**Supplementary information**

Telomere length measurements

DNA was extracted using phenol-chloroform methods. DNA (25 ng) was suspended in 10ul of either telomere or single copy gene human beta-globin (HBG) PCR reaction mixture. The telomere reaction mixture consisted of: 5 µl 2x Fast SYBR Green master mix (Life Technologies, Carlsbad, CA), 2 µl nuclease‐free water, 1 µl 1µM telomere forward primer (CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT), and 1 µl 3µM telomere reverse primer (GGCTTG CCTTACCCTTACCCTTACCCTTACCCTTACCCT). The HBG reaction consisted of: 5 µl same master mix, 2 µl water, 1 µl 3µM HBG forward primer (GCTTCTGACACAACTGTGTTCACTAGC), and 1 µl 7µM HBG reverse primer (CACCAACTTCATCCACGTTCACC). Both reactions lasted for 3min (95°C), followed by 40 cycles (15s, 95°C), for 1min at 56°C (telomere) or 58°C (HBG) on an Applied Biosystems ViiA™ 7 Real‐Time PCR System (Life Technologies Corporation, Carlsbad, CA) using 96-well plates. All primers were obtained from Integrated DNA Technologies (Singapore).

A no-template control (water, NTC) and a reference human sample (QC) were included in all plates to adjust for plate-to-plate variability (no more than ±1 cycle), and as the reference to calculate ∆∆Ct, separately. The QC sample was from a ~40-year-old male with hemochromatosis undergoing therapeutic venesection. All samples for telomere and HBG were performed in triplicate on the same plate. The ∆∆Ct was calculated as follows: ∆∆Ct = (Sample Mean HBG CT – Sample Mean Telomere CT) – (NTC (or QC) Mean HBG CT – NTC (or QC) Mean Telomere CT). The coefficients of variation (CV) of each sample was <2.5% and the inter-plate CVs of the telomere and HBG assays were 2.9% and 1.2%, respectively. The overall intra-plate CV was 1.2% for telomere length and 0.4% for HBG. Samples with CV >2.5% between replicates were repeated for both telomere and HBG measurements.

Since rLTL measurements do not reflect the actual (absolute) LTL, we were asked to test alternate commercially available methods. Although whole genome sequencing data could, in our view, provide the most accurate measure, these were unavailable to us at the time. A reference DNA sample with known absolute telomere length from a commercially available kit (ScienCell Research Laboratories, California, USA, Catalog #8918) was therefore utilized to calculate absolute telomere length in our samples. Using the qPCR method described earlier, serial dilution of the reference DNA was undertaken to develop a standard curve, which was used to extrapolate absolute telomere length based on Ct difference between telomere and single copy gene. This was used to calculate the absolute telomere length of the QC sample. We then extrapolated our rLTL QC/samples to obtain an estimated absolute telomere length for all other samples with rLTL measured.

Table S1. The comparison between all subjects and subjects without missing data

|  |  |  |  |
| --- | --- | --- | --- |
| Variables | Entire dataset, including subjects with missing data (n=5349) | Sub-group of subjects without missing data (n=4138) | P value |
| Age (years) | 57.5 ± 13.3 | 57.9 ± 13.2 | 0.159 |
| Sex (male, %) | 45.2% | 45.7% | 0.645 |
| Smoking status |  |  |  |
| Current (%) | 13.1% | 12.9% | 0.853 |
| Ever smoked (%) | 30.1% | 30.4% | 0.785 |
| Duration of diabetes (years) | 7.1 ± 6.8 | 7.0 ± 6.8 | 0.446 |
| BMI (kg/m2) | 25.3 ± 4.0 | 25.3 ± 4.1 | 0.636 |
| SBP (mmHg) | 135.1 ± 21 | 135.1 ± 21.0 | 0.960 |
| DBP (mmHg) | 75.5 ± 11.0 | 75.3 ± 11.0 | 0.355 |
| HbA1c (% / mmol/mol) | 7.6 ± 1.8 / 59.9 ± 19.7 | 7.6 ± 1.8 / 59.2 ± 19.5 | 0.108 / 0.105 |
| FPG (mmol/L) | 8.6 ± 3.3 | 8.5 ± 3.3 | 0.356 |
| TC (mmol/L) | 5.2 ± 1.2 | 5.1 ± 1.1 | **0.004** |
| HDL-C (mmol/L) | 1.3 ± 0.4 | 1.3 ± 0.4 | **0.008** |
| LDL-C (mmol/L) | 3.1 ± 1.0 | 3.1 ± 1.0 | 0.174 |
| TG (mmol/L) | 1.4 (1.0-2.1) | 1.4 (1.0-2.0) | **<0.001\*** |
| ACR (mg/mmol) | 2.3 (0.8-12.8) | 2.1 (0.8-11.7) | 0.203\* |
| eGFR (mL/min/1.73 m2) | 4.6 ± 0.6 | 4.6 ± 0.6 | 0.867 |
| Diagnosed comorbidity |  |  |  |
| Retinopathy (%) | 28.3% | 28.2% | 0.917 |
| Neuropathy (%) | 20.8% | 20.6% | 0.835 |
| Microalbuminuria (%) | 26.9% | 26.7% | 0.795 |
| Macroalbuminuria (%) | 18.1% | 17.2% | 0.256 |
| Use of medications |  |  |  |
| Lipid lowering drugs (%) | 18.8% | 20.1% | 0.131 |
| Antihypertensive drugs (%) | 47.5% | 50.2% | **0.009** |
| Oral antihyperglycemic drugs (%) | 66.8% | 71.8% | **<0.001** |
| Insulin use (%) | 17.0% | 16.8% | 0.785 |
| RAS inhibitors (ACEIs or ARBs) (%) | 22.2% | 23.0% | 0.332 |

All data are expressed as mean ± SD, median (Q1-Q3) or proportion in %. \*Natural logarithmic transformation was used in TG and ACR.

Abbreviation: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, Hemoglobin A1c; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; ACR, urinary albumin to creatinine ratio.

Table S2. The comparison of baseline characteristics between subjects with or without CVD at baseline

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Variables | Total (n=5349) | Subjects without CVD at baseline (n=4541) | subject with CVD at baseline (n=808) | P value | P value (Adjusted for age and sex) |
| Age (years) | 57.5 ± 13.3 | 56.1 ± 13.2 | 65.7 ± 10.8 | **<0.001** | - |
| Sex (male, %) | 45.2% | 44.5% | 49.1% | **0.015** | - |
| Smoking status |  |  |  |  |  |
| Current (%) | 13.1% | 13.3% | 11.6% | 0.196 | 0.861 |
| Ever smoked (%) | 30.1% | 28.8% | 37.7% | **<0.001** | **0.003** |
| Duration of diabetes (years) | 7.1 ± 6.8 | 6.7 ± 6.6 | 9.3 ± 7.6 | **<0.001** | **<0.001** |
| BMI (kg/m2) | 25.3 ± 4.0 | 25.3 ± 4.1 | 25.1 ± 3.7 | 0.159 | 0.245 |
| SBP (mmHg) | 135.1 ± 21.0 | 133.9 ± 20.2 | 142.0 ± 23.5 | **<0.001** | **<0.001** |
| DBP (mmHg) | 75.5 ± 11.0 | 75.8 ± 10.9 | 74.0 ± 11.4 | **<0.001** | **0.005** |
| HbA1c (% / mmol/mol) | 7.6 ± 1.8 / 59.9 ± 19.7 | 7.6 ± 1.8 / 59.7 ± 19.7 | 7.7 ± 1.8 / 60.8 ± 19.5 | 0.130 / 0.130 | 0.069 / 0.069 |
| FPG (mmol/L) | 8.6 ± 3.3 | 8.6 ± 3.3 | 8.6 ± 3.6 | 0.536 | 0.130 |
| TC (mmol/L) | 5.2 ± 1.2 | 5.2 ± 1.1 | 5.1 ± 1.2 | 0.372 | 0.300 |
| HDL-C (mmol/L) | 1.3 ± 0.4 | 1.3 ± 0.4 | 1.3 ± 0.4 | **0.001** | **<0.001** |
| LDL-C (mmol/L) | 3.1 ± 1.0 | 3.1 ± 1.0 | 3.0 ± 1.1 | 0.092 | 0.070 |
| TG (mmol/L) | 1.4 (1.0-2.1) | 1.5 (1.1-2.2) | 1.5 (1.1-2.1) | **<0.001\*** | **0.001\*** |
| eGFR (mL/min/1.73 m2) | 79.3 ± 26.1 | 82.1 ± 25.1 | 63.3 ± 25.8 | **<0.001** | **<0.001** |
| ACR (mg/mmol) | 2.0 (0.8-10.1) | 2.0 (0.8-10.1) | 4.9 (1.1-42.0) | **<0.001\*** | **<0.001\*** |
| Diagnosed comorbidity |  |  |  |  |  |
| Retinopathy (%) | 28.3% | 25.8% | 42.5% | **<0.001** | **<0.001** |
| Neuropathy (%) | 20.8% | 18.4% | 34.4% | **<0.001** | **<0.001** |
| Microalbuminuria (%) | 25.9% | 25.80% | 26.60% | 0.564 | 0.173 |
| Macroalbuminuria (%) | 17.5% | 15.4% | 28.8% | **<0.001** | **<0.001** |
| Use of medications |  |  |  |  |  |
| Lipid lowering drugs (%) | 18.8% | 15.5 | 37.5% | **<0.001** | **<0.001** |
| Antihypertensive drugs (%) | 47.5% | 43.1% | 72.2% | **<0.001** | **<0.001** |
| Oral antihyperglycemic drugs (%) | 66.8% | 66.9% | 66.6% | 0.879 | **0.036** |
| Insulin use (%) | 17.0% | 15.5% | 25.4% | **<0.001** | **<0.001** |
| RAS inhibitors (ACEIs or ARBs) (%) | 22.2% | 19.8% | 35.6% | **<0.001** | **<0.001** |
| rLTL | 4.6 ± 1.2 | 4.6 ± 1.2 | 4.3 ± 1.2 | **<0.001** | **<0.001** |

All data are expressed as mean ± SD, median (Q1-Q3) or proportion in %; All comparison was adjusted for the differences of age and sex by using either general linear model for continuous data or logistic regression model for categorical data; \*natural logarithmic transformation was used in TG and ACR.

Abbreviation: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, Hemoglobin A1c; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; ACR, urinary albumin to creatinine ratio; rLTL, relative leukocyte telomere length.

**Table S3. Pearson and partial correlation between** rLTL a**nd baseline characteristics**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Baseline variables | Total cohort |  | Total cohort |  | Total cohort  |  | Female |  | Male |  |
|  | N=5349 |  | adjusted for age |  | adjusted for age and sex |  | N=2930; adjusted for age |  | N=2419; adjusted for age |  |
|  | R | P value | r | P value | r | P value | r | P value | r | P value |
| Age (years) | -0.131 | **<0.001** | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| Duration of diabetes (years) | -0.098 | **<0.001** | -0.059 | **<0.001** | -0.061 | **<0.001** | -0.070 | **<0.001** | -0.052 | **0.015** |
| BMI (kg/m2) | 0.035 | **0.010** | 0.015 | 0.280 | 0.015 | 0.284 | -0.012 | 0.549 | 0.047 | **0.026** |
| SBP (mmHg) | -0.121 | **<0.001** | -0.074 | **<0.001** | -0.075 | **<0.001** | -0.080 | **<0.001** | -0.072 | **0.001** |
| DBP (mmHg) | -0.060 | **<0.001** | -0.069 | **<0.001** | -0.068 | **<0.001** | -0.066 | **0.001** | -0.070 | **0.001** |
| HbA1c (%) | -0.079 | **<0.001** | -0.082 | **<0.001** | -0.082 | **<0.001** | -0.063 | **0.001** | -0.105 | **<0.001** |
| FPG (mmol/L) | -0.085 | **<0.001** | -0.083 | **<0.001** | -0.083 | **<0.001** | -0.067 | **0.001** | -0.104 | **<0.001** |
| TC (mmol/L) | -0.070 | **<0.001** | -0.074 | **<0.001** | -0.076 | **<0.001** | -0.062 | **0.001** | -0.096 | **<0.001** |
| HDL-C (mmol/L) | 0.064 | **<0.001** | 0.061 | **<0.001** | 0.059 | **<0.001** | 0.052 | **0.007** | 0.068 | **0.001** |
| LDL-C (mmol/L) | -0.113 | **<0.001** | -0.117 | **<0.001** | -0.118 | **<0.001** | -0.109 | **<0.001** | -0.130 | **<0.001** |
| Ln(TG) | 0.004 | 0.766 | 0.006 | 0.682 | 0.005 | 0.694 | 0.009 | 0.637 | -0.003 | 0.879 |
| Ln(ACR) | -0.117 | **<0.001** | -0.082 | **<0.001** | -0.083 | **<0.001** | -0.065 | **0.001** | -0.102 | **<0.001** |
| eGFR (mL/min/1.73 m2) | 0.081 | **<0.001** | -0.016 | 0.267 | -0.017 | 0.233 | -0.006 | 0.749 | -0.028 | 0.182 |

Abbreviation: rLTL, relative leukocyte telomere length; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, Hemoglobin A1c; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; ACR, urinary albumin to creatinine ratio. Natural logarithmic transformation was used in TG and ACR.

Table S4. Cox regression analysis showing relationship between Z-score of rLTL and other risk factors for incident CVD in type 2 diabetes

|  |  |  |
| --- | --- | --- |
| Variables (Z-score) | HR (95%CI) | P |
| rLTL | 1.180 (1.111-1.254) | **<0.001** |
| HbA1c (%) | 1.195 (1.127-1.267) | **<0.001** |
| Age (years) | 1.519 (1.383-1.670) | **<0.001** |
| Sex (male) | 1.333 (1.150-1.545) | **<0.001** |
| Duration of diabetes (years) | 1.176 (1.110-1.245) | **<0.001** |
| Ever smoked | 1.363 (1.170-1.588) | **<0.001** |
| BMI (kg/m2) | 1.103 (1.034-1.176) | **0.003** |
| SBP (mmHg) | 1.097 (1.027-1.172) | **0.006** |
| LDL-C (mmol/L) | 1.105 (1.037-1.177) | **0.002** |
| eGFR (mL/min/1.73 m2) | 0.866 (0.792-0.947) | **0.002** |
| Ln (ACR) | 1.069 (1.012-1.130) | **0.017** |

HR, hazards ratio; rLTL, relative leukocyte telomere length; CVD, cardiovascular disease; BMI, body mass index; SBP, systolic blood pressure; HbA1c, Hemoglobin A1c; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; Ln(ACR), Ln transformation of urinary albumin to creatinine ratio.

**Table S5. Competing Risk Regression analysis showing subdistribution HRs of rLTL for incident CVD**

|  |  |  |
| --- | --- | --- |
| Subdistribution HRs | rLTL (each unit decrease) |  |
|  | sHR | P value |
| Model 1 | 1.233 (1.178-1.289) | **<0.001** |
| Model 2 | 1.183 (1.131-1.239) | **<0.001** |
| Model 3 | 1.164 (1.110-1.221) | **<0.001** |
| Model 4 | 1.145 (1.088-1.206) | **<0.001** |

sHR, subdistribution hazards ratio; rLTL, relative leukocyte telomere length; CVD, cardiovascular disease.

Model 1: without adjustment. Model 2: adjusted for age and sex. Model 3: Model 2 + adjusted for duration of diabetes, BMI, SBP and ever smoked. Model 4: Model 3 + adjusted for HbA1c, LDL-C, eGFR and Ln (ACR).

Table S6. Hazard ratio (95% CI) for incident cardiovascular diseases according to quartiles of rLTL

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Telomere length | Events/Total (%) | Model 1 |  | Model 2 |  | Model 3 |  | Model 4 |  |
|  |  | HR (95%CI) | P value | HR (95%CI) | P value | HR (95%CI) | P value | HR (95%CI) | P value |
| rLTL (each unit decrease) | 1140/4541 (25.1%) | 1.252 (1.195-1.311) | **<0.001** | 1.186 (1.132-1.242) | **<0.001** | 1.164 (1.110-1.221) | **<0.001** | 1.141 (1.084-1.200) | **<0.001** |
| Q1(<3.853) | 377/1071 (35.2%) | 2.144 (1.802-2.551) | **<0.001** | 1.800 (1.511-2.144) | **<0.001** | 1.689 (1.416-2.013) | **<0.001** | 1.559 (1.296-1.875) | **<0.001** |
| Q2(3.853-4.639) | 317/1116 (28.4%) | 1.680 (1.404-2.009) | **<0.001** | 1.508 (1.260-1.805) | **<0.001** | 1.417 (1.183-1.698) | **<0.001** | 1.322 (1.094-1.598) | **0.004** |
| Q3(4.640-5.326) | 252/1147 (22.0%) | 1.320 (1.095-1.592) | **0.004** | 1.177 (0.976-1.421) | **0.009** | 1.147 (0.950-1.386) | 0.154 | 1.111 (0.911-1.354) | 0.301 |
| Q4(>5.326) | 194/1207 (16.1%) | 1.000 (Reference) |  | 1.000 (Reference) |  | 1.000 (Reference) |  | 1.000 (Reference) |  |
| P trend |  |  | **<0.001** |  | **<0.001** |  | **<0.001** |  | **<0.001** |
| Female |  |  |  |  |  |  |  |  |  |
| rLTL (each unit decrease) | 559/2519 (22.2%) | 1.312 (1.227-1.404) | **<0.001** | 1.245 (1.164-1.332) | **<0.001** | 1.223 (1.141-1.310) | **<0.001** | 1.182 (1.098-1.272) | **<0.001** |
| Q1(<3.853) | 196/589 (33.4%) | 2.267 (1.773-2.898) | **<0.001** | 1.957 (1.529-2.504) | **<0.001** | 1.826 (1.424-2.341) | **<0.001** | 1.651 (1.274-2.140) | **<0.001** |
| Q2(3.853-4.639) | 149/628 (23.7%) | 1.579 (1.220-2.043) | **0.001** | 1.444 (1.115-1.870) | **0.005** | 1.327 (1.022-1.722) | **0.034** | 1.275 (0.971-1.673) | 0.080 |
| Q3(4.640-5.326) | 119/639 (18.6%) | 1.251 (0.955-1.639) | 0.103 | 1.181 (0.902-1.547) | 0.227 | 1.114 (0.848-1.462) | 0.439 | 1.105 (0.831-1.468) | 0.493 |
| Q4(>5.326) | 95/665 (14.3%) | 1.000 (Reference) |  | 1.000 (Reference) |  | 1.000 (Reference) |  | 1.000 (Reference) |  |
| P trend |  |  | **<0.001** |  | **<0.001** |  | **<0.001** |  | **<0.001** |
| Male |  |  |  |  |  |  |  |  |  |
| rLTL (each unit decrease) | 581/2022 (28.7%) | 1.194 (1.120-1.273) | **<0.001** | 1.136 (1.064-1.213) | **<0.001** | 1.115 (1.044-1.191) | **0.001** | 1.102 (1.026-1.183) | **0.008** |
| Q1(<3.853) | 181/484 (37.4%) | 2.020 (1.579-2.583) | **<0.001** | 1.658 (1.293-2.125) | **<0.001** | 1.555 (1.211-1.997) | **0.001** | 1.479 (1.136-1.926) | **0.004** |
| Q2(3.853-4.639) | 168/488 (34.4%) | 1.784 (1.391-2.289) | **<0.001** | 1.571 (1.222-2.018) | **<0.001** | 1.483 (1.153-1.907) | **0.002** | 1.369 (1.048-1.786) | **0.021** |
| Q3(4.640-5.326) | 133/508 (26.2%) | 1.390 (1.071-1.804) | **0.013** | 1.178 (0.906-1.531) | 0.220 | 1.162 (0.893-1.512) | 0.263 | 1.118 (0.846-1.477) | 0.432 |
| Q4(>5.326) | 99/542 (18.3%) | 1.000 (Reference) |  | 1.000 (Reference) |  | 1.000 (Reference) |  | 1.000 (Reference) |  |
| P trend |  |  | **<0.001** |  | **<0.001** |  | **<0.001** |  | **0.001** |

HR, hazards ratio; rLTL, relative leukocyte telomere length, categorized by quartiles of rLTL in whole cohort; CVD, cardiovascular disease; MI, myocardial infarction; CHD, coronary heart disease; CHF, congestive heart failure; CVA, cerebral vascular accident; PVD, peripheral vascular disease.

Model 1: without adjustment. Model 2: adjusted for age and sex. Model 3: Model 2 + adjusted for duration of diabetes, BMI, SBP and ever smoked. Model 4: Model 3 + adjusted for HbA1c, LDL-C, eGFR and Ln (ACR). When conducting regression models in male/female group, the category variable of sex was excluded.

Table S7. Hazards ratio of rLTL in relation to sub-group analysis according to age category and specific CVD endpoints.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Outcomes | Events/total (%) | Hazards ratio (95% CI) |  | P value\*  |
|  |  | Unadjusted model | Fully adjusted model  |  |
| Stratified by age |  |  |  |  |
| < 45 years | 110/931 (11.8%) | 1.195 (1.018-1.403) | 1.113 (0.935-1.323) | 0.227 |
| 45-59 years | 385/1704 (22.6%) | 1.233 (1.133-1.341) | 1.182 (1.080-1.295) | **<0.001** |
| 60-74 years | 520/1565 (33.2%) | 1.199 (1.120-1.284) | 1.146 (1.063-1.236) | **<0.001** |
| 75+ years | 125/341 (36.7%) | 1.113 (0.935-1.323) | 1.101 (0.942-1.289) | 0.228 |
| Specific CVD endpoints |  |  |  |  |
| MI | 318/5236 (6.1%) | 1.192 (1.091-1.303) | 1.019 (0.922-1.125) | 0.716 |
| CHD | 700/4967 (14.1%) | 1.256 (1.186-1.331) | 1.135 (1.065-1.210) | **<0.001** |
| CVA | 635/5196 (12.2%) | 1.229 (1.154-1.309) | 1.112 (1.039-1.191) | **0.002** |
| CHF | 577/5234 (11.0%) | 1.253 (1.174-1.337) | 1.092 (1.018-1.172) | **0.014** |
| PVD | 206/4996 (4.1%) | 1.128 (1.006-1.265) | 0.992 (0.873-1.127) | 0.905 |

\* P value for full adjustment; rLTL, relative leukocyte telomere length; CVD, cardiovascular disease; MI, myocardial infarction; CHD, coronary heart disease; CHF, congestive heart failure; CVA, cerebral vascular accident; PVD, peripheral vascular disease.

Full adjusted model included age, sex, duration of diabetes, BMI, SBP, ever smoked, HbA1c, LDL-C, eGFR and Ln (ACR).

Table S8. The interaction between relative telomere length and HbA1c on incident CVD

|  |  |  |
| --- | --- | --- |
| Variables | HR (95%CI) | P value |
| rLTL (each unit decrease) | 1.719 (1.373-2.152) | **<0.001** |
| HbA1c (%) | 0.870 (0.767-0.987) | **0.031** |
| Age (years)  | 1.034 (1.027-1.042) | **<0.001** |
| Sex (male)  | 1.332 (1.149-1.545) | **<0.001** |
| Duration of diabetes (years) | 1.021 (1.012-1.030) | **<0.001** |
| Ever smoked | 1.353 (1.161-1.577) | **<0.001** |
| BMI (kg/m2) | 1.020 (1.004-1.036) | **0.015** |
| SBP (mmHg) | 1.002 (0.999-1.006) | 0.185 |
| LDL-C (mmol/L) | 1.104 (1.036-1.177) | **0.002** |
| eGFR (mL/min/1.73 m2) | 0.997 (0.993-1.000) | 0.075 |
| Ln (ACR) | 1.120 (1.080-1.163) | **<0.001** |
| Interaction of rLTL and HbA1c | 1.054 (1.025-1.084) | **<0.001** |

HR, hazards ratio; rLTL, relative leukocyte telomere length; CVD, cardiovascular disease; BMI, body mass index; SBP, systolic blood pressure; HbA1c, Hemoglobin A1c; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; Ln(ACR), Ln transformation of urinary albumin to creatinine ratio.

Table S9. Cox regression showing the association between rLTL calculated based on QC materials and incident CVD

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Variables | **Model 1** |  | **Model 2** |  | **Model 3** |  | **Model 4** |  |
|  | HR (95% CI) | P | HR (95% CI) | P | HR (95% CI) | P | HR (95% CI) | P |
| rLTL (each unit decrease) | 1.331 (1.264-1.403) | **<0.001** | 1.231 (1.166-1.299) | **<0.001** | 1.208 (1.143-1.276) | **<0.001** | 1.166 (1.100-1.237) | **<0.001** |
|  |  |  | 1.045 (1.040-1.050) | **<0.001** | 1.035 (1.029-1.040) | **<0.001** | 1.034 (1.027-1.041) | **<0.001** |
| Sex (male) |  |  | 1.476 (1.314-1.658) | **<0.001** | 1.321 (1.147-1.521) | **0.024** | 1.330 (1.147-1.542) | **<0.001** |
| Duration of diabetes (years) |  |  |  |  | 1.033 (1.025-1.042) | **<0.001** | 1.021 (1.012-1.030) | **<0.001** |
| Ever smoked |  |  |  |  | 1.415 (1.222-1.637) | **<0.001** | 1.354 (1.161-1.578) | **<0.001** |
| BMI (kg/m2) |  |  |  |  | 1.023 (1.008-1.038) | **0.003** | 1.020 (1.004-1.036) | **0.013** |
| SBP (mmHg) |  |  |  |  | 1.007 (1.004-1.010) | **<0.001** | 1.002 (0.999-1.006) | 0.158 |
| HbA1c (%) |  |  |  |  |  |  | 1.088 (1.053-1.124) | **<0.001** |
| LDL-C (mmol/L) |  |  |  |  |  |  | 1.111 (1.042-1.185) | **0.001** |
| eGFR (mL/min/1.73 m2) |  |  |  |  |  |  | 0.997 (0.994-1.001) | 0.130 |
| Ln (ACR) |  |  |  |  |  |  | 1.122 (1.081-1.165) | **<0.001** |

QC, reference human sample; HR, hazards ratio; rLTL, relative leukocyte telomere length; CVD, cardiovascular disease; BMI, body mass index; SBP, systolic blood pressure; HbA1c, Hemoglobin A1c; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; Ln(ACR), Ln transformation of urinary albumin to creatinine ratio.

Model 1: without adjustment. Model 2: adjusted for age and sex. Model 3: Model 2 + adjusted for duration of diabetes, BMI, SBP and ever smoked. Model 4: Model 3 + adjusted for HbA1c, LDL-C, eGFR and Ln (ACR).

Table S10. Cox regression showing the association between estimated absolute telomere length and incident CVD

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Variables | **Model 1** |  | **Model 2** |  | **Model 3** |  | **Model 4** |  |
|  | HR (95% CI) | P | HR (95% CI) | P | HR (95% CI) | P  | HR (95% CI) | P |
| log2 (estimated absolute LTL) | 1.331 (1.264-1.403) | **<0.001** | 1.231 (1.166-1.299) | **<0.001** | 1.208 (1.143-1.276) | **<0.001** | 1.166 (1.100-1.237) | **<0.001** |
| Age (years) |  |  | 1.045 (1.040-1.050) | **<0.001** | 1.035 (1.029-1.040) | **<0.001** | 1.034 (1.027-1.041) | **<0.001** |
| Sex (male) |  |  | 1.476 (1.314-1.658) | **<0.001** | 1.321 (1.147-1.521) | **<0.001** | 1.330 (1.147-1.542) | **<0.001** |
| Duration of diabetes (years) |  |  |  |  | 1.033 (1.025-1.042) | **<0.001** | 1.021 (1.012-1.030) | **<0.001** |
| Ever smoked |  |  |  |  | 1.415 (1.222-1.637) | **<0.001** | 1.354 (1.161-1.578) | **<0.001** |
| BMI (kg/m2) |  |  |  |  | 1.023 (1.008-1.038) | **0.003** | 1.020 (1.004-1.036) | **0.013** |
| SBP (mmHg) |  |  |  |  | 1.007 (1.004-1.010) | **<0.001** | 1.002 (0.999-1.006) | 0.158 |
| HbA1c (%) |  |  |  |  |  |  | 1.088 (1.053-1.124) | **<0.001** |
| LDL-C (mmol/L) |  |  |  |  |  |  | 1.111 (1.042-1.185) | **0.001** |
| eGFR (mL/min/1.73 m2) |  |  |  |  |  |  | 0.997 (0.994-1.001) | 0.130 |
| Ln (ACR) |  |  |  |  |  |  | 1.122 (1.081-1.165) | **<0.001** |

HR, hazards ratio; estimated absolute LTL, absolute leukocyte telomere length calculated using reference DNA from the commercial kit; CVD, cardiovascular disease; BMI, body mass index; SBP, systolic blood pressure; HbA1c, Hemoglobin A1c; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; Ln(ACR), Ln transformation of urinary albumin to creatinine ratio.

Model 1: without adjustment. Model 2: adjusted for age and sex. Model 3: Model 2 + adjusted for duration of diabetes, BMI, SBP and ever smoked. Model 4: Model 3 + adjusted for HbA1c, LDL-C, eGFR and Ln (ACR)



Figure S1. Cumulative survival curve of women without cardiovascular disease based on quartiles of relative telomere length. Women were categorized by quartiles of relative telomere length in whole cohort, Q1<3.853, Q2: 3.853-4.639, Q3 4.640-5.326, and Q4>5.326.



Figure S2. Cumulative survival curve of men without cardiovascular disease based on quartiles of relative telomere length. Men were categorized by quartiles of relative telomere length in whole cohort, Q1<3.853, Q2: 3.853-4.639, Q3 4.640-5.326, and Q4>5.326.



Figure S3. Forest plot showing the association between relative telomere length and combined cardiovascular disease endpoint and according to the subgroup endpoints of cardiovascular disease (CVD), including Myocardial infarction (MI), Coronary Heart Disease (CHD), cerebral vascular accident (CVA), congestive heart failure (CHF), and Peripheral vascular disease (PVD), after adjusting for age, sex, diabetes duration, smoking status, BMI, SBP, HbA1c, LDL-C, eGFR, and Ln (ACR). Effects sizes per each unit decrease in relative telomere length are plotted as hazard ratios with 95% CI.



Figure S4. Forest plot showing the association between relative telomere length and cardiovascular disease endpoint according to age and sex category, after adjusting for age, sex, diabetes duration, smoking status, BMI, SBP, HbA1c, LDL-C, eGFR, and Ln (ACR). When conducting analysis in age category/sex, age and sex were excluded separately. Effects sizes per each unit decrease in relative telomere length are plotted as hazard ratios with 95% CI.



Figure S5: Receiver operating characteristic curve (ROC) for prediction of cardiovascular disease based on different models with or without inclusion of telomere length. rLTL: relative leukocyte telomere length; AUC: area under the curve. a) ROC with only rLTL included. AUC: 0.613(0.594-0.632); b) traditional risk models with or without inclusion of rLTL (AUC 0.731 (0.715-0.748) vs. 0.719 (0.702-0.358), P<0.001); c) risk factors from the United Kingdom Prospective Diabetes Study (UKPDS) risk engine with or without inclusion of rLTL(AUC 0.723(0.707-0.739) vs. 0.712(0.696-0.729), P<0.001); d) risk factors from the Hong Kong diabetes register (HKDR) coronary heart disease (CHD) risk equation with or without inclusion of rLTL (AUC 0.724(0.707-0.740) vs. 0.711(0.695-0.728), P<0.001).