by a recent genome-wide association study in a Chinese population. Dorajoo and colleagues observed an association between a TL-reducing allele and death due to respiratory infection.[60]

RTL attrition might be strongly triggered by the presence of elevated oxidative stress, a common condition in CKD.[61]

Figure 2 | [continued] glomerular filtration rate, urine albumin/creatinine ratio, diabetes mellitus, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, high-sensitivity C-reactive protein, albumin, smoking, body mass index, systolic blood pressure, diastolic blood pressure, and prevalent cardiovascular disease). Vertical dotted lines indicate the thresholds of RTL quartiles; the median value of RTL (0.92) is set as a reference (HR = 1; horizontal dashed line).

Several *in vitro* and *in vivo* studies showed that oxidative stress accelerates telomere attrition.[62,63] Indeed, telomeres, with their high guanine content, are highly susceptible to oxidative damage,[64] and single-strand DNA breaks induced by oxidative stress could be an important factor for telomere shortening during DNA replication.[65]

Strengths and limitations

Strengths of this investigation include the large sample size of a well-defined population with a median follow-up of 4 years with almost no loss to follow-up, homogeneity of the study population, and a centralized assessment of TL and outcome measures. The measurement of RTL especially is of utmost importance because standardization between laboratories is not easy to accomplish and therefore the process should be performed in the same laboratory under exactly the same conditions in terms of protocol, reference gene, instrument, personnel,[66] and DNA extraction procedure.[67]

There are some limitations to this study. First, RTL was measured in peripheral leukocytes. It is known that the rate of progression to senescence differs among lymphocyte subsets.[59] Unfortunately, no data were available about blood cell type composition in the German Chronic Kidney Disease study and it was therefore not possible to investigate this aspect. Knowing RTL from various kidney cell types would be of interest, but it is not possible to obtain in a large epidemiologic study as this would require tissue material from biopsies. The second limitation includes the observational design of the study, which does not allow for clarification of causality or biological mechanism. Third, the study recruited mainly CKD patients in stage G3 or A3, and the findings might not be generalizable to other stages of CKD. Fourth, the association with specific causes of death might have been limited by statistical power but was still present for the 2 specific main causes of death. Finally, although our analyses were adjusted for traditional cardiovascular risk factors as well as kidney function parameters, we cannot exclude the possibility of residual confounding by unknown or unmeasured variables. However, it was very interesting to see that the age- and sex-adjusted estimates of RTL for various outcomes were very stable with further adjustment for the other variables, indicating that RTL is relatively independent from other variables when data are adjusted for age and sex.

Conclusions

Short relative TL quantified from peripheral blood leukocytes was independently associated with all-cause mortality during 4 years of follow-up in patients with moderately severe CKD. This association was driven by death due to CVD as well as death due to infections.

METHODS

Study population

The German Chronic Kidney Disease study is an ongoing prospective multicenter observational cohort. A detailed description of the study has been published previously.[68] Briefly, 5217 patients under regular care by nephrologists were enrolled. Inclusion criteria were moderately reduced kidney function defined as eGFR of 30–60 ml/min per 1.73 m^2 (stage G3, A1–A3) or an eGFR >60 ml/min per 1.73 m^2 in the presence of overt proteinuria (stage G1–G2, A3). Exclusion criteria were non-Caucasian ethnicity, solid organ or bone marrow transplantation, active malignancy within 24 months prior to screening, New York Heart Association Stage IV heart failure, and legal attendance or inability to provide consent. Laboratory parameters presented were all measured from collected biosamples in a central laboratory as described previously.[68] Information on sociodemographic factors, medical and family history, medications, and health-related quality of life were obtained by trained personnel through standardized questionnaires. Prevalent cardiovascular disease at baseline was defined as history of non-fatal myocardial infarction, coronary artery bypass grafting, percutaneous transluminal coronary angioplasty, stroke, and interventions at the carotid arteries (carotid endarterectomy and/or carotid balloon angioplasty or stent implantation).

All participants provided written informed consent, and the study was approved by the ethics committees of all participating institutions and registered in the national registry for clinical studies (DRKS 00003971). All methods were carried out in accordance with approved guidelines and the Declaration of Helsinki. Data are collected and managed using Askimed (https://www.askimed.com) as a cloud-based web platform for collection and management of case report forms and laboratory data.

Clinical endpoints during prospective follow-up

As described recently,[69] patients are followed on a yearly basis by trained personnel alternating face-to-face visits with telephone visits. During these visits, data on hospitalizations, outcome events, and medical history are updated as part of a structured interview. Any hospital discharge reports are collected from the treating physicians and/or hospitals. Endpoints are continuously abstracted from these reports by a trained and supervised endpoint committee composed of 3 independent physicians according to a prespecified endpoint catalogue. Information on cause of death is taken from these reports as well as from death certificates collected from civil registry offices whenever study personnel are informed of the death of a study participant.

The current analysis includes all endpoints that occurred until 4-year follow-up. If a patient missed the 4-year follow-up visit, we included all endpoints until 4.5 years after the respective baseline visit. Therefore, censoring time was either the 4-year follow-up date or 4.5 years after baseline. The primary outcome was all-cause mortality. To evaluate whether identified associations could be attributed to a specific cause of death, death from cardiovascular causes and infectious diseases were considered as secondary outcomes. The group of CVD death included myocardial infarction, coronary heart disease, sudden cardiac death, congestive heart failure, pulmonary embolism, cardiac valve disease, and ischemic stroke. Other causes of death were also recorded, but these subgroups were not large enough to conduct further cause-specific analyses.

Measurement of RTL

Genomic DNA was extracted from whole blood in a central laboratory with the Chemagic Magnetic Separation Module I (PerkinElmer chemagen Technologie GmbH, Baesweiler, Germany). The current analysis was based on 4926 patients for whom measurements of RTL in baseline blood samples and data on mortality were available. RTL was measured in quadruplicate using a quantitative-polymerase chain reaction–based assay developed by Cawthon[70]

and modified as described previously.[71] DNA samples were run in 15-µl reactions containing 1x Quantifast TM SYBR Green PCR mastermix (Qiagen, Hilden, Germany), 10 ng of DNA, 1 µM of telomere primer, or 250 nm of housekeeping gene 36B4 primer. We determined the relative ratio of telomere repeat copy number (T) to single-copy gene copy number (36B4 gene, encoding ribosomal phosphoprotein PO, located on chromosome 12; S). The T/S ratios are proportional to individual RTL. The automation of this high-throughput procedure resulted in very good quality-control measures with a low inter-assay coefficient of variation of the T/S ratios. A commercially available DNA included in all the quantitative polymerase chain reaction plates was used to check the performance of the assay over the entire study. The interassay coefficient of variation of T/S ratios of this sample analyzed in 112 independent experiments was 9.6% before normalization and decreased to 4.0% after normalization (Supplementary Figure S2).

Statistical analysis

We compared baseline characteristics of participants (Table 1) using the Kruskal–Wallis test for continuous variables and chi-squared tests for categorical variables. Cumulative incidence function curves were used to estimate the cumulative incidence of the different causes of death accounting for the competing risk.[72] Cox proportional hazards regression models were performed to evaluate the associations of RTL with 3 outcomes: (i) all-cause mortality, (ii) death due to CVD, and (iii) death due to infections. For both cause-specific endpoints (ii and iii), patients were censored, if death occurred by any other cause. In addition, associations with these cause-specific endpoints were examined using competing-risks survival regression, considering all deaths from other causes as competing events. Therefore, both cause-specific HRs and subdivision HRs are reported for death due to CVD or infections. Cox proportional hazards models were fitted using 3 different levels of adjustment: model 1 adjusted for age and sex; model 2 additionally adjusted for baseline eGFR, urine albumin-creatinine ratio, prevalent CVD, and diabetes; model 3 further adjusted for the traditional CVD risk factors of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, body mass index, smoking, systolic and diastolic blood pressure, C-reactive protein, and albumin. The selection of variables adjusted for in the various models was based on the differences in the clinical characteristics between the quartiles of RTL provided in Table 1. In the regression models, RTL was analyzed continuously and as a categorical predictor (in quartiles of RTL). Given that initial results indicated a higher risk, especially for low values of RTL, the (cause-specific) HR is also reported for quartiles 1, 2, and 3 compared to quartile 4 as a reference (Supplementary Table S1). Further downstream analysis was based on visul inspection of P-spline to derive the shape of the relationship between RTL and mortality risk. We used Spearman's rank correlation coefficient to assess the association between study variables. All statistical analyses were performed using R 3.3.2 (https://www.r-project.org); P values <0.05 were considered statistically significant.

APPENDIX

List of current GCKD study investigators and collaborators

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DISCLOSURE

All the authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

FKr designed the study. JR measured relative telomere length. FF and CL performed the statistical analysis of the data. UTS, FKo, HM, BB, HW, LF, SS, IS, CW, K-UE, AK, and FKr were involved in recruitment, data acquisition, and data management the GCKD study. UTS, FKo, IS, and AK were involved in the endpoint adjudication. FF, CL, and FKr drafted the article. All the authors revised the manuscript critically for important intellectual content and gave approval of the final version.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Density plot and histogram of relative telomere length in 4926 participants in the German Chronic Kidney Disease study.

Figure S2. The very same DNA template was included in each of 112 independent qPCR plates. Relative telomere length (RTL) raw values are given on the left side. On the right side, the RTL values are provided after the normalization procedure, and the corresponding result pairs are connected by lines. Values before the normalization showed a higher variability than did those after normalization (coefficient of variation [CV] of 9.6% vs. 4.0%, respectively).

Table S1. Results of Cox model on all-cause mortality, death due to cardiovascular disease, and death due to infections for quartiles 1, 2, and 3 of RTL versus quartile 4 as a reference group.

Table S2. HRs derived from subdistribution hazard models for death due to cardiovascular disease and death due to infections for each decrease in 0.1 units of RTL.

Table S3. Results of Cox model on all-cause mortality, death due to cardiovascular disease, and death due to infections for each decrease in 0.1 RTL. Comparison between the fully adjusted model (model 3) and a model further adjusted for CKD duration.

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