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Original Article

ORIGINAL ARTICLE

Association of leukocyte telomere length with chronic kidney disease in East Asians with type 2 diabetes: a Mendelian randomization study

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ABSTRACT

Background. Chronic kidney disease (CKD) is common among people with type 2 diabetes (T2D), and increases the risk of kidney failure and cardiovascular diseases. Shorter leukocyte telomere length (LTL) is associated with CKD in patients with T2D. We previously reported single-nucleotide polymorphisms (SNPs) associated with LTL in an Asian population. In this study, we elucidated the association of these SNPs with CKD in patients with T2D using the Mendelian randomization (MR) approach.

Methods. The cross-sectional association of 16 LTL SNPs with CKD, defined as an estimated glomerular filtration rate of <60 mL/min/1.73 m^2, was assessed among 4768 (1628 cases and 3140 controls) participants in the Singapore Study of Macro-angiopathy and Micro-vascular Reactivity in T2D and Diabetic Nephropathy cohorts. MR analysis was performed using the random-effect inverse-variance weighted (IVW) method, the weighted median, MR-Egger and Radial MR adjusted for age and sex-stratified by cohorts and ethnicity (Chinese and Malays), then meta-analyzed.

Results. Genetically determined shorter LTL was associated with increased risk of CKD in patients with T2D (meta-IVW adjusted odds ratio = 1.51, 95% confidence interval 1.12–2.12, P = 0.007, $P_{het} = 0.547$). Similar results were obtained following sensitivity analysis. MR-Egger analysis (intercept) suggested no evidence of horizontal pleiotropy ($\beta = 0.010$, P = 0.751).

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Conclusions. Our findings suggest that genetically determined LTL is associated with CKD in patients with T2D. Further studies are warranted to elucidate the causal role of telomere length in CKD progression.

Keywords: chronic kidney disease, Mendelian randomization analysis, telomere length, type 2 diabetes

INTRODUCTION

Telomeres are DNA–protein structures at the ends of chromosomes that protect the genome from damage [1]. In most somatic tissues, telomeres shorten progressively with cell division [2]. When telomere lengths are critically short, it triggers apoptosis or replicative senescence [3, 4]. Therefore, telomere length is recognized as a biomarker for cellular aging [5]. Leukocyte telomere length (LTL), predominantly measured in epidemiological studies, is correlated with telomere length in multiple tissues in humans, including kidney tissues [6–8], and is inversely associated with risk of aging-related diseases including cardiovascular disease and all-cause mortality [9–11].

Diabetic kidney disease (DKD) is a leading cause of renal failure, cardiovascular disease and mortality in patients with type 2 diabetes (T2D) [12–15]. Observational studies have demonstrated inverse associations between LTL and risk of chronic kidney disease (CKD) in patients with T2D [16–19]. However, observational studies are prone to reverse causation and confounding factors. Moreover, LTL is modulated by oxidative stress as well as inflammation, obesity, and genetic and environmental factors [20, 21]. Therefore, it is uncertain whether shorter LTL is causally associated with DKD.

The Mendelian randomization (MR) approach uses single-nucleotide polymorphisms (SNPs) that are robustly associated with a risk factor to estimate the causal relationship between a risk factor and a disease [22]. Given that germline genetic variants are randomly assorted at meiosis, the MR approach is less prone to residual biases, confounding and reverse causation. For inferencing causality, it is essential that the assumptions of MR are satisfied. These are: (i) the selected SNPs are associated with exposure (telomere length); (ii) the selected SNPs are not associated with confounders; and (iii) the selected SNPs are associated with outcome exclusively through their effect on exposure (telomere length). To our knowledge, the causal effect of shorter leukocyte telomeres in CKD in patients with T2D has not been evaluated in East Asians.

A recent large-scale genome-wide association study (GWAS) in the Singapore Chinese Health Study (SCHS) cohort identified 16 SNPs associated with LTL [23]. In this study, we performed two-sample MR with summary statistics of SNP–LTL associations from the SCHS cohort and SNP–DKD association determined in this study to investigate the causal relationship between LTL and CKD in patients with T2D.

MATERIALS AND METHODS

SNP selection

Ten SNPs robustly associated ($P < 5 \times 10^{-8}$) with LTL in the Singapore Chinese population ($N = 25\,273$; mean age = 55 years) and an additional six independent SNPs identified after meta-analysis with European cohorts ($n = 37\,505$) [23] were selected as instrumental variables (IVs). These 16 SNPs are located in different regions and close to genes coding for proteins involved in telomere homeostasis, such as shelterin complex, DNA repair pathways and telomerase enzyme. The list of 16 SNPs selected as IV for LTL, and the coefficient estimate for LTL (β_{LTL}), are shown in Table 1. Together, these 16 SNPs explained ~4% of the variation in LTL in the Singaporean Chinese population [23]. The beta estimate reflects changes in standard deviation (SD) of the standardized levels of LTL adjusted for age, sex and principal components.

Table 1. SNPs selected as IV and its association with LTL and CKD

					SNP–LTL		SNP–CKD		
SNP	Chr	Position (hg19)	Gene	Test allele	β	SE (β)	$\log_e$ (OR)	SE	P-value
rs3219104	1	226562621	*PARP1*	A	−0.074	0.009	−0.013	0.048	0.780
rs11890390	2	54485682	*ACYP2*	C	−0.040	0.012	0.125	0.065	0.055
rs2293607	3	169482335	*TERC*	C	−0.120	0.009	0.030	0.058	0.406
rs10857352	4	164101482	*NAF1*	A	−0.064	0.011	−0.004	0.079	0.544
rs7705526	5	1285974	*TERT*	C	−0.118	0.009	0.044	0.050	0.386
rs7776744	7	124599749	*POT1*	G	−0.058	0.009	0.076	0.049	0.126
rs28365964	8	73920883	*TERF1*	T	−0.270	0.035	0.249	0.219	0.256
rs12415148	10	105680586	*OBFC1*	T	−0.204	0.020	0.005	0.074	0.947
rs7095953	10	101274425	*NKX2-3*	C	−0.047	0.009	0.059	0.048	0.222
rs227080	11	108247888	*ATM*	G	−0.060	0.009	0.068	0.066	0.102
rs2302588	14	73404752	*DCAF4*	G	−0.042	0.011	0.115	0.076	0.035
rs41293836	14	24721327	*TINF2*	C	−0.233	0.017	0.126	0.058	0.029
rs2967374	16	82209861	*MPHOSPH6*	G	−0.056	0.012	−0.041	0.083	0.704
rs1001761	18	662103	*TYMS*	A	−0.042	0.010	−0.101	0.090	0.256
rs7253490	19	22293706	*ZNF208, ZNF257, ZNF676*	C	−0.036	0.010	−0.051	0.051	0.317
rs41309367	20	62309554	*RTEL1*	T	−0.058	0.010	0.064	0.101	0.529

Chr, chromosome number; CKD, chronic kidney disease; LTL, leukocyte telomere length; OR odds ratio; SE, standard error.

Study design and cohorts

This is a cross-sectional study. We utilized a two-sample MR framework using two non-overlapping cohorts. We used summary statistic of a GWAS of LTL in the SCHS [23]. The association of SNPs with CKD was estimated in two independent T2D cohorts in Khoo Teck Puat Hospital: The Diabetes Nephropathy (DN) [24] and the Singapore Study of Macro-angiopathy and Micro-vascular Reactivity in T2D (SMART2D) [25] cohorts. Briefly, the DN is an on-going study including 4590 participants (age 21 years and above) recruited between March 2004 and December 2017, and the SMART2D dataset is a prospective cohort with baseline recruitment of 2052 T2D participants (age 21 years and above) between August 2011 and February 2014. Genotyping for the SMART2D and DN cohorts were carried using Illumina Humanomniexpress-24 Bead Chip and Illumina HumanOmniZhonghua Bead Chip, respectively, and quality control procedures have been described previously [26, 27]. An additional 253 Chinese and 245 Malay samples from the DN studies were genotyped using the Illumina GSA array, and quality control procedures are indicated in Supplementary data, Table S1. The estimated glomerular filtration rate (eGFR) in the DN and SMART2D datasets was calculated using CKD Epidemiology Collaboration equation and CKD was defined as eGFR <60 mL/min/1.73 m^2. In this study, only participants with information on renal condition and genotype data were included (DN: Chinese = 2459, Malay = 837; SMART2D: Chinese = 1033, Malay = 439) (Supplementary data, Figure S1). Written informed consent was obtained from each participant, and the study has been approved by the National Healthcare Group Domain Specific Review Board in Singapore.

Statistical analysis

Association of LTL shortening SNPs with CKD. The association of each IV with CKD in the Khoo Teck Puat Hospital (KTPH) cohort was determined by logistic regression adjusted for age and sex. Analysis was first performed separately in the DN and SMART2D, stratified by ethnic group and pooled using a random effect meta-analysis (βSNP–CKD). Heterogeneity in meta-analyzed data was determined using I^2 statistic and Cochran's Q P-value (P_{het}) <0.05 was determined to be significantly heterogeneous.

MR analysis. The SNP–LTL (βSNP–LTL) and SNP–CKD (βSNP–CKD) coefficients were combined using an inverse-variance weighted (IVW) method to give an overall estimate of the causal effect. This method assumes that all the SNPs included are valid instruments, and the effect size represents a weighted average of Wald ratio estimates derived from all the IVs [28]. The odds ratio (OR) from the weighted regression represents the increased risk of CKD per SD shortening in LTL. Heterogeneity in meta-analysed data was determined using I^2 statistic and P_{het} <0.05 was determined to be significantly heterogeneous.

Sensitivity analysis. The weighted median method and MR-Egger regression were performed to assess if the MR IVW estimates are biased and affected by violation of MR assumptions (i.e. horizontal pleiotropy) [29]. The weighted median method employs the weighted empirical distribution function of each SNP ratio estimate and provides a median value. This approach yields a consistent estimate of a true causal effect as long as >50% of SNPs are valid [29]. The MR-Egger regression was utilized to formally test for potential violations of MR assumptions. Intercept with $P > 0.05$ indicates no horizontal pleiotropy exists.

We also performed leave-one-out analysis, where each SNP was removed systematically, and IVW analysis was performed in the remaining 15 SNPs, to identify potentially influential SNP driving the association. All analysis was performed using R, version 3.1.2., and Stata released version 14.0 (StatCorp LP). The MendelianRandomization and RadialMR R package were used to perform MR and sensitivity analysis. P-values were two-sided, and evidence of association was declared at $P < 0.05$.

RESULTS

Among the 4768 T2D participants, the mean age [standard error (SE)] was 58.4 (11.7) years, 57.7% were male and 34.1% had CKD at baseline (Supplementary data, Table S2). The list of IVs for LTL and their pooled association with CKD from the DN and SMART2D cohorts using random-effect IVW is shown in Table 1 and Supplementary data, Table S3. Of the 16 SNPs, rs41293836 ($\beta = 0.126$, SE = 0.058, P = 0.029) and rs2302588 ($\beta = 0.115$, SE = 0.076, P = 0.035) were associated with increased risk of CKD.

Primary MR analysis using IVW method demonstrated that shorter genetically predicted LTL was associated with increased risk of CKD [OR = 1.51, 95% confidence interval (CI) 1.12–2.12, P = 0.007, $P_{het} = 0.547$] (Table 2 and Figure 1). Similar observation was obtained using the weighted median analysis (OR = 1.52, 95% CI 1.03–2.24, P = 0.035). The MR-Egger regression showed no evidence of directional pleiotropy (intercept $\beta = 0.010$, SE = 0.028, P = 0.715). Radial MR approach also did not reveal

Table 2. MR for LTL on CKD

	N SNPs	OR (95% CI)[a]	P-value	P_{het}
Inverse-variance weighted	16	1.51 (1.12–2.12)	0.007	0.547
Weighted median	16	1.52 (1.03–2.24)	0.035	–
MR-Egger	16	1.38 (0.82–2.35)	0.220	0.481
Intercept[b]	–	0.010 (0.028)	0.715	–

[a]OR per 1-SD shortening in LTL. [b]Intercept is presented as β coefficients with SEs. Model adjusted for age and sex. P_{het} represents Cochran's Q P-value after meta-analysis. N SNPs, number of SNPs.

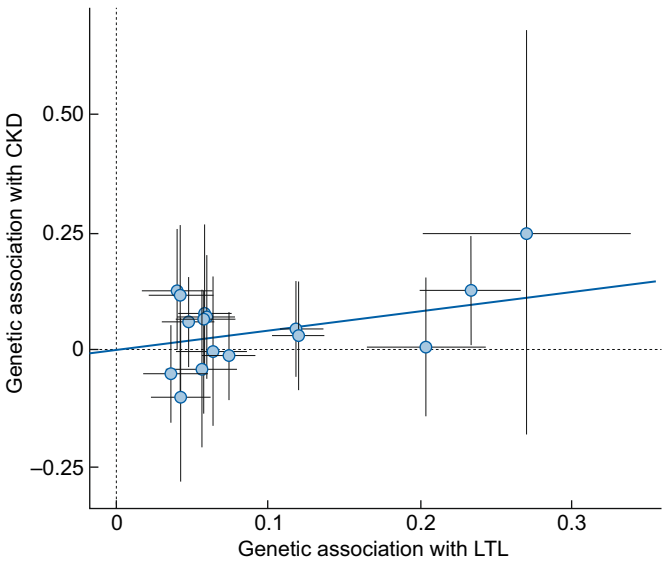

FIGURE 1: Scatter plot to visualize the causal effect of LTL on CKD.

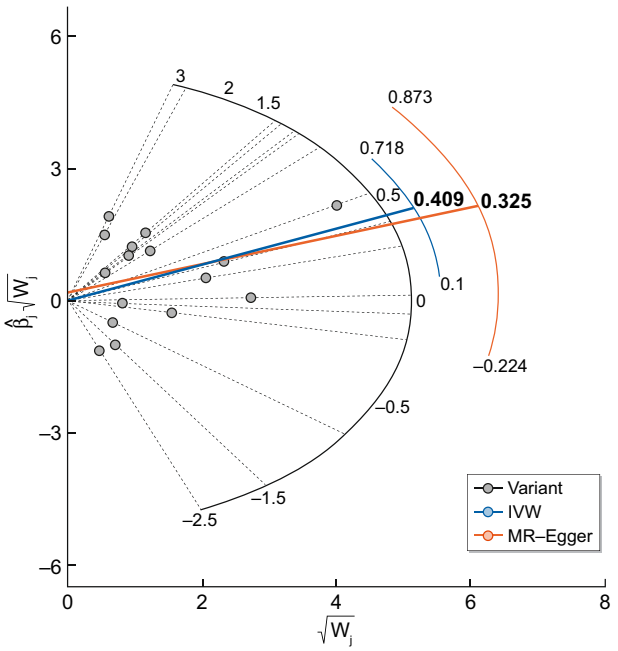

FIGURE 2: Radial plot to visualize individual outlier SNPs in the MR estimates for CKD. Black dots show valid SNPs and green dots (if any) display invalid outlier SNPs. The curved black line represents ratio estimates of each SNPs.

evidence of outlying genetic variants, in agreement with the MR-Egger regression analysis (Figure 2).

We further performed leave-one-out analysis to explore whether the associations between genetically determined LTL and CKD were driven by particular SNPs. Compared with the observed results (OR = 1.51) from 16 SNPs, the ORs fluctuated from 1.39 to 1.63, and the largest decrease and increase in OR was observed after removing rs41293836 and rs12415148, respectively. However, only removal of rs41293836 [near TERF1-interacting nuclear factor 2 (*TINF2*)] attenuated the association of LTL and CKD (P = 0.080), suggesting *TINF2* may drive the IVW point estimate (Supplementary data, Figure S2). Among the 16 SNPs, data on association for eight of these SNPs with HbA1c levels in the East Asian population were available in the MAGIC (Meta-Analysis of Glucose and Insulin-related traits Consortium) study. MR analysis found no causal relation between LTL and HbA1c levels ($\beta = 0.022$, SE = 0.035, P = 0.534). We calculated the power for this study with the assumption that the proportion of LTL variance explained by all 16 SNPs is $R^2 = 4\%$ and with type 1 error of 0.05. Using mRnd (https://shiny.cnsgenomics.com/mRnd/), this study had 82% power to detect per allele effect of LTL on CKD with corresponding OR of 1.50, at a significant level of 0.05.

DISCUSSION

In this study, we used a two-sample MR framework to demonstrate that shorter genetically predicted LTL was also associated with increased risk of CKD in patients with T2D. This finding was robust and consistent in the sensitivity analysis. Leave-one-out analysis suggests rs41293836 near *TINF2* may drive the observed association between genetically determined LTL and CKD.

Our findings are consistent with observational studies where shorter LTL was associated with renal dysfunction cross-sectionally [16, 18] and prospectively [17, 30]. Specifically, in the meta-analysis of MMKD (Mild to Moderate Kidney Disease; $n = 166$) and CRISIS (Chronic Renal Insufficiency Standards Implementation; $n = 889$) cohorts, shorter LTL was significantly associated with increased risk for CKD progression in diabetic patients but not in non-diabetic patients [17]. These results are in contrast to a study demonstrating that the association between LTL and CKD was entirely explained by age [31]. These inconsistent findings may be due to residual confounding or biased by reverse causation in conventional observational studies. To the best of our knowledge, this is the first MR analysis investigating the potential causal relationship between LTL and CKD in East Asians with T2D. A previous MR study performed in non-diabetic Europeans reported a lack of causal relationship between LTL and kidney function defined using continuous traits (creatinine, albuminin and cystatin) in the general population [32]. Moreover, the analysis with CKD was not reported. This difference might reflect different pathophysiologic mechanisms behind CKD in T2D and the general population, or could be due to different selection and strength of the IVs used in this study. We also found that the association observed in our study seems to be driven by rs41293836 near *TINF2/TGM1* loci in chromosome 14, which is monomorphic or rare in the European population but polymorphic and common in the Chinese population. Additionally, given that the diabetic condition is associated with an elevated level of oxidative stress and inflammation, factors that also accelerate telomere shortening and aging [33], it is possible that impact of telomere shortening on renal function is exacerbated in the diabetic condition when compared with non-diabetics. Further studies are warranted to elucidate the exact role of telomere length in CKD in diabetic population.

Examination of the associations between individual LTL genetic risk loci and CKD highlighted *TINF2* as the main driver of the association. Several studies have identified deleterious mutations in *TINF2* in patients with short telomere syndrome diseases such as dyskeratosis congenita and idiopathic pulmonary fibrosis [34–36]. TINF2 is a component of the telomere shelterin protein complex and regulates telomerase activity [37]. In germline and stem cells, telomerase activity is essential for the maintenance of telomere length and therefore, cell renewal capacity. However, the role of TINF2 in CKD in patients with T2D has not yet been clearly demonstrated. In human kidney, telomere length decreases more rapidly in the renal cortex than in the medulla during aging [2], contributing to the cortical scarring and glomerular senescence observed in aging kidneys. Using mice model, Westhoff *et al.* [38] showed that shorter telomere contributed to increased renal injury and decreased recovery after insult. Therefore, it is likely that shorter leukocyte telomere, as a result of increased oxidative stress and chronic inflammation, may reflect a state of compromised immune response and increased susceptibility to renal injury. Alternatively, as LTL is correlated with intrarenal telomere length ($r = 0.4$, P = 0.001) [39], it is also likely that shorter telomeres increase the likelihood of chromosomal damage, leading to cellular senescence or apoptosis and renal damage.

The strengths of this study are the robust genetic instrument identified in the same population explaining ~4% of the variance in LTL (double the phenotypic variance identified in Europeans previously) and the use of multiple MR methods with different assumptions. Moreover, we used two-sample MR where IV and the estimation of the IV with CKD in patients with T2D were derived from two independent populations, reducing bias in the causal estimate [40]. However, this study also has some limitations. First, the IV was derived for blood telomere

length and not telomere length in renal tissues. However, studies have shown that LTL correlates with telomere length in other tissues, including renal tissues ($r = 0.4$, $P = 0.001$) [6, 39, 41]. Second, pooled data using random-effect meta-analysis from the Chinese and Malay T2D participants were included in MR analysis to increase the sample size and hence statistical power. Third, the SNPs selected as IVs were derived mainly from the non-diabetic population, which may potentially reduce the validity of the measure in our study. However, we have shown that in a subset of KTPH Chinese T2D subjects ($n = 1602$), 12 SNPs were directionally consistent in T2D population (binomial $P = 0.028$) and the top hit at chromosome 14 (rs41293836) showed a statistically significant association with LTL [23]. Lastly, given that individual-level telomere length data were not available for all the participants in our study population, we were not able to assess if the IV is associated with CKD independent of its effect on telomere length. Although we performed sensitivity analysis and demonstrated the absence of directional pleiotropic effects, we cannot completely exclude the possibility.

In summary, we demonstrated a potential role of LTL in the development of CKD in East Asians with T2D. However, further studies in larger-scale East Asian T2D populations are warranted to validate our findings and elucidate the causal role of telomere length in CKD progression. With potential therapies to minimize premature leukocyte telomere shortening available [42, 43], preventing premature telomere shortening may provide a strategy to prevent CKD and reduce the public burden of diabetes-related complications.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during this study are available from the corresponding author on reasonable request.

SUPPLEMENTARY DATA

Supplementary data are available at ckj online.

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AUTHORS' CONTRIBUTIONS

R.L.G. and S.C.L. designed the study. R.L.G., Y.M., S.L., J.-J.L., Y.M.S., K.A. and S.C.L. contributed to the recruitment, sample collection and data acquisition. R.D., L.W., Y.C. and X.S. generated the genotyping data. R.L.G. and R.D. performed the data and statistical analysis. R.L.G. drafted the manuscript. All authors contributed important intellectual content, revised the manuscript critically and have approved the submission of the manuscript for publication. S.C.L. is the guarantor of this work and has full access to all the data in the study.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial and/or non-financial interests in relation to the work described. The results presented in this article have not been published previously in whole or part.

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