Telomere Length and the Risk of Atrial Fibrillation: Insights into the Role of Biological versus Chronological Aging

**Running title:** Roberts et al.; Telomere Length and Atrial Fibrillation Risk

Jason D. Roberts, MD1; Thomas A. Dewland, MD1; James Longoria, MD2; Annette L. Fitzpatrick, PhD3; Elad Ziv, MD4; Donglei Hu, PhD4; Jue Lin, PhD5; David V. Glidden, PhD6; Bruce M. Psaty, MD, PhD7,8; Esteban G. Burchard, MD, MPH9,10; Elizabeth H. Blackburn, PhD5; Jeffrey E. Olgin, MD1; Susan R. Heckbert, MD, PhD3,7,8; Gregory M. Marcus, MD, MAS1

1Section of Cardiac Electrophysiology, Division of Cardiology, Department of Medicine, 4Institute of Human Genetics & Department of Medicine, 3Department of Biochemistry & Biophysics, 9Department of Epidemiology & Biostatistics, 10Department of Medicine, 7Department of Medicine, 3Department of Bioengineering & Therapeutic Sciences, University of California San Francisco, San Francisco; 2Division of Cardiovascular Surgery, Sutter Health, Sacramento, CA; 5Department of Epidemiology, 8Cardiovascular Health Research Unit, University of Washington; 6Departments of Medicine and Health Services, University of Washington & Group Health Research Institute, Group Health, Seattle, WA

**Correspondence:**
Gregory M. Marcus, MD, MAS
University of California San Francisco
505 Parnassus Ave, M1180B
San Francisco, CA 94143-0124
Tel: (415) 476-5706
Fax: (415) 476-3505
E-mail: marcusg@medicine.ucsf.edu

**Journal Subject Codes:** [5] Arrhythmias, clinical electrophysiology, drugs
Abstract:

Background - Advanced age is the most important risk factor for atrial fibrillation (AF), however the mechanism remains unknown. Telomeres, regions of DNA that shorten with cell division, are considered reliable markers of biological aging. We sought to examine the association between leukocyte telomere length (LTL) and incident AF in a large population-based cohort using direct LTL measurements and genetic data. To further explore our findings, we compared atrial cell telomere length (ATL) and LTL in cardiac surgery patients.

Methods and Results - Mean LTL and the TERT rs2736100 single nucleotide polymorphism (SNP) were assessed as predictors of incident AF in the Cardiovascular Health Study (CHS). Among the surgical patients, within subject comparison of ATL versus LTL was assessed. Among 1639 CHS participants, we observed no relationship between mean LTL and incident AF prior to and after adjustment for potential confounders (adjusted hazard ratio [HR] 1.09; 95% CI: 0.92-1.29, p=0.299); chronologic age remained strongly associated with AF in the same model. No association was observed between the TERT rs2736100 SNP and incident AF (adjusted HR: 0.95; 95% CI: 0.88-1.04, p=0.265). In 35 cardiac surgery patients (26 with AF), ATL was longer than LTL (1.19 ± 0.20 versus 1.02 ± 0.25 [T/S ratio], p<0.001), a finding that remained consistent within the AF subgroup.

Conclusions - Our study revealed no evidence of an association between LTL and incident AF and no evidence of relative atrial cell telomere shortening in AF. Chronological aging independent of biological markers of aging is the primary risk factor for AF.

Key words: atrial fibrillation, aging, genetics, telomere genetics
Introduction

Atrial fibrillation (AF), the most common sustained cardiac arrhythmia, is a growing health epidemic associated with increased risks of heart failure, stroke, and death.1-3 The direct costs for treating the arrhythmia in the United States alone have been estimated to be $6.65 billion dollars annually.4 The clinical and economic burdens of AF are anticipated to grow dramatically in the coming years secondary to its expanding prevalence.5 The devastating impact of the arrhythmia is further exacerbated by a lack of highly effective treatment strategies, which likely stems from our limited understanding of its underlying pathophysiology.6

Advancing age is the most critical risk factor for the development of AF, reflected by its prevalence ranging from less than 0.1% among individuals younger than 55 years of age to upwards of 10% among octogenarians.7,8 Despite the dramatic impact of age on the risk of AF, the mechanisms responsible for this relationship remain unclear. Our insight into the biology governing the aging process also remains incomplete, however strong evidence has emerged supporting a role for telomeres.9 Telomeres are repetitive DNA sequences ([TTAGGG]n) located at the ends of chromosomes that shorten with advancing age as a result of repeated somatic cell division.10 These chromosomal “caps” function to prevent DNA degradation, however this ability becomes increasingly limited as telomere length shortens, resulting in cellular senescence, apoptosis, and an increased susceptibility to oxidative stress.11

Telomere length and the rate of telomeric shortening, termed telomere attrition, varies widely within the population and has been suggested to influence inter-individual variability in the aging process and susceptibility to age-related diseases.12 Links have been established between telomere length and multiple age-related cardiovascular disorders including atherosclerosis, heart failure, and left ventricular hypertrophy.13-16 Notably, telomere length is
also highly heritable and could also potentially account for a portion of the missing heritability within AF that remains unexplained following large scale genome wide association studies (GWAS) dedicated to the arrhythmia.\textsuperscript{17–22} Given the critical importance of aging in AF, we sought to investigate the impact of leukocyte telomere length (LTL) on the risk of incident AF in a large population-based cohort using both direct LTL measurements and a single nucleotide polymorphism (SNP) previously associated with reduced LTL. In order to determine the relative relationship between atrial cell telomere length (ATL) and LTL, we performed additional analyses among patients undergoing cardiac surgery.

Methods

The association between LTL and incident AF was examined in the Cardiovascular Health Study (CHS), a prospective population-based cohort study.\textsuperscript{23} A cross-sectional comparison of ATL and LTL was performed in a cohort of patients who underwent cardiac surgery and left atrial appendage excision at Sutter Hospital, Sacramento, CA.

Cardiovascular Health Study (CHS)

The CHS is a population-based cohort study designed to investigate risk factors for cardiovascular disease in the elderly. The design, recruitment, baseline characterization, and outcome ascertainment procedures for CHS have been previously described in detail.\textsuperscript{23,24} Briefly, a total of 5,201 participants aged 65 years and older were recruited in 1989-1990 from Medicare eligibility lists in four US communities: Forsyth County, North Carolina; Washington County, Maryland; Sacramento County, California; and Pittsburgh, Pennsylvania. An additional 687 participants, almost all African Americans, were recruited into the study in 1992-1993. Written informed consent was obtained, and all procedures were conducted under institutionally approved protocols for use of human subjects.
Examinations and Event Ascertainment

Participants underwent comprehensive examinations at study entry to document baseline demographics and medical co-morbidities. Self-identified race was dichotomized as white and non-white. Hypertension was defined as a reported history of physician-diagnosed hypertension and use of antihypertensive medications, or systolic blood pressure greater than or equal to 140 mm Hg, or diastolic blood pressure greater than or equal to 90. Participants were classified as diabetic if they used an anti-hyperglycemic medication or had a fasting glucose concentration greater than or equal to 126 mg/dL. Prevalent heart failure was diagnosed by participant self-report and confirmed by medical record verification, while prevalent coronary artery disease was defined as angina, previous myocardial infarction, previous coronary artery bypass grafting, or previous angioplasty, all by participant self-report and confirmed by medical record verification.

Subsequent follow-up was performed with alternating clinic visits and phone calls every six months until 1999 and semi-annual phone calls thereafter. Resting 12-lead ECGs were performed at each clinic visit. Prevalent AF at baseline was documented using the baseline ECG, while incident AF was ascertained on the basis of clinic visit ECGs and hospital discharge diagnosis codes that were supplemented with Medicare inpatient and outpatient claims data.25,26 Previous work on selected subgroups of CHS participants demonstrated that this approach to incident AF ascertainment had positive and negative predictive values of 98.6% and 99.9%, respectively.27,28

Cardiac Surgery Cohort

Consecutive consenting adult patients (≥ 18 years old) undergoing cardiac surgery with left atrial appendage excision at Sutter Hospital, Sacramento Medical Center were recruited between October 1, 2010 and November 1, 2012. Patients were excluded if they had congenital heart
disease, any history of rheumatic valve disease or mitral stenosis, if a right thoracotomy approach was employed, if they were unable to provide informed and witnessed signed consent, or if they were pregnant or incarcerated. Participant demographics and medical details were obtained using a study questionnaire and were verified with a subsequent chart review. All study participants provided informed written consent under protocols that were approved by both the University of California, San Francisco (UCSF) and Sutter Hospital, Sacramento, CA.

Telomere Analyses

Different techniques were utilized for telomere length measurements in the different cohorts, namely Southern blot analysis of terminal restriction fragment lengths in CHS and quantitative polymerase chain reaction (qPCR) in the cardiac surgery cohort. The laboratories that performed these measurements in our study had previously documented satisfactory reproducibility and correlation of both methods.29

CHS

Details regarding LTL measurements within CHS have been previously described.30,31 Briefly, 1675 participants were randomly selected for LTL analysis among a subgroup of the cohort that had completed the 1992-93 and 1997-98 clinic examinations, had stored genetic samples collected at these visits, and had signed consent for DNA analysis. LTL measurement was performed on blood collected at the 1992–93 clinic visit using Southern blot analysis of terminal restriction fragment lengths and reported in kilobases.32 Telomere measurements were performed in duplicate, and the mean was used for statistical analyses. The Pearson’s Correlation Coefficient for duplicates was 0.96, with an average coefficient of variation for paired sets of 2.5%. The laboratory conducting the LTL measurements was blinded to all characteristics of participants.
Cardiac Surgery Cohort

Lymphocyte DNA was purified from the buffy coat using the Gentra Puregene Blood Kit (Qiagen Inc., Valencia, CA) obtained from phlebotomy performed prior to surgery. Intraoperatively, left atrial appendage samples were immediately flash frozen in liquid nitrogen. All samples were shipped to UCSF in dry ice, stored in a -80°C freezer, and analyses were batched. Genomic DNA was isolated from atrial tissue using the AllPrep DNA/RNA Mini Kit (Qiagen Inc.), Telomere length analysis for all samples was adapted from the qPCR technique originally described by Cawthon. The qPCR reaction conditions and primers utilized for the telomere and single copy gene (human beta-globin) have been previously reported. The qPCR assays were conducted using the Roche Lightcycler 480 real-time PCR machine (Roche, Indianapolis, IN). Telomere lengths are reported as the T/S ratio, the ratio of mean telomere repeat sequence copy number (T) to the reference single-copy gene copy number (S).

LTL SNPs and CHS Genotyping

In order to further investigate the potential relationship between LTL and AF, we sought to examine for associations between single nucleotide polymorphisms (SNPs) linked to reduced LTL and incident AF. Review of 9 SNPs previously associated with LTL through genome wide association studies revealed a single SNP that had also been directly genotyped within the CHS cohort using a DNA microarray. In a recent large meta-analysis of genome wide association studies, the minor allele (C>A) was shown to be associated with reduced telomere length (beta coefficient: -0.078, p-value = 4.38 x 10^{-19}). The genotyping methodology for rs2736100 within CHS has been previously described. SNP genotyping was performed using the Illumina 370 CNV DNA microarray and analyzed using the BeadStudio variant calling algorithm (Illumina, San Diego, CA). Genetic analyses were restricted to individuals of Western European ancestry.
Statistical Analysis

Normally distributed continuous variables are presented as means ± standard deviation and were compared using the Student’s t-test. Comparison of categorical values was performed using the Chi-squared test. In CHS, time-to-event analyses using Cox proportional hazards models were employed to evaluate the association of LTL with incident AF. The primary predictor, LTL, was treated as both an ordinal and a categorical variable divided into tertiles (to maintain consistency with previous literature). Multivariable Cox regression analyses were performed to adjust for potential confounding. Covariates added to these models included baseline age, sex, self-reported race, hypertension, diabetes, body mass index, coronary artery disease, and congestive heart failure. Similar analyses restricted to a subgroup of participants within the overall cohort that had no baseline hypertension, diabetes, coronary artery disease, or congestive heart failure were also performed. Examinations for an interaction between age and LTL were performed using baseline age dichotomized by its median value in both unadjusted and multivariate models. An additive genetic model was employed for the genetic SNP analyses. Linear regression analysis was performed in order to examine for an association between the SNP and LTL.

Bivariate and multivariable Cox regression models, as described above, were utilized to examine for an association between the SNP and incident AF. For the adjusted survival curves, categorical covariates were set at 0 and continuous covariates were set at their mean values. For the surgical cohort, ATL and LTL were compared using mixed effects regression models adjusting for age and gender. Assessment for association and correlation between ATL and LTL was performed using linear regression adjusting for the same covariates and the Pearson pairwise correlation coefficient, respectively. Two-tailed p-values < 0.05 were considered statistically significant. Statistical analyses were performed using Stata version 12 (College Station, TX).
Results

CHS Participant Characteristics

A total of 1675 individuals from the CHS cohort underwent LTL measurement. Among this group, 36 had prevalent AF and were excluded from the analysis. At baseline, the mean age of the cohort was 72.2 years, 41.3% were male, and 71.3% were classified as white. The remaining baseline clinical characteristics of the cohort, stratified by the presence or absence of incident AF, are summarized in Table 1. During a mean follow-up period of 11.6 years, a total of 476 of the 1639 individuals were diagnosed with incident AF.

LTL and the Risk of Incident AF in CHS

Mean LTL within the cohort was 6.33 kb. Each decade of advancing age was associated with a significant 21% lower mean LTL (p<0.001). Bivariate analysis treating LTL as a continuous variable revealed no statistically significant association between longer telomere length and incident AF (hazard ratio [HR] 0.91 for each kilobase increase in LTL, 95% CI: 0.78-1.06, p=0.226). Following adjustment for pre-specified covariates, again no statistically significant relationship between telomere length and AF was observed, with a HR close to 1 (HR 1.09 for each kilobase increase in LTL, 95% CI: 0.92-1.29, p=0.299). Similar results were obtained when the analysis was restricted to 705 individuals with no baseline risk factors for AF including hypertension, diabetes, coronary artery disease, and congestive heart failure (unadjusted HR: 0.95, 95% CI: 0.74-1.22, p=0.684; adjusted HR: 1.12, 95% CI: 0.87-1.45, p=0.378).

The results examining LTL in tertiles as an ordinal and a categorical variable are presented in Figures 1 and 2, respectively. No significant associations were observed. Of note, in each multivariable model, age remained a statistically significant predictor of incident AF following adjustment for LTL. Examination for an impact of age on the association of LTL with
incident AF revealed no evidence of an interaction in both unadjusted (p=0.522) and adjusted (p=0.765) models when dichotomizing age based on the median value of 72 years. Consistent with these results, the HRs for incident AF among individuals less than 72 years (unadjusted HR: 0.96 [95% CI: 0.77-1.20; p = 0.719], adjusted HR: 1.09 [95% CI: 0.86-1.38; p = 0.484]) were nearly identical to those among individuals older than 72 years (unadjusted HR: 0.96 [95% CI: 0.77-1.19; p = 0.719], adjusted HR: 1.09 [95% CI: 0.86-1.38; p = 0.484]).

**LTL associated SNP and the Risk of Incident AF in CHS**

A total of 3794 individuals of Western European ancestry underwent successful SNP genotyping of rs2736100. Within this subgroup, 1235 had also undergone LTL measurement. Linear regression analysis using an additive genetic model confirmed an association between rs2736100 and LTL (β-coefficient: -0.065, 95% CI: -0.111 - -0.018, p=0.006). Consistent with previous work, our findings also demonstrated that the minor allele was associated with a reduced LTL. Among the 3794 individuals that had undergone successful genotyping, Cox regression analysis revealed no association between the SNP and incident AF (unadjusted HR: 0.96 [95% CI: 0.89-0.96, p = 0.522], adjusted HR: 1.09 [95% CI: 0.86 - 1.39; p = 0.475]) (Figure 3).

**Cardiac Surgery Cohort Participant Characteristics**

A total of 35 patients undergoing cardiac surgery with left atrial appendage excision at Sutter Hospital, Sacramento provided both atrial tissue and peripheral blood for telomere length analysis. Within this cohort, 74% of individuals had a history of AF and 60% were undergoing cardiac surgery for a minimally invasive AF ablation. The remaining baseline characteristics are summarized in Table 2.

**Atrial Cell Telomere Length and Leukocyte Telomere Length**

Among the surgical cohort of patients, the mean atrial cell telomere T/S ratio was 1.19 ± 0.20 in
comparison with a mean leukocyte telomere T/S ratio of 1.02 ± 0.24 (Figure 4). Mixed effects regression modeling adjusting for age and gender revealed that ATL was significantly longer than LTL (β-coefficient 0.17, 95% CI: 0.09-0.25, p<0.001). In the multivariate model, neither age (β-coefficient -0.001, 95% CI: -0.006-0.004, p=0.649) nor gender (β-coefficient -0.012, 95% CI: -0.15-0.122, p=0.862) exhibited statistically significant associations with telomere length. Multivariate linear regression analysis revealed a statistically significant association between ATL and LTL (β-coefficient 0.30, 95% CI: 0.03-0.57, p=0.030), while their calculated pairwise correlation coefficient (R²) was 0.37. Within the subgroup of patients with AF, the mean atrial cell telomere T/S ratio (1.19 ± 0.21) was again longer than LTL (1.03 ± 0.25) after adjusting for age and gender (p=0.001). Similar results were also observed for the 9 patients without AF (1.21 ± 0.16 in the atria versus 1.00 ± 0.25 in leukocytes; adjusted p=0.08).

Discussion

Our investigation involving 1639 elderly subjects from a population-based cohort study identified no association between LTL and the risk of incident AF. Further strengthening this finding, we also found no association between rs2736100, a SNP associated with reduced LTL, and incident AF among 3794 individuals from the same cohort. In an effort to advance our insights into atrial biology with respect to telomere length, we compared ATL and LTL among patients with and without AF undergoing cardiac surgery. ATL was longer than LTL in both the overall group and among the subgroup of patients with AF. Because ATL correlated with LTL, it appears unlikely that ATL alone is important in the development of AF. Collectively, these findings suggest that telomere shortening does not account for the increased risk of AF associated with aging.

The importance of advancing age in the pathogenesis of AF is highlighted by its
dramatically increased prevalence within older populations. Although extremely rare among individuals less than 50 years of age, the arrhythmia affects upwards of 10% of octo- and nonagenerians. This age-dependent increase in its frequency, in association with our aging population, is leading to a surge in its overall prevalence. Indeed, a recent study projected that the number of affected Americans may grow nearly 8-fold from approximately 2.3 million in the year 2000 to nearly 16 million by 2050. This worrisome forecast, further aggravated by our lack of preventive strategies and definitive therapies for the arrhythmia, emphasizes the need for an improved understanding into the pathophysiology of the arrhythmia. Insight into the pathophysiological mechanisms through which age predisposes to the arrhythmia has the potential to unveil critical biological pathways that could serve as targets for novel therapeutic interventions.

Telomeres, repetitive DNA elements located at the ends of chromosomes, have been implicated as potential mediators of biological aging. Consistent with this hypothesis, reduced telomere length has been associated with an increased risk for multiple age-dependent cardiovascular conditions. Telomere shortening is felt to contribute to biological aging through an increased vulnerability to oxidative stress and subsequent fibrosis secondary to reduced genomic stability. Notably, atrial fibrosis is considered critical for the initiation and maintenance of AF through its role as a substrate that promotes regional conduction velocity heterogeneity. Despite this apparent overlapping pathophysiology, we identified no evidence of an association between LTL and the risk of incident AF among CHS participants. Given the large size of our study and the persistence of statistically significant relationships between known risk factors (such as age) and AF despite adjustment for LTL, we have provided strong evidence suggesting the absence of an independent clinically significant impact of LTL on the
risk of AF.

In an effort to further explore for a potential link between LTL and AF, we examined for an association between a SNP associated with reduced LTL and the arrhythmia. The rs2736100 SNP lies in the vicinity of the TERT gene which encodes for the enzyme telomerase reverse transcriptase. Telomerase reverse transcriptase functions to elongate telomeres following cellular division in an effort to preserve telomere length over time. For this analysis, the TERT SNP effectively functions as an instrumental variable in the relationship between LTL and AF, thus serving to minimize the potential impact of confounding variables on the analysis. The lack of association between the SNP and incident AF further reinforces our initial findings that LTL does not influence the risk of the AF in this population.

Following these results, further exploration into the relationship between telomere length and atrial biology was performed through comparison of ATL and LTL in a separate cohort of individuals that had undergone left atrial appendage excision during cardiac surgery. ATL was noted to be longer than LTL among the entire cohort and within the subgroup of individuals with AF. Because ATL correlated with LTL, extrapolation of these observations in combination with the LTL findings above argues against an important telomere length phenomenon localized to the atria. This relative preservation of ATL may potentially account for the apparent lack of impact of telomere length on the risk of AF.

Of note, a longer telomere length within atrial cells relative to leukocytes is consistent with our understanding of both atrial biology and the mechanism of telomere attrition. Telomere length shortens progressively with age secondary to an inability of the telomerase enzyme to fully replicate telomeres following repeated somatic cell division. Atrial tissue samples are composed of a combination of cell types including myocytes, fibroblasts, endothelial and
vascular smooth muscles cells. Notably, atrial and ventricular myocytes undergo very limited cell division following embryogenesis, which may potentially protect them from the adverse consequences of telomere shortening. To our knowledge, our study is the first to document that telomere length is longer in atrial tissue relative to leukocytes.

Our findings suggest that aging increases the risk of AF through biological pathways that are independent of LTL or through a mechanism restricted to chronological aging. Examining for a potential role of telomere biology in the pathogenesis of AF was particularly important given the critical, yet largely unexplained impact of advancing age on the risk of the arrhythmia. Future work can now focus on other biological pathways associated with aging that may predispose to the development of AF. As telomere length is generally considered the primary marker of biological aging, our results may also suggest that there is something specific to chronological, rather than biological, age that is inherently important. As a hypothetical example, perhaps there is a cumulatively growing probability of AF induction as more and more premature atrial contractions (known to be an important AF risk factor) are introduced at different times into the atrial substrate. An improved understanding of the mechanisms governing the relationship between advanced age and the risk of AF will likely be critical for the development of preventive and therapeutic treatment modalities necessary to combat this growing health epidemic.

Our study has several limitations. Because our population-based analysis was restricted to individuals ≥ 65 years of age, it does not rule out an impact of LTL in younger age groups. Although this may limit the generalizability of our findings, we would highlight that the vast majority of individuals affected by AF are elderly, emphasizing the importance of furthering our understanding of the arrhythmia within this subgroup of the population. Second, it is
conceivable that our study was inadequately powered to detect an association between LTL and AF. It should be noted that we failed to detect an association between hypertension and AF in our multivariate model (HR: 1.10, 95% CI: 0.90-1.35, p=0.342), though an association was observed on unadjusted analysis (HR: 1.19, 95% CI: 1.07-1.32, p=0.001). The magnitude of association between hypertension and AF in our study was comparable to previous work involving the CHS cohort and the association on multivariate analysis would likely have been statistically significant had our cohort had been larger. Despite these observations, we feel that it is unlikely that inadequate power was responsible for our lack of association between LTL and AF given that the direction of association identified was opposite the expectation that older biological age increased AF risk. Third, the rs2736100 SNP is a modest predictor of LTL variability and should not be viewed as definitive genetic evidence to rule out an association between LTL and the risk of AF. Fourth, we cannot rule out a potential association between the rate of telomere attrition and AF given that telomere length was only measured at a single time point. Finally, although ATL was clearly and consistently longer than LTL, we were not adequately powered to examine for an association between ATL (alone) and the risk of AF in our relatively small surgical cohort. Although the correlation between ATL and LTL would support the notion that ATL is unlikely to be critical to AF development, the modest R² value of 0.37 suggests we cannot exclude the possibility that ATL might yet be important in AF.

Our study has several limitations. Because our population-based analysis was restricted to individuals ≥ 65 years of age, it does not rule out an impact of LTL in younger age groups. Although this may limit the generalizability of our findings, we would highlight that the vast majority of individuals affected by AF are elderly, emphasizing the importance of furthering our understanding of the arrhythmia within this subgroup of the population. Second, it is
conceivable that our study was inadequately powered to detect an association between LTL and AF. It should be noted that we failed to detect an association between hypertension and AF in our multivariate model (HR: 1.10, 95% CI: 0.90-1.35, p=0.342), though an association was observed on unadjusted analysis (HR: 1.19, 95% CI: 1.07-1.32, p=0.001). The magnitude of association between hypertension and AF in our study was comparable to previous work involving the CHS cohort and the association on multivariate analysis would likely have been statistically significant had our cohort had been larger. Given that the point estimate for LTL as a predictor of AF was in the opposite direction of what would be expected if older biological age increased AF risk, we feel it is unlikely that inadequate power was responsible for our negative findings. Third, we cannot rule out a potential association between the rate of telomere attrition and AF given that telomere length was only measured at a single time point. Finally, although ATL was clearly and consistently longer than LTL, we were not adequately powered to examine for an association between ATL (alone) and the risk of AF in our relatively small surgical cohort. Although the correlation between ATL and LTL would support the notion that ATL is unlikely to be critical to AF development, the modest R² value of 0.37 suggests we cannot exclude the possibility that ATL might yet be important in AF.

Conclusions

Our study found no evidence that LTL influences the risk of incident AF. ATL was noted to be longer than LTL among subjects with and without AF undergoing cardiac surgery. Reduced telomere attrition secondary to limited somatic cell division among atrial myocytes may account for the lack of association between LTL and the risk of AF. Our findings suggest that other factors associated with biological aging or properties inherent to chronological aging
independent of cell division or telomere shortening are responsible for the association between advancing age and the risk of AF.

**Funding Sources:** This work was made possible by an American Heart Association National Innovative Research Grant (G.M.M) and the Joseph Drown Foundation (G.M.M.). J.D.R. is supported by a Research Fellowship Grant from the Heart & Stroke Foundation of Canada. Grants for the Cardiovascular Health Study include: R01 HL80698-01, NHLBI contracts HHSN268201200036C, HHSN268200800007C, N01 HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, and grants HL080295 and HL102214, with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided by AG023629 from the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org.

**Conflict of Interest Disclosures:** None.

**References:**


Table 1: Baseline Characteristics of CHS Participants with and without Incident AF

<table>
<thead>
<tr>
<th></th>
<th>Incident AF</th>
<th>No AF</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 476</td>
<td>n = 1163</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>72.8 ± 5.1</td>
<td>71.9 ± 5.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male (%)</td>
<td>203 (42.6)</td>
<td>470 (40.4)</td>
<td>0.404</td>
</tr>
<tr>
<td>White Race (%)</td>
<td>434 (91.2)</td>
<td>989 (85.0)</td>
<td>0.009</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>163 (34.2)</td>
<td>351 (30.2)</td>
<td>0.358</td>
</tr>
<tr>
<td>Diabetes Mellitus (%)</td>
<td>79 (16.6)</td>
<td>158 (13.6)</td>
<td>0.113</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>27.0 ± 4.8</td>
<td>26.8 ± 4.5</td>
<td>0.434</td>
</tr>
<tr>
<td>Coronary Artery Disease (%)</td>
<td>122 (25.6)</td>
<td>182 (15.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Congestive Heart Failure (%)</td>
<td>29 (6.1)</td>
<td>33 (2.8)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Data are n (%) or mean ± standard deviation
Table 2: Clinical Demographics of the Surgical Cohort with Atrial and Leukocyte Telomere Length Measurements

<table>
<thead>
<tr>
<th></th>
<th>AF n = 26</th>
<th>No AF n = 9</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64.6 ± 12.0</td>
<td>78.4 ± 8.2</td>
<td>0.003</td>
</tr>
<tr>
<td>Male</td>
<td>16 (61.5)</td>
<td>6 (66.7)</td>
<td>0.784</td>
</tr>
<tr>
<td>White Race</td>
<td>25 (96.2)</td>
<td>8 (88.9)</td>
<td>0.363</td>
</tr>
<tr>
<td>Hypertension</td>
<td>14 (53.9)</td>
<td>6 (66.7)</td>
<td>0.503</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>5 (19.2)</td>
<td>1 (11.1)</td>
<td>0.577</td>
</tr>
<tr>
<td>Coronary Artery Disease</td>
<td>5 (19.2)</td>
<td>4 (44.4)</td>
<td>0.136</td>
</tr>
<tr>
<td>Congestive Heart Failure</td>
<td>3 (11.5)</td>
<td>1 (11.1)</td>
<td>0.972</td>
</tr>
<tr>
<td>Indication For Surgery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AF Ablation</td>
<td>21 (80.8)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Coronary Artery Bypass Grafting</td>
<td>2 (7.7)</td>
<td>4 (44.4)</td>
<td>0.012</td>
</tr>
<tr>
<td>Aortic Valve Replacement</td>
<td>1 (3.9)</td>
<td>4 (44.4)</td>
<td>0.003</td>
</tr>
<tr>
<td>Mitral Valve Surgery</td>
<td>2 (7.7)</td>
<td>2 (22.2)</td>
<td>0.238</td>
</tr>
</tbody>
</table>

Data are n (%) or mean ± standard deviation
Figure Legends:

**Figure 1:** Adjusted Survival Curves of Incident AF among Tertiles of Leukocyte Telomere Length. HR denotes hazard ratio. The calculated HR is for tertiles of leukocyte telomere length treated as an ordinal variable.

**Figure 2:** Impact of Leukocyte Telomere Length Tertiles on the Risk of Incident AF in a Multi-Variable Cox Regression Model. Leukocyte telomere length tertile was treated as a categorical variable. HR denotes hazard ratio; BMI denotes body mass index; CAD denotes coronary artery disease; CHF denotes congestive heart failure. Error bars denote 95% confidence intervals.

**Figure 3:** Adjusted Survival Curves of Incident AF by rs2736100 Genetic Carrier Status Utilizing an Additive Genetic Model. HR denotes hazard ratio.

**Figure 4:** Box plot comparisons of Atrial and Leukocyte Telomere Length among the Surgical Cohort With and Without AF. Boxes represent 25th-75th quartiles and lines within boxes represent median values. Outliers are displayed by distinct dots.
HR: 1.07, 95% CI: 0.95-1.21, p = 0.256
<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Hazard Ratio for Incident AF</th>
<th>HR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte Telomere Length</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shortest (3.49-6.03 kb)</td>
<td></td>
<td>1.0</td>
<td>Reference</td>
</tr>
<tr>
<td>Middle (6.03-6.51 kb)</td>
<td></td>
<td>0.95 (0.75-1.20)</td>
<td>0.651</td>
</tr>
<tr>
<td>Longest (6.51-8.67 kb)</td>
<td></td>
<td>1.15 (0.91-1.45)</td>
<td>0.246</td>
</tr>
<tr>
<td>Decade of Life</td>
<td></td>
<td>1.92 (1.59-2.31)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>1.09 (0.90-1.33)</td>
<td>0.372</td>
</tr>
<tr>
<td>White Race</td>
<td></td>
<td>1.67 (0.89-3.13)</td>
<td>0.109</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td>1.01 (0.98-1.03)</td>
<td>0.560</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td>1.11 (0.90-1.35)</td>
<td>0.327</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td>1.41 (1.09-1.84)</td>
<td>0.010</td>
</tr>
<tr>
<td>Prevalent CAD</td>
<td></td>
<td>1.78 (1.41-2.23)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prevalent CHF</td>
<td></td>
<td>1.95 (1.28-2.99)</td>
<td>0.002</td>
</tr>
</tbody>
</table>
Freedom from Atrial Fibrillation

Follow-Up (Years)

CC  CA  AA

C = major allele  A = minor allele

HR: 0.95, 95% CI: 0.88-1.04, p = 0.265
Telomere Length and the Risk of Atrial Fibrillation: Insights into the Role of Biological versus Chronological Aging


Circ Arrhythm Electrophysiol. published online November 8, 2014;
Circulation: Arrhythmia and Electrophysiology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3149. Online ISSN: 1941-3084

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circep.ahajournals.org/content/early/2014/11/08/CIRCEP.114.001781

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Arrhythmia and Electrophysiology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Arrhythmia and Electrophysiology is online at:
http://circep.ahajournals.org//subscriptions/