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Leukocyte telomere length and conduction system ageing

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Abstract:	Objective: Deterioration of the cardiac conduction system is an important manifestation of cardiac ageing. Cellular ageing is accompanied by telomere shortening and telomere length is often regarded as a marker of biological ageing, potentially adding information regarding conduction

disease over and above chronological age. We therefore sought to evaluate the association between leukocyte telomere length (LTL) on two related, but distinct aspects of the cardiac conduction system: ECG measures of conduction (PR interval and QRS duration) and incident pacemaker implantation in a large population-based cohort.
Methods: In the UK Biobank, we measured PR interval and QRS duration from signal-averaged electrocardiogram waveforms in 59,868 and 62,266 participants, respectively. Incident pacemaker implantation was ascertained using hospital episode data from 420,071 participants. Associations with LTL were evaluated in (Cox) multivariable regression analyses adjusted for potential confounders. Putative causal effects of LTL were investigated by mendelian randomisation (MR).
Results: Mean PR interval and QRS duration were 144.2ms (± 20.4) and 92.3ms (± 7.8), respectively, and there were 7,169 (1.7%) incident pacemaker implantations, during a median follow-up period of 13.6 (IQR 1.5) years. LTL was significantly associated with PR interval (0.19ms (95% CI: 0.03-0.35ms), per 1 SD shorter LTL, p=0.021), but not QRS duration. After adjusting for age, sex, and cardiovascular risk factors, shorter LTL remained associated with an increased risk for incident pacemaker implantation (hazard ratio per SD decrease in LTL: 1.03 (95% CI: 1.01-1.06), p=0.012). Mendelian randomisation analysis showed a trend towards an association of shorter LTL with longer PR interval and higher risk of pacemaker implantation but was likely to be underpowered.
Conclusions: Shorter LTL was significantly and possibly causally associated with prolongation of atrioventricular conduction and pacemaker implantation, independent of traditional cardiovascular risk factors. Our findings support further research to explore the role of ageing on cardiac conduction beyond chronological age.

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Leukocyte telomere length and conduction system ageing

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ABSTRACT

Objective: Deterioration of the cardiac conduction system is an important manifestation of cardiac ageing. Cellular ageing is accompanied by telomere shortening and telomere length is often regarded as a marker of biological ageing, potentially adding information regarding conduction disease over and above chronological age. We therefore sought to evaluate the association between leukocyte telomere length (LTL) on two related, but distinct aspects of the cardiac conduction system: ECG measures of conduction (PR interval and QRS duration) and incident pacemaker implantation in a large population-based cohort.

Methods: In the UK Biobank, we measured PR interval and QRS duration from signal-averaged electrocardiogram waveforms in 59,868 and 62,266 participants, respectively. Incident pacemaker implantation was ascertained using hospital episode data from 420,071 participants. Associations with LTL were evaluated in (Cox) multivariable regression analyses adjusted for potential confounders. Putative causal effects of LTL were investigated by mendelian randomisation (MR).

Results: Mean PR interval and QRS duration were 144.2ms (± 20.4) and 92.3ms (± 7.8), respectively, and there were 7,169 (1.7%) incident pacemaker implantations, during a median follow-up period of 13.6 (IQR 1.5) years. LTL was significantly associated with PR interval (0.19ms (95% CI: 0.03-0.35ms), per 1 SD shorter LTL, p=0.021), but not QRS duration. After adjusting for age, sex, and cardiovascular risk factors, shorter LTL remained associated with an increased risk for incident pacemaker implantation (hazard ratio per SD decrease in LTL: 1.03 (95% CI: 1.01-1.06), p=0.012). Mendelian randomisation analysis showed a trend towards an association of shorter LTL with longer PR interval and higher risk of pacemaker implantation but was likely to be underpowered.

Conclusions: Shorter LTL was significantly and possibly causally associated with prolongation of atrioventricular conduction and pacemaker implantation, independent of traditional cardiovascular risk factors. Our findings support further research to explore the role of ageing on cardiac conduction beyond chronological age.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Deterioration of the cardiac conduction system is an important manifestation of cardiac ageing. Whilst ageing is commonly defined by chronological age, a great heterogeneity in cardiac ageing trajectories occurs in individuals of the same age.

WHAT THIS STUDY ADDS

- This is largest population-based study to date to examine the impact of cellular ageing, measured by leukocyte telomere length, on the deterioration of cardiac conduction. We found evidence that shorter telomere length was associated with longer ECG PR interval and future de-novo pacemaker insertion, independent of other cardiovascular risk factors.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- Our findings indicate a potential role for cellular aging in the pathogenesis and clinical presentation of atrio-ventricular conduction disease.

INTRODUCTION

Deterioration of the cardiac conduction system is an important manifestation of cardiac ageing which includes increased incidence of sinus node dysfunction, conduction delay or block at the atrioventricular node (AVN), and/or within the His Purkinje system[1–4]. Failure of atrio-ventricular (AV) conduction may result in syncope and significant associated injuries. Thus, high grade AV block is an indication for permanent pacemaker implantation.

Whilst ageing is commonly defined by chronological age, a great heterogeneity in ageing trajectories and health outcomes occurs in individuals of the same age[5]. Measures of biological age may provide added information about the impacts of ageing independent of chronological age[6]. This knowledge could be helpful in defining potential indicators of conduction system ageing, which is key for improved risk stratification and understanding of disease mechanisms. Telomeres are repetitive DNA sequences located at the ends of chromosomes which progressively shorten in somatic cells with increasing number of cell divisions and have therefore been regarded as a marker of biologic ageing. Previous work has shown that telomere length (TL) may provide insights into ageing across key organ systems beyond chronological age[7,8]. Whether variation in TL is also associated with deterioration of the cardiac conduction system remains unclear.

In this study, we therefore examined the association between leukocyte telomere length (LTL), a practical indicator of telomere length that correlates well across various tissues[9], and cardiac conduction in the large population-based cohort of UK Biobank. Specifically, we studied two related, but distinct aspects of the cardiac conduction system – one reflected by electrical measures of cardiac conduction (electrocardiographic PR interval and QRS duration) and the other reflected by a significant clinical outcome of cardiac conduction deterioration: incident pacemaker implantation. Prolongation of both PR interval and QRS duration are associated with increased risk of future permanent pacemaker insertion in the general population[4,10].

METHODS

Participants

From the 473,811 participants in UK Biobank with valid measurements of LTL[11], we excluded all participants with missing leukocyte count, mismatches between reported and genetic sex, and unknown ancestry (Figure 1). We also excluded participants with evidence of pre-existing pacemakers at baseline as this could render invalid inferences regarding ECG conduction measures and because we were specifically interested in incident pacemaker implantation. From the remaining participants (n = 452,997), we created two cohorts: one to study ECG measurements of cardiac conduction, and another to study incident pacemaker implantation (Figure 1). Please note that both cohorts were not mutually exclusive but were constructed to study different aspects of the cardiac conduction system in parallel.

In the ECG cohort, we included 69,625 participants (Figure 1) with single-lead (Lead I) ECG scans (CAM-USB 6.5, Cardiosoft v6.51) available at baseline. Signal-averaged waveforms recorded during a 15 second resting period were analysed for PR interval and QRS duration. Following exclusions for poor quality and extreme measurements (PR interval < 110ms or >200ms; QRS duration < 80ms or >120ms), and genetic relatedness (Figure 1), there were 59,868 and 62,266 participants included for analysis of PR interval and QRS duration, respectively. Genetically unrelated samples were obtained by randomly excluding one from each pair based on a kinship coefficient of K>0.088. In the pacemaker implantation cohort, there were 420,071 genetically unrelated individuals with hospital follow-up data included in the analysis (Figure 1). Cases of incident pacemaker implantation for a bradyarrhythmia indication were ascertained using International Classification of Disease (ICD) and OPCS Classification of Surgical Operations and Procedures version 4 codes from the hospital episode statistics (HES). Details are provided in Supplemental Table 1 and 2.

Leucocyte telomere length measurement

Details on the process of measuring of LTL, the extensive quality checks, and adjustment for technical factors have been reported previously[11]. 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 PCR method. LTL values were $\log_e$-transformed and Z-standardised for all analyses.

Statistical analyses

The descriptive statistics are presented as mean ± standard deviation [SD] for continuous variables and frequency (percentage) for categorical variables. The trends across PR and QRS quintiles were examined by Cuzick's extension[12] 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. The associations between $\log_e$ LTL residuals and ECG conduction measures were evaluated in multivariable linear regression models adjusted for sex, heart rate, height, and body-mass-index (BMI). The association between $\log_e$ LTL residuals and incident pacemaker implantation was evaluated using Cox proportional hazards regression adjusted for sex. Time-to-event duration was obtained from the admission date recorded in the hospital episode statistics. The censor date for hospital episode statistics data was 31 October 2022, the median (inter quartile range) follow up time was 13.6 [IQR: 1.5] years. Proportional hazards assumptions were assessed and met (Supplemental Figure 1). Significant associations for ECG measures and pacemaker implantation were additionally adjusted for traditional cardiovascular risk factors, including current smoking, hypertension, type 2 diabetes mellitus, and prevalent coronary artery disease (definitions provided in Supplemental Table 3), to assess their potential confounding effects. The effect sizes and hazard ratios were represented per SD LTL shortening. A p-value less than 0.05 was considered statistically significant. All analyses were conducted in R version 4.2.0[13].

Sensitivity analysis

As cardiac conduction can be affected by vagal tone or antiarrhythmic medication, post-hoc sensitivity analyses were performed to explore whether associations were affected by these parameters. The influence of vagal tone was explored in a subgroup of individuals with ECG recordings who also participated in an exercise test immediately after conduction measurements were taken. Vagal tone was measured by the speed of heart rate recovery after exercise[14] and included as an additional covariate in the multivariable linear regression models. The influence of antiarrhythmic medication was explored by excluding participants exposed to beta and calcium blockers.

Mendelian randomisation analysis

To examine potential causality of LTL with observationally associated cardiac conduction traits (PR interval and pacemaker implantation), we conducted single-sample univariable MR using two-sample methods shown to be robust in large-scale biobanks[15]. The inverse-variance weighted method[16] was implemented to test for the possible causal effect of LTL on PR interval and pace-maker implantation based on 130 independent and pleiotropically pruned variants associated with LTL[8]. The PR interval estimates were obtained from previously performed meta-analysis by our group including 293,051 individuals of European ancestry[17]. For pacemaker implantation, effect estimates were obtained by performing logistic regression between pacemaker implantation and the allele dosage information available in UK Biobank. Sensitivity analyses for MR were performed using (i) MR-Egger regression to estimate unmeasured pleiotropy in the intercept[18], (ii) weighted median estimator to assess the robustness to extreme SNP–outcome associations[19], and (iii) a contamination-mixture method to explore potential presence of multiple pathways[20].

RESULTS

Baseline population characteristics

In the ECG cohort (N=59,868 and 62,266 for PR interval and QRS duration, respectively), the average age ± SD was: 56.8 ± 8.1 years for participants with PR interval and QRS duration available and approximately 46% were men (Table 1 and 2). The average PR interval and QRS duration were 144.2 ± 20.4ms and 92.3 ± 7.8ms, respectively. Individuals in the higher PR interval quintiles were more likely to be chronologically older and male with higher prevalence of cardiovascular risk factors (Table 1). A similar trend was observed for QRS duration. LTL decreased with increasing PR interval and QRS duration quintiles (Figure 2A and 2B). In the pacemaker cohort, there were 7,169 (1.7%) incident pacemaker implantations. Individuals who received a pacemaker implantation, as compared to those who did not, were older, more likely to be male (Table 3), and had shorter LTL (Figure 2C).

Table 1. Baseline characteristics according to quintiles of PR interval

Variable	Full cohort (59,868)	PR interval quintile levels					P value
		1st (<126 ms)	2nd (126 - 136ms)	3rd (136 - 148ms)	4th (148 - 162ms)	5th (>162ms)	
Chronical age (years)	56.8 (8.1)	56.0 (8.3)	56.3 (8.2)	56.7 (8.0)	57.1 (7.9)	58.0 (7.8)	<0.001
Men (%)	46.4	36.9	42.5	46.2	51.3	57.3	<0.001
WBC (mmol/l)	7.1 (2.1)	7.2 (2.1)	7.1 (2.1)	7.1 (1.9)	7.0 (1.8)	7.0 (2.4)	<0.001
Height (cm)	168.7 (9.3)	167.4 (9.1)	168.1 (9.1)	168.5 (9.2)	169.5 (9.4)	170.3 (9.2)	<0.001
BMI	27.5 (4.7)	26.6 (4.4)	27.3 (4.6)	27.6 (4.6)	27.9 (4.7)	28.3 (4.8)	<0.001
LTL	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	-0.1 (1.0)	<0.001
Heart rate (bpm)	71.2 (11.8)	73.7 (11.7)	72.7 (11.6)	71.4 (11.4)	69.7 (11.5)	68.0 (11.9)	<0.001
Coronary artery disease (%)	3.3	2.1	2.5	2.9	3.8	5.6	<0.001
Hypertension (%)	53.6	49.9	51.6	53.6	54.6	59.0	<0.001
Active smoker (%)	9.3	10.2	10.3	9.2	8.4	8.3	<0.001
Diabetes mellitus (%)	5.5	4.6	4.7	5.8	6.1	6.4	<0.001
Ancestry (%)							
Asian	3.2	3.9	3.4	3.5	2.6	2.3	<0.001
Black	3.0	2.7	2.5	2.7	3.3	3.7	<0.001
Chinese	0.4	0.6	0.4	0.5	0.4	0.3	0.005
Mixed	0.9	1.0	0.9	0.7	0.8	0.9	0.266
Other	1.5	1.4	1.5	1.7	1.3	1.5	0.799
White	91.1	90.4	91.3	90.9	91.6	91.3	0.006

Continuous values given as mean (standard deviation). WBC, white blood cell count; BMI, body-mass-index; bpm, beats/min; LTL, loge-transformed leukocyte telomere length; SD, standard deviation

Table 2. Baseline characteristics according to quintiles of QRS duration.

Variable	Full cohort (62,266)	QRS duration quintile levels					P value
		1st (<86 ms)	2nd (86 - 90ms)	3rd (90 - 94ms)	4th (94 - 98ms)	5th (>98ms)	
Chronical age (years)	56.8 (8.1)	56.7 (8.1)	56.5 (8.1)	56.6 (8.1)	56.6 (8.2)	57.4 (8.2)	<0.001
Men (%)	46.7	34.5	44.4	49.2	54.7	59.1	<0.001
WBC (mmol/l)	7.1 (2.0)	7.1 (2.1)	7.1 (2.0)	7.0 (1.8)	7.0 (1.8)	7.0 (2.3)	<0.001
Height (cm)	168.9 (9.2)	166.2 (8.8)	168.4 (9.1)	169.4 (9.1)	170.6 (9.2)	171.6 (9.1)	<0.001
BMI	27.4 (4.7)	27.1 (4.6)	27.5 (4.7)	27.5 (4.7)	27.4 (4.7)	27.5 (4.9)	<0.001
LTL	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	<0.001
Heart rate (bpm)	71.2 (12.0)	73.4 (12.2)	71.9 (11.8)	71.0 (11.7)	70.0 (11.8)	68.3 (11.8)	<0.001
Coronary artery disease (%)	3.4	2.2	2.9	3.1	3.6	5.7	<0.001
Hypertension (%)	53.1	50.0	52.9	54.2	53.6	56.4	<0.001
Active smoker (%)	9.4	8.9	9.3	9.8	9.9	9.5	0.051
Diabetes mellitus (%)	5.5	5.0	5.3	5.5	5.5	6.2	<0.001
Ancestry (%)							
Asian	3.1	4.0	3.4	2.9	2.4	2.0	<0.001
Black	2.9	3.8	3.4	2.9	2.3	1.4	<0.001
Chinese	0.4	0.6	0.6	0.4	0.4	0.2	<0.001
Mixed	0.8	1.0	1.0	0.8	0.7	0.6	<0.001
Other	1.5	1.8	1.6	1.5	1.3	0.9	<0.001
White	91.3	88.9	90.0	91.6	92.8	94.9	<0.001

Continuous values given as mean (standard deviation). WBC, white blood cell count; BMI, body-mass-index; bpm, beats/min; LTL, loge-transformed leukocyte telomere length; SD, standard deviation

Table 3. Baseline characteristics for the pacemaker implantation cohort.

		Incident pacemaker implantation		
	Full cohort (420,071)	**Yes (7,169)**	**No (412,902)**	**P value**
Chronical age (years)	56.5 (8.0)	56.4 (8.0)	62.0 (6.0)	<0.001
Men (%)	46.0	45.6	68.4	<0.001
WBC (mmol/l)	6.9 (2.0)	6.9 (2.0)	7.1 (1.9)	<0.001
LTL	0.0 (1.0)	0.0 (1.0)	-0.2 (1.0)	<0.001
Coronary artery disease (%)	3.8	3.7	13.7	<0.001
Hypertension (%)	53.2	52.8	75.2	<0.001
Active smoker (%)	10.6	10.6	9.8	0.032
Diabetes mellitus (%)	5.3	5.2	12.2	<0.001
Ancestry (%)				
Asian	2.0	2.0	2.2	0.145
Black	1.5	1.6	0.6	<0.001
Chinese	0.3	0.3	0.2	0.014
Mixed	0.6	0.6	0.3	0.003
Other	0.9	0.9	0.7	0.067
White	94.7	94.6	96.0	<0.001

Continuous values given as mean (standard deviation).WBC, white blood cell count; SD, standard deviation

Observational associations between LTL, ECG conduction measurements, and incident pacemaker implantation

In the minimally adjusted model (sex, height, body-mass-index (BMI), and heart rate), LTL was significantly associated with PR interval (beta = 0.18ms per 1 SD decrease in $\log_e$ LTL, 95% confidence interval (CI): 0.02 – 0.34ms, p = 0.029), but not QRS duration (Table 4). The association between LTL and PR interval was not attenuated and remained statistically significant after additional adjusting for cardiovascular risk factors and coronary artery disease (Table 4). In the sensitivity analyses, no important changes were observed in the magnitude and direction of the effect when additionally adjusting for vagal tone or excluding individuals exposed to beta and calcium blockers (Supplemental Table 4 and 5). In the pacemaker cohort, shorter LTL was associated with a higher risk of future pacemaker implantation when adjusting for age and sex (hazard ratio (HR) per 1 SD decrease in $\log_e$ LTL: 1.03; 95% CI, 1.01-1.05; p = 0.029). The association remained significant with little attenuation after additional adjusting for cardiovascular risk factors (T2DM, current smoking, and hypertension), and coronary artery disease (Table 4). No evidence was found that the association was affected by beta and calcium blockers in the sensitivity analysis (Supplemental Table 5). To put the effect sizes for

LTL in perspective, we also calculated the effect size of (chronological) age on PR interval: After adjusting for the same cardiovascular risk factors, 1 year increase in age was found to have a similar effect size for PR interval compared to 1 SD reduction in LTL (0.19ms per 1-year increase in age, 95% CI: 0.17 – 0.21, p < 0.001). For pacemaker implantation, the HR was 1.10 per 1-year increase in age (95% CI: 1.10-1.11, p < 0.001). The HR of 1 SD decrease in LTL (HR 1.03) was therefore comparable with ~3.8 months older age.

Table 4. Multivariable regression results for the association between leukocyte telomere length and PR interval, QRS duration, and incident pacemaker implantation

PR interval			
Model	beta	95% CI	p value
Adjusted for sex, age, height, BMI, and heart rate	0.18	0.02 - 0.34	0.029
... + T2DM, current smoking, hypertension, and CAD	0.19	0.03 - 0.35	0.021

QRS duration			
Model	beta	95% CI	p value
Adjusted for sex, age, height, BMI, and heart rate	0.04	-0.02 - 0.11	0.152

Incident pacemaker implantation			
Model	Hazard ratio	95% CI	p value
Adjusted for sex and age	1.04	1.02 - 1.07	<0.001
... + T2DM, current smoking, hypertension, and CAD	1.03	1.01 - 1.06	0.012

Effect sizes and hazard ratios are expressed per 1 SD decrease in leukocyte telomere length. *CAD, prevalent coronary artery disease.*

Mendelian randomisation analyses

Using 130 genetic variants independently associated with LTL as instruments[8] in the MR analysis, we found nominal significant associations for a direct effect from LTL to PR interval and pacemaker implantation with consistent directions of effect (0.74ms increase in PR interval per 1 SD shorter LTL, 95% CI (0.01 – 1.47); and HR of 1.16 for pacemaker implantation per 1 SD shorter LTL, 95% CI (1.00 – 1.34), Table 5). This HR was comparable with 18.7 months older age (as shown above, HR of age is 1.10 per 1-year increase). No evidence of horizontal pleiotropy was found in the MR-Egger analyses and while effect sizes remain consistent across the sensitivity analyses the nominal level of significance was lost when applying the weighted median and contamination mixture methods in the sensitivity analysis (Table 5).

Table 5. Mendelian randomisation associations between leukocyte telomere length (LTL) and PR interval and pacemaker implantation

MR method	PR interval		Pacemaker implantation	
	Beta (95% CI)	P	HR (95% CI)	P
IVW	0.74 (0.01, 1.47)	0.048	1.16 (1.00 - 1.34)	0.046
Weighted Median	0.73 (-0.19, 1.65)	0.122	1.19 (0.97 - 1.48)	0.100
ConMix	0.46 (-0.16, 1.05)	0.144	1.21 (0.97 - 1.42)	0.088
Egger Intercept		0.936		0.599

Associations per 1 SD shorter LTL. MR, Mendelian Randomisation; IVW, inverse variance weighted; ConMix: contamination mixture, HR hazard ratio; CI confidence interval

DISCUSSION

This work represents the largest population-based observational study to date to examine the impact of cellular ageing, measured by LTL, on the deterioration of cardiac conduction in humans. Our main findings are that in a middle-aged population: (1) LTL is associated with PR interval, but not QRS duration; and (2) LTL is associated with incident pacemaker implantation, and (3) evidence from mendelian randomisation analysis suggest the observed associations might be causal, where shorter LTL increases PR interval and the risk of pacemaker implantation.

Association of LTL with ECG measures of cardiac conduction

Our work confirms previous data of prolongation of PR interval and QRS duration with increasing aging[2,21]. However, only a very small number of studies have investigated the implications of biological ageing. Most recently, Von Falkenhausen et al.[22] found no effect of LTL on PR interval and QRS duration in 2,575 subjects from the community-based KORA study after accounting for age, sex, height, and body mass-index. With ~60,000 subjects, our study was considerably larger and better powered to detect any potential association. It is likely that this has enabled us to detect the association between LTL and PR interval, as the adjusted effect size was very small (e.g. <1ms increase in PR interval per 1 SD LTL shortening). In addition, Von Falkenhausen et al. did not adjust for heart rate which might have potentially diluted their results as PR interval is known to vary with heart rate[23]. Our direction of effect was consistent with that reported in the KORA study. Like Von Falkenhausen, we did not find evidence that LTL was associated with QRS duration. There may be different explanations compatible with these findings. For example, age-related processes might be different for atrioventricular and intraventricular conduction. These processes may include diminished dromotropic effect of catecholamines on atrioventricular junctional tissues. For example, aging has been associated with diminished chronotropic and inotropic responses to catecholamines[24,25]. However, in the sensitivity analysis, we found no evidence to suggest that vagal tone could explain the association between LTL and PR interval. Alternative explanations include structural changes that occur within the atrioventricular junction with advanced age[26], whereas QRS duration may depend more on age-related functional and structural changes of the ventricular myocardium, for example due to fibrosis[27,28]. Interestingly, we have recently demonstrated a (causal) association between LTL and cardiac dimensions and function using cardiac imaging[29], it might be that these processes have little impact on ventricular conduction and QRS duration. It is also worth noting that PR interval

represents the total transmission time through both atria, the AV node, His bundle, and bundle branches to the onset of ventricular activation via the Purkinje system, and increased conduction delay can occur at any of these sites[30]. Alternatively, the lack of association may also simply reflect that fact that QRS duration shows less variation with age compared to PR interval, making it harder to measure the association with LTL.

Association of LTL with incident pacemaker implantation

We further enhance our understanding of the role of cellular aging in the deterioration of the cardiac conduction system by demonstrating that shorter LTL is associated with higher risk of incident pacemaker implantation. This provides an additional line of evidence that shows that cellular aging may not only be associated with atrioventricular conduction (PR interval) as a continuous variable, but also the actual clinically significant pathophysiology that underlies the deterioration of the cardiac conduction system which requires pacemaker intervention. The exact mechanisms remain unclear, but our results suggest that they may include processes linked to accelerated aging.

Causal relationship between LTL and cardiac conduction

In this work, we also explored the causal relationship between LTL and cardiac conduction disease for the first time. Results from the MR analysis hint at a potential trend indicating that a reduction in LTL may cause an increase in the PR interval and a higher risk of incident pacemaker implantation. However, the observed associations did not retain statistical significance in sensitivity analyses, likely due to the limited study power (<10% power to detect any association). This contrast with our findings for other age-related cardiovascular diseases such as coronary artery disease and heart failure[8,29]. Whether the LTL associations are causal or not, the (causal) mechanisms for the biological age-associated increase in PR interval and a higher risk of pacemaker implantation remain therefore to be further investigated.

Clinical implications

Although associations between LTL and cardiac conduction were statistically significant, it is important to interpret this in the context of the study's high power to detect even modest associations. The observed effect sizes in this work were small and may therefore not necessarily imply clinical importance. For example, the observed 3% increased risk of pacemaker implantation for 1 SD shortening in LTL was estimated to be equivalent to a 3.8 month increase in (chronological) age within the observational analysis (see Results), and yet

in our MR this estimate increases to an estimated 18.7 months older age. However, the observational associations between LTL and PR interval and pacemaker implantation were independent of other cardiovascular risk factors potentially suggesting that telomere biology, and perhaps cellular aging in general, may contribute uniquely to the pathophysiology of the cardiac conduction system. This may offer potential for improved risk stratification and novel insights into disease mechanisms beyond (chronological) age, and other traditional cardiovascular risk factors. Results also support the growing body of evidence linking telomere biology to cardiovascular health outcomes[8,29]. Further research is needed to explore these associations in more detail, particularly to understand the underlying mechanisms and to investigate other markers of aging in relation to cardiac conduction system pathophysiology.

Strengths & limitations

Our study has several strengths, including access to the largest sample size to date with long-term follow-up to study the association between LTL, PR interval, QRS duration, and pacemaker implantation. Nevertheless, several limitations need to be acknowledged. First, analogous to previous studies, this is a post-hoc analysis on a database where conduction disease was not the primary outcome of interest. We applied a rather conservative case definition for pacemaker implantation, which may have caused an underestimation of the actual rate and therefore attenuated the effect estimations. Second, there is a "healthy volunteer" selection bias in the UK Biobank with the participants being older and healthier than the UK general population. Third, the majority of our cohort (97%) is of white ancestry, which may limit the generalisability of our findings in under-represented ethnicities. Fourth, we cannot fully rule out that the observed associations might be affected by residual confounding from undetected cardiovascular disease or unmeasured medication use. However, given that our cohort was rather healthy and that there was no supportive evidence for confounding by beta and calcium blocker medication use, we believe the impact is likely to be limited. Finally, telomere length was quantified in blood leukocytes which may not reflect cell or conduction-tissue telomere length.

In conclusion, in this long-term prospective cohort study we studied the association of LTL with two related but distinct aspects of the cardiac conduction system. One aspect reflected measures of electrical conduction where we demonstrate that shorter LTL is associated with increased atrioventricular conduction delay. The other aspect reflected on incident pacemaker implantation for a bradyarrhythmia indication, a significant clinical outcome of cardiac conduction disorder, where we demonstrate that shorter LTL is associated with a higher risk of pacemaker implantation. Combined, results suggest a potential role of cellular aging as a mechanistic pathway for age-related conduction disease, providing insights into novel risk stratification approaches and therapeutic targets for conduction disease. In this context, future work may focus on alternative measures of biological ageing.

ACKNOWLEDGMENTS

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George's University Hospitals NHS Foundation Trust and St George's University of London. SEP acknowledges the BHF for funding the manual analysis to create a cardiovascular magnetic resonance imaging reference standard for the UK Biobank imaging resource in 5000 CMR scans. SEP acknowledges support from the SmartHeart EPSRC programme grant. NA acknowledges the 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. ZRE recognises the NIHR Integrated Academic Training programme, which supports her Academic Clinical Lectureship post, and was also supported by BHF Clinical Research Training Fellowship number FS/17/81/33318.

Conflict of interest

JD 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.

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FIGURE LEGENDS

Figure 1. Exclusion diagrams for PR interval, QRS duration, and pacemaker implantation cohorts. WBC, white blood cell count; ECG, electrocardiogram.

Figure 2. Leukocyte telomere length (LTL) plotted as function of PR interval quintiles (A), QRS duration (B), and do-novo pacemaker implantation (C). We removed the confounding influence of chronological age, white blood cell count, and self-reported ethnicity by taking the residuals of $\log_e$ LTL regressed on these variables. Trends across PR interval quintiles, QRS duration quintiles, and pacemaker implantation outcome were both significant ($P < 0.001$).

Reviewer(s)' Comments to Author (if any):

Reviewer: 1

Comments to the Author
In this manuscript by Dr Duijvenboden association of telomere length and conduction related ECG parameters and pacemaker implantation rate in a large general population cohort. The results show that shorter leucosyte length (surrogate for higher biological age) were associated with longer PR interval and pacemaker implantation rate, but was not associated with QRS prolongation.
The analyses are solid and the results are clear. However, there are a few conceptual issues related to the study desing and conclusions.
PR interval represents the conduction in the AV node which is mostly affected by autonomic tone than actual conduction system disease i.e. vagal tone prolongs PR inveral. Possibly due to this reason PR interval has not been associated with progressive conduction system disease or pacemaker implantation rate in the general population in previous studies. QRS interval is a more reliable variable in illustrating conduction system disease and there was no association with telomere length in the current study.

Thank you for raising this interesting point. Previous findings from large community-based populations such as the Framingham Heart Study do indicate that prolonged PR and QRS intervals are both independently associated with risk of future pacemaker implantation (for example see Cheng S et al. *JAMA.* 2009;301(24):2571-2577. doi:10.1001/jama.2009.888 and Cheng S et al. Am J Cardiol. 2010 Jul 23;106(5):668–672. doi: 10.1016/j.amjcard.2010.04.021). Nonetheless, we fully agree with your notion that changes in autonomic (vagal) tone may play an important role in this context. To explore this possibility, we took advantage of the fact that most individuals in our study also completed an exercise test immediately after the resting ECG that was used to derive PR interval. The protocol included measurements of the speed of heart rate recovery after exercise, which is a highly reproducible marker of vagal tone[1]. This allowed us to assess the role of vagal tone, measured by heart rate recovery, in the observed association between leukocyte telomere length (LTL) and PR interval. In this sensitivity analysis, we included 47,755 participants (80.0%) of the original PR interval cohort who had heart rate recovery measured. As shown in the table below, additionally adjusting for heart rate recovery did not influence the magnitude or direction of the association effect of telomere length on PR interval. Our data therefore does not suggest that increased vagal tone accounts for the observed association between telomere length and PR interval.

PR interval (N=47,755 individuals with heart rate recovery measured)

Model	beta	95% CI
Adjusted for sex, age, height, BMI, resting heart rate, T2DM, current smoking, hypertension, and CAD	0.13	-0.06 - 0.31
…+ heart rate recovery	0.12	-0.06 - 0.30

Another major issue is the absence of data which could affect PR interval and pacemaker implantation rate in the population such as underlying, undiagnosed, cardiac disease and/or AV node affecting medication (i.e. b-blockers).

We agree that is it possible that residual confounding due to undiagnosed cardiac disease might have affected our results and that this is a limitation of our study. However, it is worth noting that the prevalence of existing cardiovascular disease was low (3.3%) and the UK Biobank population is known to be generally healthy. We therefore believe the impact of undiagnosed cardiac disease is likely to be limited. Medication use was recorded at the time of the study assessment, and we agree it would be

important to further explore this given the profound effect that some antiarrhythmic agents have on AV node conduction. To address this comment, we performed another sensitivity analysis where we excluded individuals who were on either beta-blockers or calcium channel blockers, which are most commonly used to control heart rate and blood pressure but also affect AV node conduction. As shown in the tables below, approximately 7 and 9% of the study cohorts were using this medication, for PR interval and pacemaker implantation, respectively. As shown below, excluding these individuals did not result in important changes in effect size for telomere length.

PR interval	Original analysis, N=59,868		After excluding for beta-blocker & calcium antagonists, N=55,829	
Model	Beta	95% CI	beta	95% CI
Adjusted for sex, age, height, BMI, resting heart rate, T2DM, current smoking, hypertension, and CAD	0.19	0.03 - 0.36	0.17	0.01 - 0.34

Incident pacemaker implantation	Original analysis, N = 420,071		After excluding beta-blocker and calcium antagonists, N = 385,811	
Model	Hazard ratio	95% CI	Hazard ratio	95% CI
Adjusted for sex, age, T2DM, current smoking, hypertension, and CAD	1.03	1.01 - 1.06	1.04	1.01 - 1.07

With respect to pacemaker implantation, one potential limitation is that the effect of the medication might be time varying (e.g. individuals may stop or start medication during follow up). Unfortunately, medication was not assessed routinely but this also applies to other covariates used in the model. Whilst we agree that the role of residual confounding due to either undiagnosed heart disease or medication use is possible, results seem to suggest that the impact is likely to be limited.

Following the comments and our responses and the additional analyses described above, we have made the following changes to manuscript, which we are hope are satisfactory:

Introduction, page 5: We have now cited papers which show the association between PR length and QRS duration and future pacemaker implantation:

“Prolongation of both PR interval and QRS duration are associated with increased risk of future permanent pacemaker insertion in the general population [4,10].”

Methods, page 7:

“Sensitivity analysis
As cardiac conduction can be affected by vagal tone or antiarrhythmic medication, post-hoc sensitivity analyses were performed to explore whether associations were affected by these parameters. The influence of vagal tone was explored in a subgroup of individuals with ECG recordings who also participated in an exercise test immediately after conduction measurements were taken. Vagal tone was measured by the speed of heart rate recovery after exercise [14] and included as an additional covariate in the multivariable linear regression models. The influence of

antiarrhythmic medication was explored by excluding participants exposed to beta and calcium blockers.

Results, page 11:
"In the sensitivity analyses, no important changes were observed in the magnitude and direction of the effect when additionally adjusting for vagal tone or excluding individuals exposed to beta and calcium blockers (Supplemental Table 4 and 5)."

"No evidence was found that the association was affected by beta and calcium blockers in the sensitivity analysis (Supplemental Table 5)."

Discussion, page 14:
"These processes may include diminished dromotropic effect of catecholamines on atrioventricular junctional tissues. For example, aging has been associated with diminished chronotropic and inotropic responses to catecholamines[23,24]. However, in the sensitivity analysis, we found no evidence to suggest that vagal tone could explain the association between LTL and PR interval."

Limitations, page 16:
"Fourth, we cannot fully rule out that the observed associations might be affected by residual confounding from undetected cardiovascular disease or unmeasured medication use. However, given that our cohort was rather healthy and that there was no supportive evidence for confounding by beta and calcium blocker medication use, we believe the impact is likely to be limited."

Statistical Reviewer(s)' Comments to Author (if any):

This study is based on large population-based sample. With overwhelmingly large power, the p value should not be used as "significant" criteria. Instead, the author should focus on the clinical significance. For example, per 1 SD decrease in leukocyte telomere length, the HR for pacemaker implantation is 1.04 95% CI (1.02 - 1.07). Will such a small HR with 1 SD decrease of leukocyte telomere length be clinically significant?

We appreciate the Reviewer's point regarding the use of p-values in studies with large sample sizes and agree that while the very small effect sizes were statistically significant, they do not necessarily imply clinical importance. Though modest, we believe the findings still offer important novel insights in the role of cellular aging in cardiac conduction disease. They also suggest further explore cellular aging in conjunction with other biomarkers of aging may further deepen our understanding of the role of aging in cardiac conduction disease. We made the following changes to the text to emphasise your point that p-values should not be used as significant criteria.

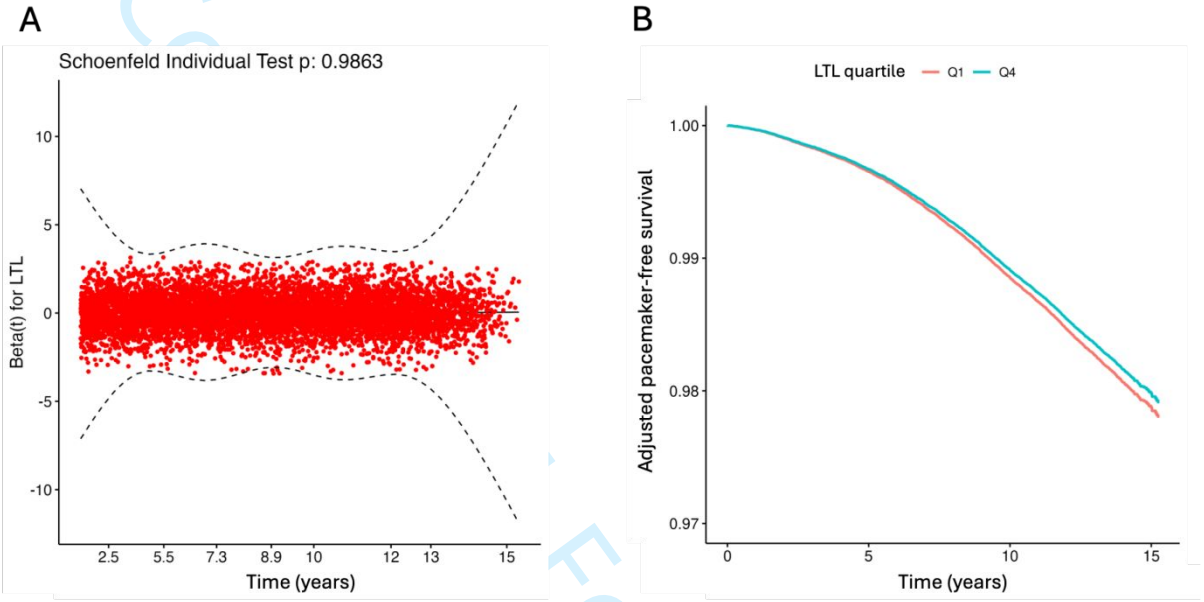
"Clinical implications, page 15:
Although associations between LTL and cardiac conduction were statistically significant, it is important to interpret this in the context of the study's high power to detect even modest associations. The observed effect sizes in this work were small and may therefore not necessarily imply clinical importance."

The author did not report the proportional hazard assumption assessment result. Although the method has been mentioned as the following "Schoenfeld residuals were visually checked and met the proportional hazards assumption". The author should also present adjusted survival curve by first and third quartiles (or mean-0.5*SD vs. mean+0.5*SD) of leukocyte telomere length.

Thank you for your suggestion, we have added the residual plot and the requested figure to the supplement to support our statement that the proportional hazard assumption holds.

Methods, page 7: Proportional hazards assumptions were assessed and met (Supplemental Figure 1).

Supplemental Figure 1: Assessment of proportional hazards assumptions, leukocyte telomere length (LTL) – incident pacemaker implantation.



References

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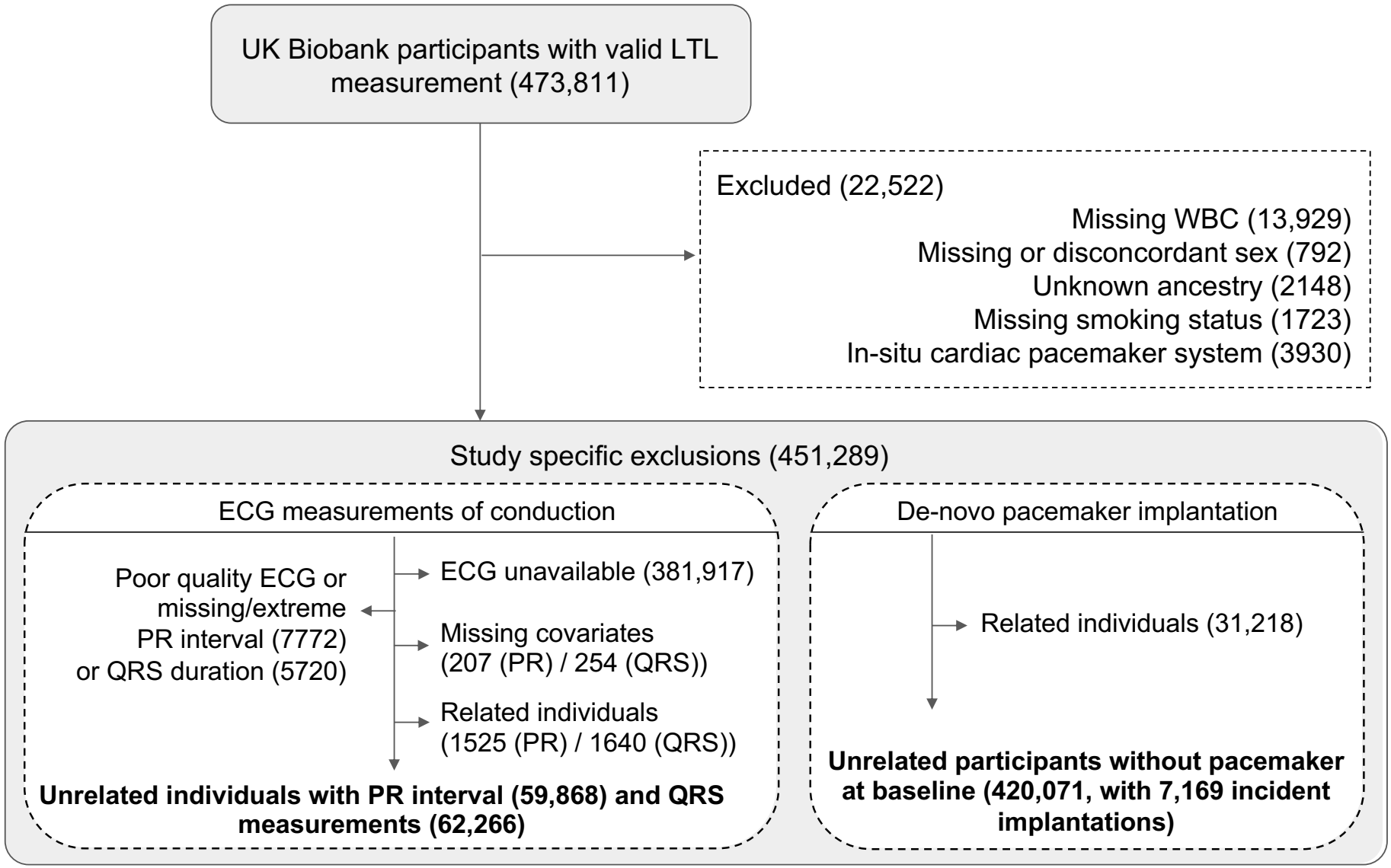
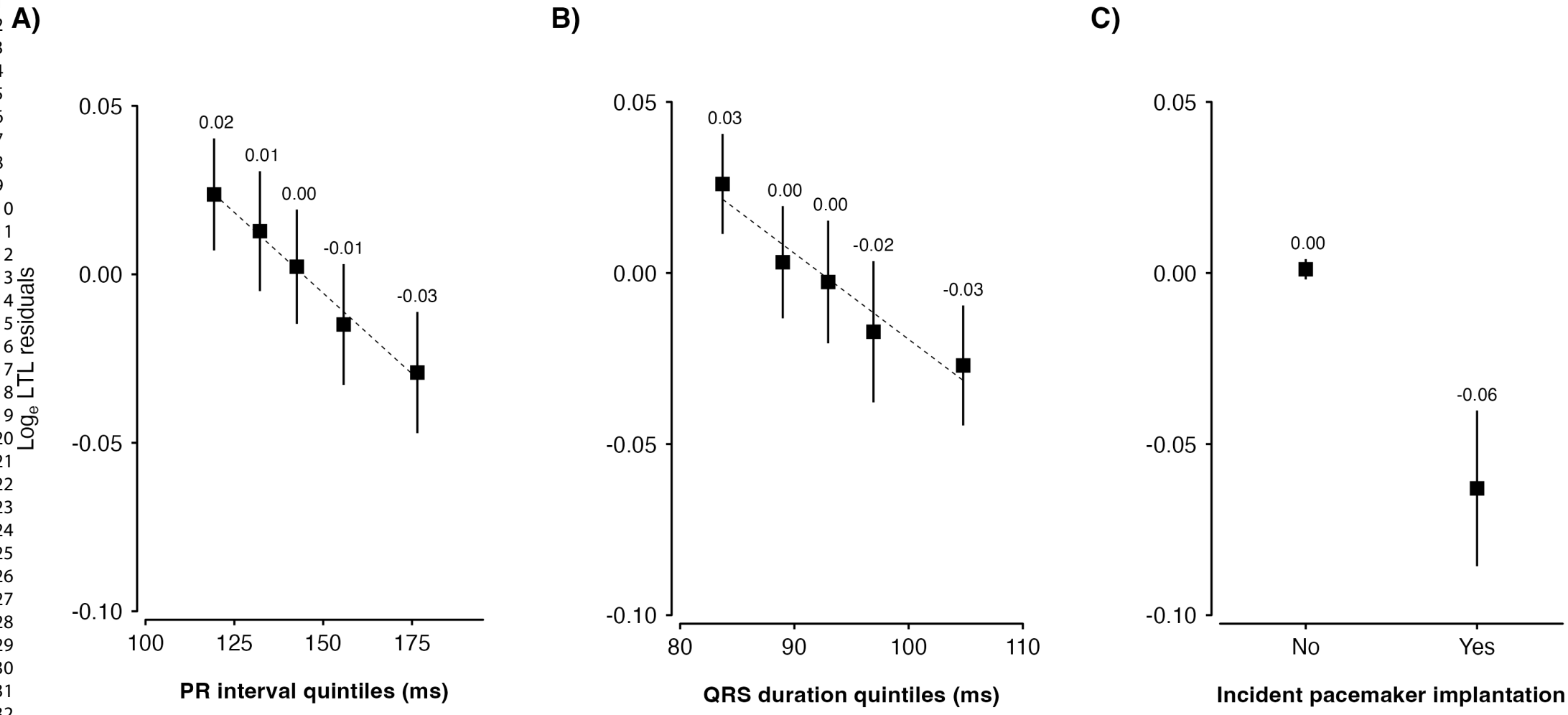
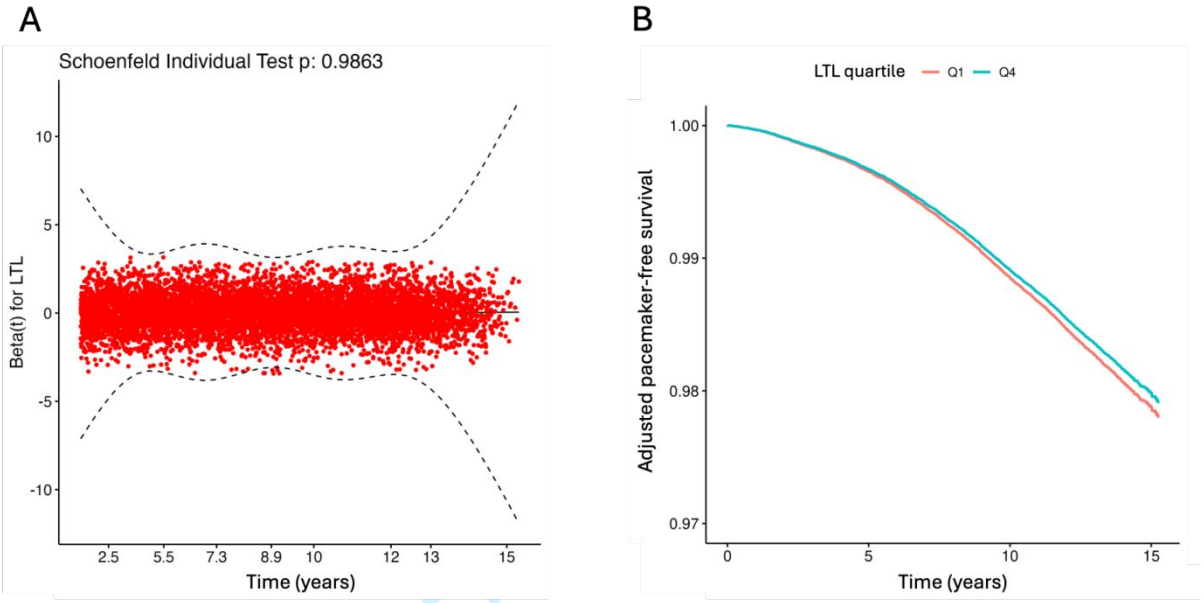

UK Biobank participants with valid LTL measurement (473,811)
Excluded (22,522)
Missing WBC (13,929)
Missing or disconcordant sex (792)
Unknown ancestry (2148)
Missing smoking status (1723)
In-situ cardiac pacemaker system (3930)
Study specific exclusions (451,289)
ECG measurements of conduction
Poor quality ECG or missing/extreme PR interval (7772) or QRS duration (5720)
ECG unavailable (381,917)
Missing covariates (207 (PR) / 254 (QRS))
Related individuals (1525 (PR) / 1640 (QRS))
Unrelated individuals with PR interval (59,868) and QRS measurements (62,266)
De-novo pacemaker implantation
Related individuals (31,218)
Unrelated participants without pacemaker at baseline (420,071, with 7,169 incident implantations)


A)
0.05
0.00
-0.05
-0.10
0.02
0.01
0.00
-0.01
-0.03
100
125
150
175
PR interval quintiles (ms)
Log$_e$ LTL residuals
B)
0.05
0.00
-0.05
-0.10
0.03
0.00
0.00
-0.02
-0.03
80
90
100
110
QRS duration quintiles (ms)
C)
0.05
0.00
-0.05
-0.10
0.00
-0.06
No
Yes
Incident pacemaker implantation

Supplemental Figure 1. Assessment of proportional hazard assumptions: leukocyte telomere length (LTL) – incident pacemaker implantation



Supplemental Table 1: Codes used to identify individuals with pre-existing pacemaker.

source	code	description
OPSC-4	K60.1	Implantation of intravenous cardiac pacemaker system NEC
OPSC-4	K60.2	Resiting of lead of intravenous cardiac pacemaker system
OPSC-4	K60.3	Renewal of intravenous cardiac pacemaker system
OPSC-4	K60.4	Removal of intravenous cardiac pacemaker system
OPSC-4	K60.5	Implantation of intravenous single chamber cardiac pacemaker system
OPSC-4	K60.6	Implantation of intravenous dual chamber cardiac pacemaker system
OPSC-4	K60.8	Other specified cardiac pacemaker system introduced through vein
OPSC-4	K60.9	Unspecified cardiac pacemaker system introduced through vein
OPSC-4	K61.1	Implantation of cardiac pacemaker system NEC
OPSC-4	K61.2	Resiting of lead of cardiac pacemaker system NEC
OPSC-4	K61.3	Renewal of cardiac pacemaker system NEC
OPSC-4	K61.4	Removal of cardiac pacemaker system NEC
OPSC-4	K61.5	Implantation of single chamber cardiac pacemaker system
OPSC-4	K61.6	Implantation of dual chamber cardiac pacemaker system
OPSC-4	K61.8	Other specified cardiac pacemaker system
OPSC-4	K61.9	Unspecified cardiac pacemaker system
ICD10	Z45.0	Adjustment and management of cardiac pacemaker
ICD10	Z95.0	Presence of cardiac pacemaker
ICD9	V450	Cardiac pacemaker in situ

OPSC-4: Office of Population Censuses and Surveys Classification of Interventions and Procedures version 4; ICD: International Classification of Diseases

Supplemental Table 2: Codes used to identify individuals with de-novo pacemaker implantation.

source	code	description
OPSC-4	K60.1	Implantation of intravenous cardiac pacemaker system NEC
OPSC-4	K60.5	Implantation of intravenous single chamber cardiac pacemaker system
OPSC-4	K60.6	Implantation of intravenous dual chamber cardiac pacemaker system
OPSC-4	K60.8	Other specified cardiac pacemaker system introduced through vein
OPSC-4	K60.9	Unspecified cardiac pacemaker system introduced through vein
OPSC-4	K61.1	Implantation of cardiac pacemaker system NEC
OPSC-4	K61.5	Implantation of single chamber cardiac pacemaker system
OPSC-4	K61.6	Implantation of dual chamber cardiac pacemaker system
OPSC-4	K61.8	Other specified cardiac pacemaker system
OPSC-4	K61.9	Unspecified cardiac pacemaker system

Individuals who had their pacemaker excluded during follow-up where excluded from the analysis unless they had a re-implantation. *OPSC-4: Office of Population Censuses and Surveys Classification of Interventions and Procedures version 4*

Supplemental Table 3: Definition of coronary artery disease

Code type	Code	Definitions
ICD10	I21	Acute myocardial infarction
ICD10	I22	Subsequent myocardial infarction
ICD10	I23	Certain current complications following acute myocardial infarction.
ICD10	I24	Other acute ischaemic heart diseases.
ICD10	I25	Chronic Ischemic Heart Disease
ICD9	410	Acute myocardial infarction
ICD9	411	Other acute and subacute forms of ischemic heart disease
ICD9	412	Old myocardial infarction
ICD9	414	Other forms of chronic ischemic heart disease
OPCS4	K40	Saphenous vein graft replacement of coronary artery
OPCS4	K41	Other autograft replacement of coronary artery
OPCS4	K42	Allograft replacement of coronary artery
OPCS4	K43	Prosthetic replacement of coronary artery
OPCS4	K44	Other replacement of coronary artery
OPCS4	K45	Connection of thoracic artery to coronary artery
OPCS4	K46	Other bypass of coronary artery12
OPCS4	K49	Transluminal balloon angioplasty of coronary artery
OPCS4	K501	Percutaneous transluminal laser coronary angioplasty
OPCS4	K75	Percutaneous transluminal balloon angioplasty and insertion of stent into coronary artery
Self-reported medical conditions	1	Heart Attack
Self-reported operations	1070	coronary angioplasty (ptca) +/- stent
Self-reported operations	1095	coronary artery bypass grafts (cabg)
Self-reported operations	1523	triple heart bypass

OPSC-4: Office of Population Censuses and Surveys Classification of Interventions and Procedures version 4; ICD: International Classification of Diseases

Supplemental Table 4: Multivariable regression results for the association between leukocyte telomere length and PR interval after additionally adjusting for vagal tone (hear rate recovery) in post-hoc sensitivity analysis

Model	beta	95% CI
Adjusted for sex, age, height, BMI, resting heart rate, T2DM, current smoking, hypertension, and CAD	0.13	-0.06 - 0.31
…+ heart rate recovery	0.12	-0.06 - 0.30

N=47,755 individuals with heart rate recovery measured.

Supplemental Table 5: Multivariable regression results for the association between leukocyte telomere length, PR interval, and pacemaker implantation after excluding for beta and calcium antagonists in post-hoc sensitivity analyses.

PR interval	Original analysis, N=59,868		After excluding for beta-blocker & calcium antagonists, N=55,829	
Model	Beta	95% CI	beta	95% CI
Adjusted for sex, age, height, BMI, resting heart rate, T2DM, current smoking, hypertension, and CAD	0.19	0.03 - 0.36	0.17	0.01 - 0.34

Incident pacemaker implantation	Original analysis, N = 420,071		After excluding beta-blocker and calcium antagonists, N = 385,811	
Model	Hazard ratio	95% CI	Hazard ratio	95% CI
Adjusted for sex, age, T2DM, current smoking, hypertension, and CAD	1.03	1.01 - 1.06	1.04	1.01 - 1.07

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6/7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	7
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6
		(b) Give reasons for non-participation at each stage	6
		(c) Consider use of a flow diagram	Fig. 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	6
		(c) Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	Report numbers of outcome events or summary measures over time	9

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	11,12 Table 1/2
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	16
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15,16
Generalisability	21	Discuss the generalisability (external validity) of the study results	15,16
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.