Association of longer leukocyte telomere length with cardiac size, function, and heart failure

Nay Aung, MBBS, PhD[a,b,c], Qingning Wang, PhD[d,e], Stefan van Duijvenboden, PhD[a,b,f], Richard Burns, MSc[g], Svetlana Stoma, PhD[d], Zahra Raisi-Estabragh, MBBS, PhD[a,b,c], Selda Ahmet, MSc, MBBS, MRCP[c], Elias Allara, MD, PhD[h,i,j], Angela Wood, PhD[h,i,j,k,l,m], Emanuele Di Angelantonio, FMedSci[h,i,j,k,l,n], John Danesh, FMedSci[h,i,j,k,l,o], Patricia B Munroe, PhD[a,b], Alistair Young, PhD[g], Nicholas C Harvey, PhD[p,q], Veryan Codd, PhD[d], Christopher P Nelson, PhD[d], Steffen E Petersen, MD, DPhil, MSc, MPH[a,b,c], Nilesh J Samani, MD[d]

Running Title: leukocyte telomere length and cardiovascular imaging phenotypes

Affiliations

[a] William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom

[b] National Institute for Health and Care Research Barts Cardiovascular Biomedical Research Centre, Queen Mary University of London, London, United Kingdom

[c] Barts Heart Centre, St Bartholomew's Hospital, Barts Health NHS Trust, West Smithfield, London, United Kingdom

[d] Department of Cardiovascular Sciences, University of Leicester and NIHR Leicester Biomedical Research Centre, Glenfield Hospital, Leicester, United Kingdom

[e] National Institute for Health and Care Research Leicester Biomedical Research Centre, Glenfield Hospital, Leicester, UK

[f] Nuffield Department of Population Health, University of Oxford, Oxford, United Kingdom

[g] School of Biomedical Engineering and Imaging Sciences, King's College London

[h] British Heart Foundation Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom

[i] Victor Phillip Dahdaleh Heart and Lung Research Institute, University of Cambridge, Cambridge, United Kingdom

[j] National Institute for Health and Care Research Blood and Transplant Research Unit in Donor Health and Genomics, University of Cambridge, Cambridge, United Kingdom

[k] British Heart Foundation Centre of Research Excellence, University of Cambridge, Cambridge, United Kingdom

[l] Health Data Research UK Cambridge, Wellcome Genome Campus and University of Cambridge, Cambridge, United Kingdom

[m] Cambridge Centre of Artificial Intelligence in Medicine, Cambridge, United Kingdom

[n] Health Data Science Centre, Human Technopole, Milan, Italy

[o] Department of Human Genetics, Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, UK

[p] MRC Lifecourse Epidemiology Centre, University of Southampton, Southampton, United Kingdom

[q] NIHR Southampton Biomedical Research Centre, University of Southampton and University Hospital Southampton NHS Foundation Trust, Southampton, United Kingdom

Date of revision: 18th May 2023

Word count: 2,873

Corresponding author

Dr Nay Aung

William Harvey Research Institute, NIHR Barts Biomedical Research Centre, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ, United Kingdom.

Email: n.aung@qmul.ac.uk

Telephone: 02073777000 (ext : 10656)

Twitter: @NayAungMD

Key Points

Question: Is leukocyte telomere length (LTL) associated with alterations in cardiovascular structure and function?

Findings: Longer LTL is associated with higher left ventricular mass, larger ventricular and atrial sizes and higher stroke volumes. Mendelian Randomisation analysis demonstrates the causal genetic association between LTL on left ventricular mass, ventricular size and left ventricular stroke volume. We also provide confirmatory evidence that longer LTL is associated with a lower risk of incident heart failure after accounting for potential confounders.

Meaning: These findings highlight that modulation of LTL dynamics may have a role in improving cardiovascular structure and function which could potentially explain the observed lower future risk of heart failure.

Tweet: "Longer telomere length is associated with a larger heart with better cardiac function and a lower risk of heart failure."

Abstract

Importance: Longer leukocyte telomere length (LTL) is associated with a lower risk of adverse cardiovascular outcomes. The extent to which variation in LTL is associated with intermediary cardiovascular phenotypes is unclear.

Objective: To evaluate the relationships between LTL and a diverse set of cardiovascular imaging phenotypes

Design: This is a cross-sectional study of UK Biobank participants recruited from 2006 to 2010. LTL was measured using a quantitative polymerase chain reaction method. Cardiovascular measurements were derived from cardiovascular magnetic resonance (CMR) using machine learning. The median (interquartile range) duration of follow-up was 12.0 (1.4) years. The associations of LTL with imaging measurements and incident heart failure (HF) were evaluated by multivariable regression models. Genetic associations between LTL and significantly associated traits was investigated by Mendelian Randomisation.

Setting: Population-based cohort study

Participants: UK Biobank participants with CMR and LTL data

Exposure: LTL

Main Outcomes and Measures: Cardiovascular imaging traits and heart failure

Results: The mean age of the cohort (n = 40,459) was 55 ± 7.6 years; 48.3% were men. Longer LTL was independently associated with a pattern of positive cardiac remodelling (higher left ventricular mass (LVM), larger global ventricular size and volume, and higher ventricular and atrial stroke volumes) and a lower risk of incident HF (Hazard ratio, 95% confidence interval [CI]: 0.86, 0.81 – 0.91 for LTL 4^{th} quartile vs 1^{st} quartile). Mendelian Randomisation analysis suggested a causal association between LTL and LVM, global ventricular volume and left ventricular stroke volume.

Conclusions and Relevance: Longer LTL is associated with a larger heart with better cardiac function in middle age, which could potentially explain the observed lower risk of incident heart failure.

Key words:

Leukocyte telomere length, cardiovascular remodelling, heart failure

Abbreviations

LTL, leukocyte telomere length

CAD, coronary artery disease

HF, heart failure

LVM, left ventricular mass

UKB, UK Biobank

CMR, cardiovascular magnetic resonance

LVSV, left ventricular stroke volume

LVMVR, left ventricular mass to end-diastolic volume ratio

RVSV, right ventricular stroke volume

HF, heart failure

LA, left atrium/atrial

RA, right atrium/atrial

WBC, white blood cell

SBP, systolic blood pressure

MET, metabolic equivalent of task

MR, Mendelian Randomisation

GWAS, genome-wide association study

CVD, cardiovascular disease

Background

Telomeres are protective caps at the end of chromosomes that progressively shorten with each cell division.[1–3] When telomeres reach a critical length, cells enter senescence; hence telomere length is a marker of cellular replicative capacity and history.[2] At population level, there is a considerable inter-individual variation in mean telomere length, usually measured in leukocytes (leukocyte telomere length, LTL), but also present in other tissues and manifest from an early age.[3] In epidemiological studies, we and others have shown that shorter LTL is associated with risk of incident coronary artery disease (CAD) as well as heart failure (HF).[4–6] Mendelian Randomisation (MR) analyses have strongly suggested that the association of shorter LTL with CAD is genetically causal although evidence for an association with HF is less certain.[4]

Cardiac imaging measurements such as left ventricular mass (LVM) are intermediary phenotypes whose variability has also been shown to influence adverse cardiovascular outcomes, including CAD and HF.[7] Two previous studies have investigated the association of LTL with LVM.[8,9] The first by Vasan et al[8] investigated 850 Framingham Heart Study participants and the second by Kuznetsova and colleagues[9] examined 334 volunteers from the Flemish Study on Environment, Genes and Health Outcomes. Both studies used the LVM estimated by measurements from M-mode echocardiography and reported a positive association between LTL and LVM. However, neither study examined whether the relationship was consistent with a causal association.

UK Biobank (UKB) is a large population cohort established between 2006 and 2010 of participants aged 40-69 years at recruitment.[10] Participants have been characterised in detail

using questionnaires, physical measurements, urinary and plasma biomarker measurements, genomic assays and longitudinal linkage with multiple health record systems.[11] A sub-set of participants have also undergone cardiovascular magnetic resonance (CMR) scans. We have recently completed a large-scale measurement of LTL in UKB participants and identified a large number of genetic variants associated with LTL which are useful for causal inference (Mendelian Randomisation) analyses.[4,12] We have also derived measurements of cardiac structure and function from the CMR scans using automated artificial intelligence-based protocols.[13,14] Here, using these datasets we have examined (i) observational associations between LTL and cardiac morphology, function and geometry including LVM, global ventricular volume and size, left and right ventricular stroke volume (LVSV and RVSV), LVM to end-diastolic volume ratio (LVMVR), atrial maximum volume and atrial emptying volume, (ii) the genetic association between LTL and observationally associated CMR measurements using Mendelian Randomisation, and (iii) the relationship between LTL and future development of HF.

Methods

Subjects

From participants with valid LTL measurements in UKB (n=474,074)[12], we excluded genetically related samples, randomly excluding one from each pair based on a kinship coefficient of K>0.088 and samples with no genetic data or those that failed quality control. We also excluded participants who lacked information on ethnicity or white blood cell (WBC) count, which are both associated with LTL[12] and were used together with age and sex to adjust the trait associations. Ethnicity was self-reported by the participants using a

touchscreen questionnaire at the assessment centre. Among the remaining subjects with LTL measurements (n=422,797), 40,459 individuals participated in the ongoing CMR sub-study (**Figure 1**). We followed the reporting guidelines outlined in the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement from the EQUATOR (Enhancing the QUAlity and Transparency Of health Research) network. This study received the overall ethical approval for UK Biobank studies from the NHS National Research Ethics Service on 17th June 2011 (Ref 11/NW/0382) which was extended on 18 June 2021 (Ref 21/NW/0157). All study participants provided written informed consent.

Measurement of leukocyte telomere length

Details of LTL measurements including extensive quality checks and the adjustment for technical factors in the UK Biobank participants have been described previously by our group[12]. In brief, LTL was measured as the ratio of telomere repeat copy number (T) relative to that of a single copy gene (S, HBG) from the peripheral blood leukocyte DNA, extracted from blood collected at baseline, using a multiplex quantitative polymerase chain reaction (PCR) method. LTL measurements (T/S ratios) were $\log_e$-transformed due to non-normality ($\log_e$-LTL) and Z-standardised for all analyses (UKB field code: 22192).

Derivation of CMR parameters and arterial stiffness

Detailed CMR protocol and analysis methods have been described in prior publications[13–16]. Out of ~500,000 original UK Biobank participants, those living within a reasonable travelling distance to one of the four imaging assessment centres were invited back for imaging enhancement substudy with a target sample size of 100,000 individuals. The CMR scans

available for the current study (n~40,000) were obtained on average 9.0 ± 1.7 years after the baseline visit. Segmentation of the left and right ventricular and atrial cavities and left ventricular myocardium and aortic luminal area were performed by automated machine learning algorithms as detailed previously[13]. Global ventricular volume was defined as the sum of the right and left ventricular end-diastolic volumes. LVSV, RVSV and atrial stroke volumes were calculated by the difference between respective end-diastolic volume and end-systolic volume. LVMVR was derived by dividing LVM with LV end-diastolic volume. End-diastolic bi-ventricular shape models were obtained as described previously[17]. These models were compiled into a statistical shape atlas through principal component analysis, with principal components capturing the largest sources of variation in cardiac shape amongst the cohort. Through plotting these principal components, we can estimate biological features that they represent. The first principal component (PC1) represents the overall size of the heart (higher scores having larger hearts) which was the greatest source of variation in heart shape amongst individuals (**eFigure 1**).

Statistical analyses

The descriptive statistics are presented as mean ± standard deviation [SD] for continuous variables and number (percentage) for categorical variables. The trends across the LTL quartiles were examined by Cuzick's extension of the Wilcoxon rank-sum test for continuous variables and the Chi-square test for trend for ordinal variables. We removed the confounding influence of chronological age at baseline, white blood cell count and self-reported ethnicity by taking the residuals of $\log_e$ LTL regressed on these variables. Participants with missing data were excluded from the analysis. The relationships between $\log_e$ LTL residuals (independent variable) and CMR measures were evaluated in multivariable linear regression

models adjusted for age at the imaging visit, sex, height and weight. Significant associations were additionally adjusted for traditional cardiovascular risk factors (systolic blood pressure [SBP], diabetes mellitus, dyslipidaemia, smoking status and physical activity expressed in total metabolic equivalent of task [MET] minutes per week) to interrogate the potential confounding effects. Given the time lag between LTL sampling and CMR data acquisition, a sensitivity analysis investigating the interaction between the time lag between these two dates and LTL was conducted. We sought to identify the association between LTL and incident HF by performing survival analyses using Cox proportional hazards models adjusted for age, sex, body mass index (BMI), hypertension, hyperlipidaemia, diabetes mellitus, and smoking status. We also explored the mediating effect of LTL and LVM on incident HF by introducing an interaction term. The effect sizes were represented by one SD increase in $\log_e$ LTL residuals. All analyses were conducted in R version 4.0.2[18].

Mendelian Randomisation

To investigate the causality and directionality of the relationships of LTL with observationally associated imaging traits and with HF, we undertook Mendelian Randomisation (MR) analysis, using large–scale genome-wide association study (GWAS) datasets.[4,14,19] To assess whether the associations between LTT and imaging parameters and HF were consistent with a causal association, we used 130 conditionally independent, non-pleiotropic genetic variants that we have recently reported to be associated with LTL in UK Biobank.[4]

For each analysis, we used the inverse-variance weighted MR method[20] allowing for a random effect and also reported the p-value for the intercept from MR Egger[21] as a check for

horizontal pleiotropy. As sensitivity analyses, we undertook MR analyses using the Weighted Median method[22] which is additionally robust in the presence of outliers and the MR Raps method[23] which overcomes challenges related to measurement error, weak or invalid (due to pleiotropy) measurements and selection bias (due to weak instrument). We also applied Steiger filtering implemented in the steiger_filtering() function in the R package 'TwoSampleMR' to our genetic instruments which removed variants that explain more variance in the outcome (i.e. imaging measurements or heart failure) than the exposure (LTL) to minimise the risk of reverse causality. A combination of these approaches provides the best evidence for the presence of a genetic association consistent with a causal relationship.

Results

The baseline characteristics of the study cohort (N = 40,459) stratified by the LTL quartiles are presented in **Table 1**. The average age ± SD was 55 ± 7.6 years and 48.3% were men. Individuals in the higher LTL quartiles were more likely to be chronologically younger and female with a more favourable traditional cardiovascular risk profile. The vast majority of study cohort had CMR measurements within normal ranges[24]; the proportion of LVH was 2%. LVM trended downwards across the LTL quartiles in the overall cohort but when stratified by sex, LVM was higher in the higher LTL quartiles for both men and women (**eTables 1 and 2**). Our study cohort of UK Biobank participants who had CMR assessment were marginally younger, slightly more likely to be male and white and had a lower prevalence of cardiometabolic risk factors than those participants who did not receive CMR examination (**eTable 3**).

Observational associations between LTL and cardiovascular measurements

After accounting for the differences in age, sex, height and weight, a positive association was observed between LTL and LVM (β = 0.47 mg, 95% confidence interval [CI]: 0.34 – 0.60 mg per 1SD increment in $\log_e$ LTL, p = 4.0 x 10^{-12}) (**Table 2**). Similarly, longer LTL was associated with larger global ventricular volume (β [95% CI] = 1.33 [0.87 – 1.79] ml, p = 1.8 x 10^{-8}), larger overall ventricular size based on shape modelling (β [95% CI] = 0.01 [0.006 – 0.02], p = 1.2 x 10^{-4}), higher LVSV (β [95% CI] = 0.35 [0.19 – 0.50] ml, p = 8.7 x 10^{-6}), higher RVSV (β [95% CI] = 0.34 [0.18 – 0.50] ml, p = 3.2 x 10^{-5}), larger LA maximal volume (β [95% CI] = 0.23 [0.05 – 0.41] ml, p = 1.4 x 10^{-2}), higher LA emptying volume (β [95% CI] = 0.12 [0.02 – 0.23] ml, p = 2.2 x 10^{-2}). Additional adjustment with cardiovascular risk factors (SBP, diabetes, dyslipidaemia, smoking status and physical activity level) slightly attenuated the effect sizes while retaining the statistical significance (**Table 2**). In contrast, there were no significant associations of LTL with LVMVR, an adverse remodelling phenotype, after adjusting for age, sex, height and weight. A sensitivity analysis investigating the interaction between LTL and the time lag (between LTL sampling date and imaging visit date) did not find any significant results.

Longitudinal association between LTL and incident HF

Among 406,602 UKB participants with valid LTL measurements free from prevalent cardiovascular diseases, 7,827 individuals had incident HF over a median (interquartile range) follow-up of 12.0 (1.4) years. In Cox proportional hazards analysis adjusted for age, sex and other cardiovascular risk factors, longer LTL was associated with a lower future risk of heart failure (LTL 4^{th} quartile hazard ratio [HR] = 0.86, 95% CI = 0.81 – 0.91, p = 1.8 x 10^{-6}) (**Figure 2**). Formal mediation analysis of LTL on the relationships between LVM (or

other imaging traits) and HF was not feasible due to the low event rates in the CMR sub-cohort (total N ~ 40K, event N ~ 100) at this stage. Our exploratory interaction analysis of LTL and LVM on incident HF showed an association with lower risk (interaction HR = 0.87, p = 0.038).

Mendelian randomisation analyses

Using 130 genetic variants independently associated with LTL as instruments (**eTable 4**), we observed genetic associations of LTL with LVM, LVSV, global ventricular volume and biventricular overall size from shape model with inverse variance weighted [IVW] β [95% CI] = 0.13 [0.07 – 0.19], p = <0.0001, β [95% CI] = 0.08 [0.02 – 0.14], p = 0.013, β [95% CI] = 0.08 [0.02 – 0.14], p = 0.014 and β [95% CI] = 0.04 [0.0002 – 0.07], p = 0.049, respectively (**Figure 3**). Other imaging traits and heart failure did not achieve a statistically significant association with LTL although the overall effect directions were concordant with observational results. There was no evidence of confounding by directional horizontal pleiotropy (MR-Egger intercept p > 0.05). Sensitivity analyses with the weighted median and MR RAPS methods gave similar estimates as our primary MR IVW models. Furthermore, Steiger filtering which removed genetic variants that explain more variance in the outcomes did not materially alter our findings (**eTable 5**).

Discussion

This is the first and largest study to investigate the relationship between LTL and a comprehensive set of cardiac structure and function, robustly measured with CMR. Our principal findings are that in a middle-aged population: (i) longer LTL is associated with

higher LVM, larger global ventricular volume and overall size, and higher ventricular and atrial stroke volumes, (ii) confirmation that longer LTL is associated with a lower risk of incident HF even after accounting from traditional cardiovascular risk factors, and (iii) the genetic associations between LTL and LVM, LVSV and global ventricular volume are concordant with the observational results.

Our findings of an association of longer LTL with increased LVM are consistent with two previous reports[8,9] which assessed LVM using echocardiography and build on these findings. We advanced this insight by highlighting that longer LTL is also associated with larger global ventricular volume and size and higher LVSV. Our finding of better LV systolic function with longer LTL in a general population parallels the data from two small prior studies which reported the associations between shorter LTL and reduced LV ejection fraction a hypertensive mouse model[25] and in a human heart failure cohort[26]. The overall pattern of cardiac morpho-functional differences observed with longer LTL (higher LVM, larger global ventricular volume, static LVMVR, larger atria and higher ventricular and atrial stroke volumes) closely resembles beneficial "balanced" myocardial remodelling frequently seen with the physiological adaptation to exercise (i.e. athlete's heart)[27]. We also provide compelling genetic evidence, based on multiple MR approaches, that the associations of LTL with LVM, global ventricular volume and LVSV are consistent with a causal association.

The impact of LTL on cardiac structure and function could have clinical relevance. We demonstrated in this work that longer LTL is associated with a reduced observed incidence of HF in UKB (HR 0.86, 95% CI = 0.81 – 0.91). The MR analysis, although trending in the same direction, was non-significant (MR odds ratio 0.96 (95% CI 0.89-1.03) per 1SD longer LTL) possibly related to low power. However, no firm conclusion can be drawn based on this

data and future studies using information from larger GWAS are needed. Other studies have shown that LTL is shorter in patients with HF and is associated with poor prognosis.[6,28–30] Experimental studies also directly support a role of telomere dynamics in cardiac structure and function. With ageing, telomerase knockout mice hearts showed shortening of telomeres, attenuated proliferation and increased apoptosis of cardiomyocytes, and greater cardiac remodelling and left ventricular failure[31,32]. On the other hand, enhanced expression of telomerase reverse transcriptase in rat cardiomyocytes preserved telomere length, and induced cardiomyocyte proliferation, hypertrophy and survival[33]. While it is recognised that LV hypertrophy and LV dilatation in isolation are associated with adverse outcomes, through access to a more comprehensive set of imaging features, our study demonstrated a more global positive pattern of cardiac remodelling in association with longer LTL, which could explain the lower incidence of HF.

Limitations

Our study benefited from several important advantages including: (i) access to the largest sample size to date of LTL data and the diversity and accuracy of cardiovascular imaging measurements using the reference standard CMR and (ii) application of Mendelian Randomisation for causal inference analysis using the data from recent large genome-wide association studies. Nevertheless, several limitations need to be acknowledged. First, there is a "healthy volunteer" selection bias in the UK Biobank with the participants being older, more affluent and having a healthier lifestyle with fewer comorbid conditions than the UK general population.[34] The imaging substudy cohort is even slightly healthier than the overall UK Biobank cohort. In line with this observation, the majority of our study cohort had imaging measurements within normal physiological ranges and the applicability of our

findings in disease states leading to left ventricular hypertrophy is uncertain. Second, the majority of our cohort (97%) is of European descent which may limit the generalisability of our findings in under-represented ethnicities. Third, telomere length was quantified in blood leukocytes which may not reflect cell or tissue-specific telomere length. Finally, the LTL and CMR measurements were obtained at different time points. The impact of this on the findings is uncertain but, if anything, is likely to have blunted the magnitude of the observed associations. Furthermore, our findings from Mendelian randomisation which circumvents the issues of confounding, measurement errors and reverse causation in observational studies, provide concordant results for the key findings.

Conclusion

Longer LTL is associated with higher LVM, larger global ventricular size and better cardiac function and a lower risk of incident heart failure. Further investigations into the prognostic relevance of LTL in adverse cardiac remodelling and the related mechanistic pathways could provide insights into the novel risk stratification approaches and therapeutic targets for heart failure.

Data Sharing Statement

The individual-level data can be requested from the UK Biobank via the standard access request process (https://www.ukbiobank.ac.uk/enable-your-research/apply-for-access). the Additional supporting information (statistical/analytic code) are available upon request.

Acknowledgements

N.A. acknowledges the National Institute for Health and Care Research (NIHR) Integrated Academic Training programme which supports his Academic Clinical Lectureship post and the funding support from the Academy of Medical Sciences Clinical Lecturer Starter Grant. N.A. and S.v.D recognise the support from the QMUL Impact Acceleration Account funded by Engineering and Physical Sciences Research Council. Z.R.E recognizes the National Institute for Health and Care Research (NIHR) Integrated Academic Training programme which supports her Academic Clinical Lectureship post and was also supported by British Heart Foundation Clinical Research Training Fellowship No. FS/17/81/33318. Cambridge University investigators are supported by core funding from the: British Heart Foundation (RG/13/13/30194; RG/18/13/33946); NIHR Cambridge Biomedical Research Centre (BRC-1215-20014; NIHR203312) [*]; and Cambridge BHF Centre of Research Excellence (RE/18/1/34212). E.A was supported by the EU/EFPIA Innovative Medicines Initiative Joint Undertaking BigData@Heart grant 116074 and is currently funded by a British Heart Foundation programme grant (RG/18/13/33946). A.W is part of the BigData@Heart Consortium, funded by the Innovative Medicines Initiative-2 Joint Undertaking under grant agreement No 116074. A.W is supported by the BHF-Turing Cardiovascular Data Science Award (BCDSA\100005). J.D holds a British Heart Foundation Professorship and a NIHR Senior Investigator Award [*]. *The views expressed are those of the authors and not necessarily those of the NIHR or the Department of Health and Social Care. N.C.H acknowledges support from the UK Medical Research Council [MC_PC_21003; MC_PC_21001] and NIHR Southampton Biomedical Research Centre, University of Southampton, and University Hospital Southampton. S.E.P and P.B.M acknowledge support from the National Institute for Health and Care Research (NIHR) Biomedical Research Centre at Barts. S.E.P acknowledge the British Heart Foundation for funding the manual

analysis to create a cardiovascular magnetic resonance imaging reference standard for the UK Biobank imaging resource in 5,000 CMR scans (www.bhf.org.uk; PG/14/89/31194). S.E.P acknowledges support from the SmartHeart EPSRC programme grant (www.nihr.ac.uk; EP/P001009/1).

Telomere length measurements were funded by the UK Medical Research Council (MRC), Biotechnology and Biological Sciences Research Council and British Heart Foundation (BHF) through MRC grant MR/M012816/1 to N.J.S. C.P.N is funded by the BHF (SP/16/4/32697). Q.W., L.L., V.C., C.P.N. and N.J.S. are supported by the National Institute for Health and Care Research (NIHR) Leicester Cardiovascular Biomedical Research Centre (BRC-1215-20010). Cambridge University investigators are supported by the BHF (RG/13/13/30194; RG/18/13/33946), Health Data Research UK, NIHR Cambridge BRC (BRC-1215-20014), NIHR Blood and Transplant Research Unit in Donor Health and Genomics (NIHR BTRU-2014-10024) and MRC (MR/L003120/1). J.N.D. holds a BHF Personal Professorship and NIHR Senior Investigator Award.

This research has been conducted using the UK Biobank Resource under Application 2964. We thank all UK Biobank participants and staff.

The funders had no role in design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication. N.A. and Q.W. had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Disclosures

S.E.P provides consultancy to Circle Cardiovascular Imaging, Inc., Calgary, Alberta, Canada. J.D. serves on scientific advisory boards for AstraZeneca, Novartis, and UK Biobank, and has received multiple grants from academic, charitable and industry sources outside of the submitted work. Other authors have no disclosures to make.

References

1. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. *Nature*. 1990;345(6274):458-460. doi:10.1038/345458a0

2. Allsopp RC, Vaziri H, Patterson C, et al. Telomere length predicts replicative capacity of human fibroblasts. *Proc Natl Acad Sci U S A*. 1992;89(21):10114-10118.

3. Demanelis K, Jasmine F, Chen LS, et al. Determinants of telomere length across human tissues. *Science*. 2020;369(6509):eaaz6876. doi:10.1126/science.aaz6876

4. Codd V, Wang Q, Allara E, et al. Polygenic basis and biomedical consequences of telomere length variation. *Nat Genet*. 2021;53(10):1425-1433. doi:10.1038/s41588-021-00944-6

5. Haycock PC, Heydon EE, Kaptoge S, Butterworth AS, Thompson A, Willeit P. Leucocyte telomere length and risk of cardiovascular disease: systematic review and meta-analysis. *BMJ*. 2014;349:g4227. doi:10.1136/bmj.g4227

6. van der Harst P, van der Steege G, de Boer RA, et al. Telomere length of circulating leukocytes is decreased in patients with chronic heart failure. *J Am Coll Cardiol*. 2007;49(13):1459-1464. doi:10.1016/j.jacc.2007.01.027

7. de Simone G, Gottdiener JS, Chinali M, Maurer MS. Left ventricular mass predicts heart failure not related to previous myocardial infarction: the Cardiovascular Health Study. *Eur Heart J*. 2008;29(6):741-747. doi:10.1093/eurheartj/ehm605

8. Vasan RS, Demissie S, Kimura M, et al. Association of leukocyte telomere length with echocardiographic left ventricular mass: the Framingham heart study. *Circulation*. 2009;120(13):1195-1202. doi:10.1161/CIRCULATIONAHA.109.853895

9. Kuznetsova T, Codd V, Brouilette S, et al. Association Between Left Ventricular Mass and Telomere Length in a Population Study. *Am J Epidemiol*. 2010;172(4):440-450. doi:10.1093/aje/kwq142

10. Sudlow C, Gallacher J, Allen N, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med*. 2015;12(3):e1001779. doi:10.1371/journal.pmed.1001779

11. Bycroft C, Freeman C, Petkova D, et al. The UK Biobank resource with deep phenotyping and genomic data. *Nature*. 2018;562(7726):203-209. doi:10.1038/s41586-018-0579-z

12. Codd V, Denniff M, Swinfield C, et al. *A Major Population Resource of 474,074 Participants in UK Biobank to Investigate Determinants and Biomedical Consequences of Leukocyte Telomere Length*.; 2021:2021.03.18.21253457. doi:10.1101/2021.03.18.21253457

13. Bai W, Sinclair M, Tarroni G, et al. Automated cardiovascular magnetic resonance image analysis with fully convolutional networks. *J Cardiovasc Magn Reson*. 2018;20(1):65. doi:10.1186/s12968-018-0471-x

14. Aung Nay, Vargas Jose D., Yang Chaojie, et al. Genome-Wide Analysis of Left Ventricular Image-Derived Phenotypes Identifies Fourteen Loci Associated With Cardiac Morphogenesis and Heart Failure Development. *Circulation*. 2019;140(16):1318-1330. doi:10.1161/CIRCULATIONAHA.119.041161

15. Petersen SE, Matthews PM, Francis JM, et al. UK Biobank's cardiovascular magnetic resonance protocol. *J Cardiovasc Magn Reson*. 2016;18(1):8. doi:10.1186/s12968-016-0227-4

16. Biasiolli L, Hann E, Lukaschuk E, et al. Automated localization and quality control of the aorta in cine CMR can significantly accelerate processing of the UK Biobank population data. Grisan E, ed. *PLOS ONE*. 2019;14(2):e0212272. doi:10.1371/journal.pone.0212272

17. Mauger C, Gilbert K, Lee AM, et al. Right ventricular shape and function: cardiovascular magnetic resonance reference morphology and biventricular risk factor morphometrics in UK Biobank. *J Cardiovasc Magn Reson*. 2019;21(1):41. doi:10.1186/s12968-019-0551-6

18. R Core Team. *R: A Language and Environment for Statistical Computing*.; 2020. https://www.R-project.org/

19. Shah S, Henry A, Roselli C, et al. Genome-wide association and Mendelian randomisation analysis provide insights into the pathogenesis of heart failure. *Nat Commun*. 2020;11(1):163. doi:10.1038/s41467-019-13690-5

20. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol*. 2013;37(7):658-665. doi:10.1002/gepi.21758

21. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol*. 2015;44(2):512-525. doi:10.1093/ije/dyv080

22. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. *Genet Epidemiol*. 2016;40(4):304-314. doi:10.1002/gepi.21965

23. Zhao Q, Wang J, Hemani G, Bowden J, Small DS. Statistical inference in two-sample summary-data Mendelian randomization using robust adjusted profile score. Published online January 1, 2019. doi:10.48550/arXiv.1801.09652

24. Petersen SE, Aung N, Sanghvi MM, et al. Reference ranges for cardiac structure and function using cardiovascular magnetic resonance (CMR) in Caucasians from the UK Biobank population cohort. *J Cardiovasc Magn Reson*. 2017;19(1):18. doi:10.1186/s12968-017-0327-9

25. Brandt M, Dörschmann H, Khraisat S, et al. Telomere Shortening in Hypertensive Heart Disease Depends on Oxidative DNA Damage and Predicts Impaired Recovery of Cardiac Function in Heart Failure. *Hypertension*. 2022;79(10):2173-2184. doi:10.1161/HYPERTENSIONAHA.121.18935

26. Sharifi-Sanjani M, Oyster NM, Tichy ED, et al. Cardiomyocyte-Specific Telomere Shortening is a Distinct Signature of Heart Failure in Humans. *J Am Heart Assoc*. 6(9):e005086. doi:10.1161/JAHA.116.005086

27. De Innocentiis C, Ricci F, Khanji MY, et al. Athlete's Heart: Diagnostic Challenges and Future Perspectives. *Sports Med Auckl NZ*. 2018;48(11):2463-2477. doi:10.1007/s40279-018-0985-2

28. Haver VG, Mateo Leach I, Kjekshus J, et al. Telomere length and outcomes in ischaemic heart failure: data from the COntrolled ROsuvastatin multiNAtional Trial in Heart Failure (CORONA). *Eur J Heart Fail*. 2015;17(3):313-319. doi:10.1002/ejhf.237

29. van der Harst P, de Boer RA, Samani NJ, et al. Telomere length and outcome in heart failure. *Ann Med*. 2010;42(1):36-44. doi:10.3109/07853890903233887

30. Romaine SPR, Denniff M, Codd V, et al. Telomere length is independently associated with all-cause mortality in chronic heart failure. *Heart*. 2022;108(2):124-129. doi:10.1136/heartjnl-2020-318654

31. Leri A, Franco S, Zacheo A, et al. Ablation of telomerase and telomere loss leads to cardiac dilatation and heart failure associated with p53 upregulation. *EMBO J*. 2003;22(1):131-139. doi:10.1093/emboj/cdg013

32. Wong LSM, Oeseburg H, de Boer RA, van Gilst WH, van Veldhuisen DJ, van der Harst P. Telomere biology in cardiovascular disease: the TERC−/− mouse as a model for heart failure and ageing. *Cardiovasc Res*. 2009;81(2):244-252. doi:10.1093/cvr/cvn337

33. Bär C, Bernardes de Jesus B, Serrano R, et al. Telomerase expression confers cardioprotection in the adult mouse heart after acute myocardial infarction. *Nat Commun*. 2014;5:5863. doi:10.1038/ncomms6863

34. Fry A, Littlejohns TJ, Sudlow C, et al. Comparison of Sociodemographic and Health-Related Characteristics of UK Biobank Participants With Those of the General Population. *Am J Epidemiol*. 2017;186(9):1026-1034. doi:10.1093/aje/kwx246

Figure Legend

Figure 1. Sample selection flowchart

UKB, UK Biobank; LTL, leukocyte telomere length; WBC, white blood cell count; LVM, left ventricular mass; LVSV, left ventricular stroke volume; LVMVR, left ventricular mass to end-diastolic volume ration; LA, left atrial; RA, right atrial; RVSV, right ventricular stroke volume

Figure 2. Longitudinal association between LTL and incident heart failure

LTL, leukocyte telomere length; SD, standard deviation; BMI, body mass index

Figure 3. Mendelian Randomisation associations between LTL and cardiac imaging traits and heart failure

MR, Mendelian Randomisation; LTL, leukocyte telomere length; LV, left ventricular; RV, right ventricular, LA, left atrial; HF, heart failure

599
600 Table 1. Study cohort characteristics

		LTL quartiles				
	Full cohort	**1st**	**2nd**	**3rd**	**4th**	**P trend**
N	40459	10115	10115	10114	10115	
Age telomere visit (year)	55.1 (7.6)	56.9 (7.4)	55.6 (7.4)	54.7 (7.5)	53.3 (7.5)	<0.0001
Age imaging visit (year)	64.2 (7.8)	65.8 (7.6)	64.6 (7.6)	63.8 (7.7)	62.4 (7.7)	<0.0001
Male sex	19529 (48.3)	5558 (54.9)	5132 (50.7)	4655 (46.0)	4184 (41.4)	<0.0001
Height (cm)	169.2 (9.3)	169.6 (9.2)	169.3 (9.3)	169.1 (9.3)	168.7 (9.2)	<0.0001
Weight (kg)	75.9 (15.0)	76.9 (15.0)	76.1 (15.1)	75.7 (15.1)	74.8 (15.0)	<0.0001
Ethnicity						
Asian	416 (1.0)	94 (0.9)	110 (1.1)	101 (1.0)	111 (1.1)	0.3545
Black	258 (0.6)	32 (0.3)	54 (0.5)	60 (0.6)	112 (1.1)	<0.0001
Chinese	115 (0.3)	25 (0.2)	16 (0.2)	25 (0.2)	49 (0.5)	0.0007
Mixed	189 (0.5)	40 (0.4)	40 (0.4)	46 (0.5)	63 (0.6)	0.0145
Other	204 (0.5)	39 (0.4)	43 (0.4)	48 (0.5)	74 (0.7)	0.0006
White	39277 (97.1)	9885 (97.7)	9852 (97.4)	9834 (97.2)	9706 (96.0)	<0.0001
SBP (mmHg)	139.1 (18.7)	140.1 (18.7)	139.5 (18.5)	138.9 (18.6)	137.7 (18.8)	<0.0001
Diabetes mellitus	2359 (5.8)	731 (7.2)	624 (6.2)	523 (5.2)	481 (4.8)	<0.0001
Hyperlipidaemia	14191 (35.1)	3920 (38.8)	3696 (36.5)	3447 (34.1)	3128 (30.9)	<0.0001
Smoking status	1323 (6.4)	328 (6.2)	345 (6.7)	345 (6.7)	305 (5.9)	0.5363
Physical activity (Total MET minutes per week)	2750.4 (2432.8)	2706.1 (2408.5)	2796.3 (2474.2)	2759.4 (2441.1)	2740.1 (2406.7)	0.2028
WBC (mmol/L)	6.5 (1.5)	6.6 (1.5)	6.6 (1.5)	6.5 (1.5)	6.4 (1.5)	<0.0001
LV mass (g)	86.0 (22.4)	87.3 (22.3)	86.4 (22.5)	85.7 (22.4)	84.7 (22.3)	<0.0001
Indexed LV mass (g/m^2)	45.3 (8.6)	45.6 (8.7)	45.5 (8.7)	45.2 (8.5)	45.0 (8.6)	<0.0001
Global ventricular volume (ml)	303.2 (68.6)	304.3 (68.0)	303.4 (68.7)	303.2 (68.9)	302.1 (68.8)	0.0011
Indexed global ventricular volume (ml/m^2)	160.6 (28.0)	159.8 (28.0)	160.5 (28.2)	160.8 (27.9)	161.3 (28.0)	0.0003

LVMVR (g/ml)	0.59 (0.09)	0.59 (0.09)	0.59 (0.09)	0.58 (0.09)	0.58 (0.09)	<0.0001
LVSV (ml)	87.0 (19.2)	86.9 (19.0)	87.1 (19.3)	86.9 (19.3)	87.0 (19.0)	0.4253
Indexed LVSV (ml/m^2)	46.2 (8.5)	45.8 (8.4)	46.2 (8.6)	46.2 (8.5)	46.6 (8.4)	<0.0001
Overall ventricular size from shape model	0.01 (0.98)	0.05 (0.98)	0.03 (0.98)	0.01 (0.99)	-0.03 (0.98)	<0.0001
RVSV (ml)	88.5 (20.2)	88.5 (20.2)	88.6 (20.1)	88.6 (20.4)	88.3 (20.0)	0.1665
Indexed RVSV (ml/m^2)	47.0 (8.9)	46.6 (9.0)	47.0 (8.9)	47.1 (9.0)	47.3 (8.8)	<0.0001
LA maximum volume (ml)	44.3 (17.1)	44.5 (17.5)	44.2 (17.2)	44.4 (17.2)	44.2 (16.5)	0.2063
Indexed LA maximum volume (ml/m^2)	23.8 (9.1)	23.7 (9.2)	23.7 (9.2)	23.9 (9.2)	24.0 (8.9)	<0.0001
LA emptying volume (ml)	28.0 (9.8)	27.8 (9.9)	27.9 (9.9)	28.1 (9.9)	28.1 (9.6)	<0.0001
Indexed LA emptying volume (ml/m^2)	15.1 (5.4)	14.8 (5.3)	15.0 (5.4)	15.2 (5.4)	15.3 (5.4)	<0.0001
RA maximum volume (ml)	49.8 (20.6)	50.2 (21.4)	49.6 (20.0)	49.8 (20.8)	49.7 (20.2)	0.6762
Indexed RA maximum volume (ml/m^2)	26.9 (11.2)	26.8 (11.4)	26.7 (10.9)	26.9 (11.4)	27.0 (11.1)	0.0096
RA emptying volume (ml)	24.4 (10.8)	24.3 (11.1)	24.3 (10.6)	24.4 (10.8)	24.5 (10.8)	0.0061
Indexed RA emptying volume (ml/m^2)	13.2 (6.0)	13.1 (6.1)	13.1 (5.9)	13.2 (6.1)	13.4 (6.1)	<0.0001

SBP, systolic blood pressure; MET, metabolic equivalent of task; WBC, white blood cell count; LV, left ventricle; LVMVR, LV mass to end-diastolic volume ratio; LVSV, LV stroke volume; RVSV, right ventricular stroke volume; LA, left atrium; RA, right atrium; Other ethnicity category refers participants who selected “Other ethnic group” in the self-reported questionnaire.

Table 2. Multivariable regression results for the association between LTL and cardiovascular measurements

	Model 1			Model 2		
	beta	**95% CI**	**p value**	**beta**	**95% CI**	**p value**
LV mass (g)	0.47	0.34 to 0.60	3.97E-12	0.37	0.24 to 0.50	2.31E-08
Global ventricular volume (ml)	1.33	0.87 to 1.79	1.84E-08	1.17	0.70 to 1.63	9.37E-07
Overall ventricular size from shape model	0.01	0.006 to 0.02	1.23E-04	0.01	0.004 to 0.02	7.98E-04
LVSV (ml)	0.35	0.19 to 0.50	8.67E-06	0.30	0.15 to 0.46	1.26E-04
LVMVR (g/ml)	6.90×10^{-4}	-1.05×10^{-4} to 1.49×10^{-3}	8.90E-02	-	-	-
RVSV (ml)	0.34	0.18 to 0.50	3.15E-05	0.27	0.11 to 0.43	8.76E-04
LA maximum volume (ml)	0.23	0.05 to 0.41	1.36E-02	0.19	0.01 to 0.38	3.68E-02
LA emptying volume (ml)	0.12	0.02 to 0.23	2.24E-02	0.09	-0.01 to 0.20	8.6E-02
RA maximum volume (ml)	0.15	-0.06 to 0.35	1.62E-01	-	-	-
RA emptying volume (ml)	0.09	-0.02 to 0.20	9.19E-02	-	-	-

LV, left ventricle; LVSV, LV stroke volume, LVMVR, LV mass to end-diastolic volume ratio; RVSV, right ventricular stroke volume; LA, left atrial; RA, right atrial; CI, confidence interval

Model 1: Adjusted for age, sex, height and weight

Model 2: Adjusted for age, sex, height, weight, systolic blood pressure, diabetes mellitus, hyperlipidaemia, current smoking, total MET minutes per week

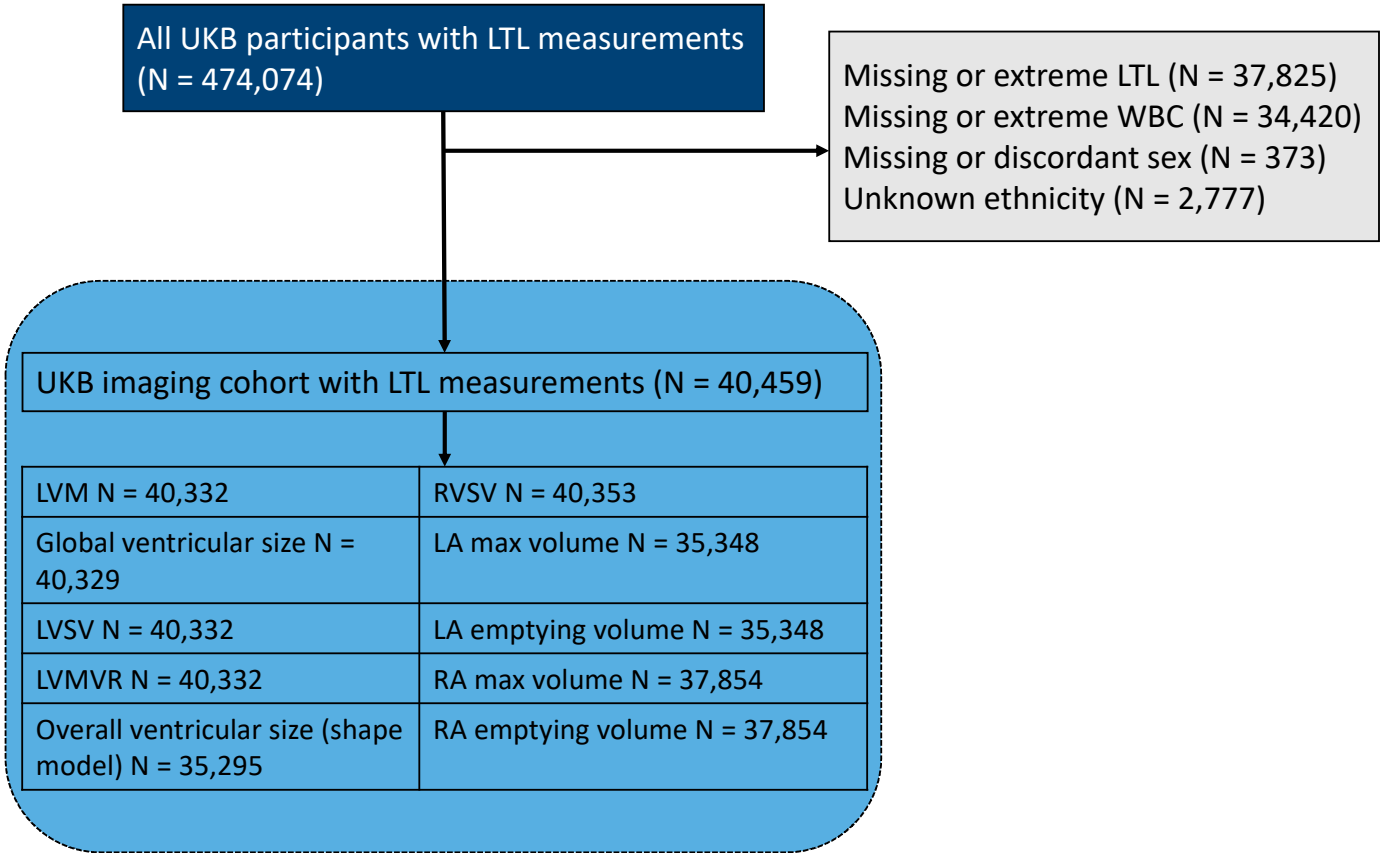


Figure 1. Sample selection flowchart

UKB, UK Biobank; LTL, leukocyte telomere length; WBC, white blood cell count; LVM, left ventricular mass; LVSV, left ventricular stroke volume; LVMVR, left ventricular mass to end-diastolic volume ration; RVSV, right ventricular stroke volume; LA, left atrial; RA, right atrial

Variable		N	Hazard ratio	p
LTL quartiles	Q1 (shortest)	101668	Reference	
	Q2	101618	0.93 (0.88, 0.99)	0.03
	Q3	101652	0.88 (0.83, 0.94)	<0.001
	Q4 (longest)	101664	0.86 (0.81, 0.91)	<0.001
Age (per SD)		406602	2.26 (2.20, 2.33)	<0.001
Sex	female	226913	Reference	
	male	179689	1.65 (1.58, 1.73)	<0.001
BMI (per SD)		406602	1.39 (1.36, 1.42)	<0.001
Hypertension	no	302831	Reference	
	yes	103771	1.61 (1.53, 1.69)	<0.001
Hyperlipidaemia	no	300840	Reference	
	yes	105762	0.99 (0.94, 1.04)	0.66
Diabetes mellitus	no	386753	Reference	
	yes	19849	1.74 (1.63, 1.86)	<0.001
Ever smoked	no	167391	Reference	
	yes	239211	1.21 (1.16, 1.27)	<0.001

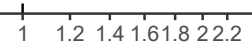


Figure 2. Longitudinal association between LTL and incident heart failure

LTL, leukocyte telomere length; SD, standard deviation; BMI, body mass index

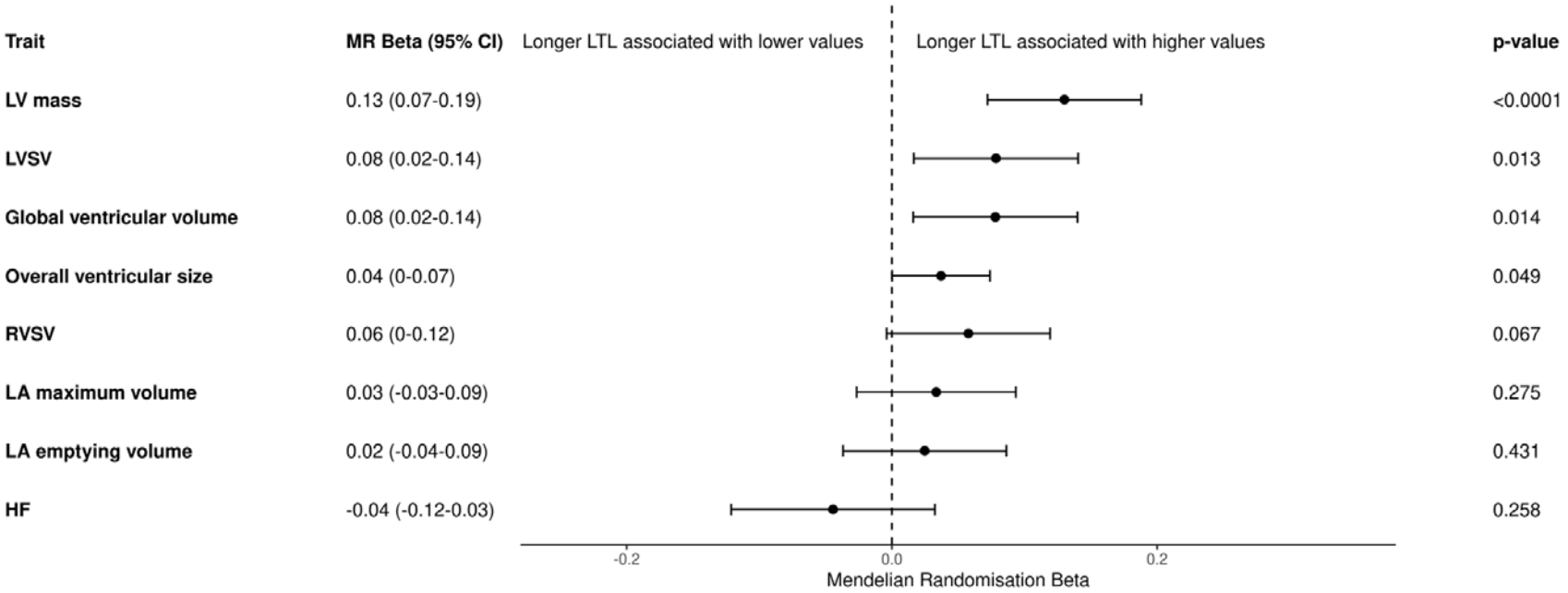
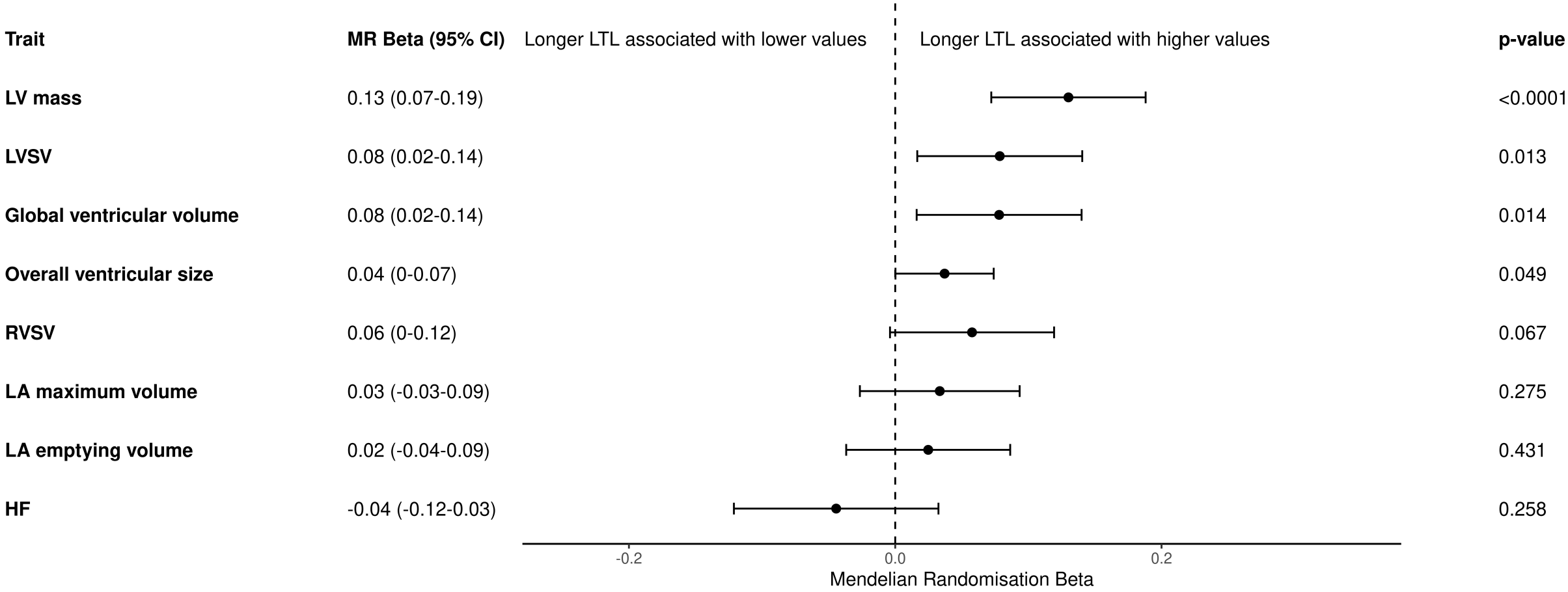
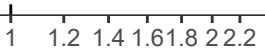


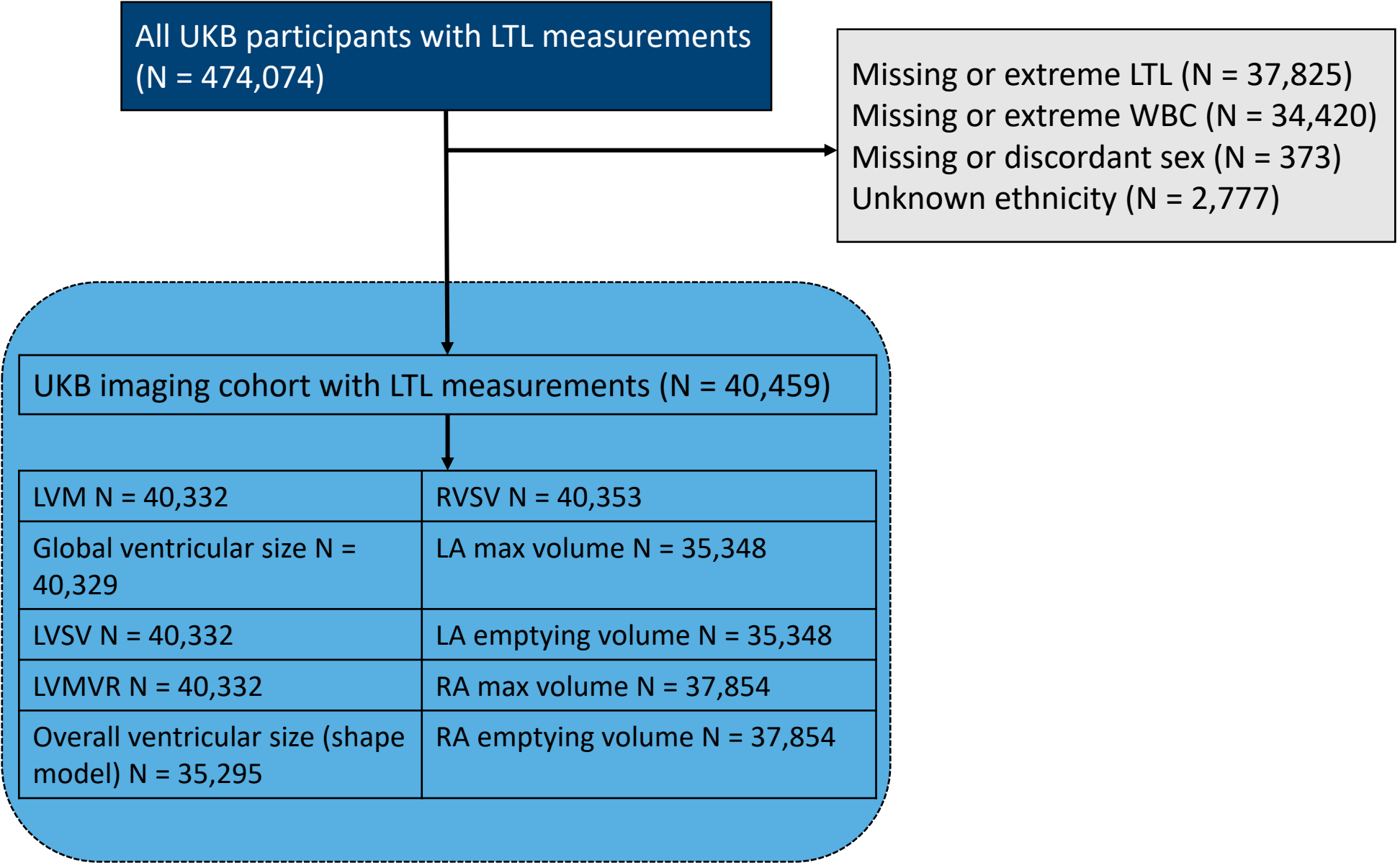
Figure 3. Mendelian Randomisation associations between LTL and cardiac imaging traits and heart failure

MR, Mendelian Randomisation; LTL, leukocyte telomere length; LV, left ventricular; RV, right ventricular, LA, left atrial; HF, heart failure


Trait
MR Beta (95% CI)
Longer LTL associated with lower values
Longer LTL associated with higher values
p-value
LV mass
0.13 (0.07-0.19)
<0.0001
LVSV
0.08 (0.02-0.14)
0.013
Global ventricular volume
0.08 (0.02-0.14)
0.014
Overall ventricular size
0.04 (0-0.07)
0.049
RVSV
0.06 (0-0.12)
0.067
LA maximum volume
0.03 (-0.03-0.09)
0.275
LA emptying volume
0.02 (-0.04-0.09)
0.431
HF
-0.04 (-0.12-0.03)
0.258
-0.2
0.0
0.2
Mendelian Randomisation Beta

Variable		N	Hazard ratio	p
LTL quartiles	Q1 (shortest)	101668	Reference	
	Q2	101618	0.93 (0.88, 0.99)	0.03
	Q3	101652	0.88 (0.83, 0.94)	<0.001
	Q4 (longest)	101664	0.86 (0.81, 0.91)	<0.001
Age (per SD)		406602	2.26 (2.20, 2.33)	<0.001
Sex	female	226913	Reference	
	male	179689	1.65 (1.58, 1.73)	<0.001
BMI (per SD)		406602	1.39 (1.36, 1.42)	<0.001
Hypertension	no	302831	Reference	
	yes	103771	1.61 (1.53, 1.69)	<0.001
Hyperlipidaemia	no	300840	Reference	
	yes	105762	0.99 (0.94, 1.04)	0.66
Diabetes mellitus	no	386753	Reference	
	yes	19849	1.74 (1.63, 1.86)	<0.001
Ever smoked	no	167391	Reference	
	yes	239211	1.21 (1.16, 1.27)	<0.001




All UKB participants with LTL measurements (N = 474,074)
Missing or extreme LTL (N = 37,825)
Missing or extreme WBC (N = 34,420)
Missing or discordant sex (N = 373)
Unknown ethnicity (N = 2,777)
UKB imaging cohort with LTL measurements (N = 40,459)
LVM N = 40,332
RVSV N = 40,353
Global ventricular size N = 40,329
LA max volume N = 35,348
LVSV N = 40,332
LA emptying volume N = 35,348
LVMVR N = 40,332
RA max volume N = 37,854
Overall ventricular size (shape model) N = 35,295
RA emptying volume N = 37,854