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

**Methods.** The cross-sectional association of 16 leukocyte telomere length SNPs with CKD, defined as an estimated glomerular filtration rate of less than 60ml/min/1.73m$^2$ was assessed among 4,768 (1,628 cases, 3,140 controls) participants in the Singapore Study of Macro-angiopathy and Micro-vascular Reactivity in Type 2 Diabetes 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-analysed.

**Results.** Genetically determined shorter leukocyte telomere length 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; $P = 0.007$; $P_{\text{het}} = 0.547$]). Similar results were obtained following sensitivity analysis. MR-Egger analysis (intercept) suggested no evidence of horizontal pleiotropy ($\beta = 0.010$, $P = 0.751$).

**Conclusions.** Our findings suggest that genetically determined leukocyte telomere length 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 randomisation analysis, Telomere length, type 2 diabetes
INTRODUCTION

Telomeres are DNA-protein structures at the ends of chromosomes and 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 ageing (5). Leukocyte telomere length, 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 ageing-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 T2D (12-15). Observational studies have demonstrated inverse associations between leukocyte telomere length 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, leukocyte telomere length is modulated by oxidative stress as well as inflammation, obesity, genetic and environmental factors (20, 21). Therefore, it is uncertain whether shorter leukocyte telomere length is causally associated with DKD.

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, 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: 1) the selected SNPs are associated with exposure (telomere length); 2) the selected SNPs are not associated with confounders; and 3) 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 studies (GWAS) in the Singapore Chinese Health Study (SCHS) cohort identified sixteen SNPs associated with Leukocyte telomere length (23). In the present study, we performed two-sample MR with summary statistics of SNP-leukocyte telomere length associations from the SCHS cohort and SNP-DKD association determined in this study to investigate the causal relationship between leukocyte telomere length and CKD in patients with T2D.
MATERIALS AND METHODS

SNP selection

Ten SNPs robustly associated ($P < 5 \times 10^{-8}$) with leukocyte telomere length in Singapore Chinese population (N=25,273; mean age= 55 years) and additional six independent SNPs identified after meta-analysis with European cohorts (n= 37,505) (23) were selected as instrument variables (IVs). These sixteen 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 sixteen SNPs selected as IV for Leukocyte telomere length and the coefficient estimate for leukocyte telomere length ($\beta_{\text{LTL}}$) are shown in Table 1. Together, these sixteen SNPs explained approximately 4% of the variation in Leukocyte telomere length in the Singaporean Chinese population (23). The beta estimate reflects changes in standard deviation (SD) of the standardized levels of leukocyte telomere length adjusted for age, sex and principal components.

Study design and cohorts

This is a cross-sectional study. We utilized two-sample MR framework using two non-overlapping cohorts. We used summary statistic of a GWAS of leukocyte telomere length in the Singapore Chinese Health Study (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 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation and CKD was defined as eGFR less than 60ml/min/1.73m$^2$. In this study, only participants with information on renal condition and genotype

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data were included (DN: Chinese = 2459, Malay = 837; SMART2D: Chinese = 1033, Malays = 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 (DSRB) in Singapore.

Statistical Analysis

Association of leukocyte telomere length shortening SNPs with CKD

The association of each IV with CKD in the 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 (\( \beta_{\text{SNP-CKD}} \)). Heterogeneity in meta-analyzed data was determined using \( I^2 \) statistic and Cochran’s Q P-value (\( P_{\text{het}} \)) < 0.05 was determined to be significantly heterogenous.

Mendelian randomization analysis

The SNP-leukocyte telomere length (\( \beta_{\text{SNP-LTL}} \)) and SNP-CKD (\( \beta_{\text{SNP-CKD}} \)) 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 leukocyte telomere length. Heterogeneity in meta-analyzed data was determined using \( I^2 \) statistic and Cochran’s Q P-value (\( P_{\text{het}} \)) < 0.05 was determined to be significantly heterogenous.

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 more than 50% of SNPs are valid (29). The MR-Egger regression was utilised 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 fifteen SNPs, to identify potentially influential SNP driving the association. All analysis was performed using R, version 3.1.2. and Stata released 14.0 (StatCorp LP). The MendelianRandomisation and RadialMR R package were
used to perform MR and sensitivity analysis. *P* values were 2-sided, and evidence of association was declared at *P* < 0.05.

**RESULTS**

Among the 4,768 T2D participants, the mean age [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 instrumental variables for leukocyte telomere length and their pooled association with CKD from the DN and SMART2D cohorts using random-effect inverse-variance weighted are shown in Table 1 and Supplementary data, Table S3. Of the sixteen SNPs, rs41293836 (β = 0.126, SE = 0.058, *P* = 0.029) and rs2302588 (β = 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 leukocyte telomere length was associated with increased risk of CKD (OR = 1.51, 95%CI 1.12 - 2.12, *P* = 0.007; *P* _het_ = 0.547) (Table 2 and Fig.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 β = 0.010, SE = 0.028, *P* = 0.715). Radial MR approach also did not reveal evidence of outlying genetic variants, in agreement with the MR-Egger regression analysis (Fig.2).

We further performed leave-one-out analysis to explore whether the associations between genetically determined leukocyte telomere length and CKD was 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 TINF2) attenuated the association of leukocyte telomere length and CKD (*P* = 0.080), suggesting TINF2 may drive the IVW point estimate (Supplementary data, Figure S2). Among the sixteen SNPs, data on association for eight of these SNPs with HbA1c levels in East Asian population was available in the MAGIC study. MR analysis found no causal relation between leukocyte telomere length and HbA1c levels (β = 0.022, SE = 0.035, *P* = 0.534). We calculated the power for this study with the assumption that the proportion of leukocyte telomere length variance explained by all sixteen SNPs is R² = 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 leukocyte telomere length 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 leukocyte telomere length was also associated with increased risk of CKD in patients with T2D. This finding was robust and consistent in the sensitivity analysis. Leave-out-one analysis suggests rs41293836 near TINF2 may drive the observed association between genetically determined leukocyte telomere length and CKD.

Our findings is consistent with observational studies where shorter leukocyte telomere length was associated with renal dysfunction cross-sectionally (16, 18) and prospectively (17, 30). Specifically, in the meta-analysis of MMKD (n = 166) and CRISIS cohort (n = 889), shorter leukocyte telomere length 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 leukocyte telomere length 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 analyses investigating the potential causal relationship between leukocyte telomere length and CKD in East Asians with T2D. A previous MR study performed in non-diabetic Europeans reported a lack of causal relationship between leukocyte telomere length and kidney function defined using continuous traits (creatinine, albumin and cystatin) in the general population (32). Moreover, the analysis with CKD was not reported. This difference might reflect different pathophysiologic mechanism behind CKD in T2D, and 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 European population but polymorphic and common in the Chinese population. Additionally, given that diabetes condition is associated with an elevated level of oxidative stress and inflammation, factors that also accelerate telomere shortening and ageing (33), it is possible that impact of telomere shortening on renal function is exacerbated in diabetic condition as compared to non-diabetic. Further studies are warranted to elucidate the exact role of telomere length in CKD in diabetic population.

Examination of the associations between individual leukocyte telomere length genetic risk loci and CKD highlighted TERF1 interacting nuclear factor 2 (TINF2) as the main driver of the association. Several studies have identified deleterious mutations in TINF2 in patients with short telomere length.
syndrome diseases such as dyskeratosis congenita (DKC) 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 ageing (2), contributing to the cortical scarring and glomerular senescence observed in ageing kidneys. Using mice model, Westhoff et al. showed that shorter telomere contributed to increased renal injury and decreased recovery after insult (38). Therefore, it is likely that shorter leukocyte telomere, as a result of increased oxidative stress and chronic inflammation, may reflects a state of compromised immune response and increased susceptibility to renal injury. Alternatively, as leukocyte telomere length 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 approximately 4% of the variance in leukocyte telomere length (doubled 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 was derived from two independent populations, reducing bias in the causal estimate (40). However, this study also has some limitations. Firstly, the IV was derived for blood telomere length and not telomere length in renal tissues. However, studies have shown that leukocyte telomere length correlates with telomere length in other tissues, including renal tissues \( r = 0.4, P = 0.001 \) (6, 39, 41). Secondly, 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. Thirdly, the SNPs selected as IVs were derived mainly from 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 type 2 diabetic subjects \( n = 1602 \), twelve SNPs were directionally consistent in type 2 diabetic population (binomial \( P = 0.028 \)) and the top hit at chromosome 14 (rs41293836) showed statistically significant association with leukocyte telomere length (23). Lastly, given that individual-level telomere length data was not available for all the participants in our study population, we were not able to assess if the instrumental variable 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 leukocyte telomere length in the development of CKD in East Asians with T2D. However, further studies in larger-scale East Asian T2D population is warranted to validate our findings and elucidate the causal role of telomere length in CKD progression. With potential therapies to minimise 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.

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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 genotyping data; R.L.G and R.D performed data and statistical analysis. R.L.G drafted the manuscript. All authors contributed important intellectual content and revised the manuscript critically. All authors 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 paper have not been published previously in whole or part.
DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.
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Table 1. SNPs selected as instrumental variable and its association with leukocyte telomere length and CKD

| SNP       | Chr | Position (hg19) | Gene     | Test Allele | SNP-LTL β   | SE (β) | log_e (OR) | SE   | P     |
|-----------|-----|-----------------|----------|-------------|--------------|--------|------------|------|-------|
| 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 |
| rs777644  | 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       | NIKX2-3  | 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 |

*LTL* leukocyte telomere length, *Chr* chromosome number, *TA* test allele, *OR* odds ratio, *SE* standard error, *CKD* chronic kidney disease.
Figure 1. Scatter plot to visualise the causal effect of Leukocyte telomere length on CKD.

Each data point (black dot) represents a single-nucleotide polymorphism selected as instrumental variable. Vertical and horizontal lines centred at each data point represent 95% confidence intervals for each association. The slope of the (bold) line is the instrumental variable regression estimate of the effect of leukocyte telomere length on CKD risk. **CKD** chronic kidney disease, **LTL** leukocyte telomere length.
Table 2. Mendelian randomization for leukocyte telomere length on CKD

|                      | N SNPs | *OR (95% CI)      | P   | 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*            |        | 0.010 (0.028)     | 0.715 |         |

*Odds ratio per 1-SD shortening in Leukocyte telomere length. *Intercept is presented as β coefficients with SEs. Model adjusted for age and sex. P_{het} represent Cochran’s Q P-value after meta-analysis. N SNPs number of SNPs, OR odds ratio; and P_{het}, Cochrane’s Q stats P-value.
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. (-----) represents ratio estimates of each SNPs. IVW inverse variance weighted.
