ARIC Manuscript Proposal # 3270

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2. Proposed Title: The association of leukocyte telomere length to longitudinal change in lung function and respiratory infection: The NHLBI Pooled Cohorts Study

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6. Who/what group will do the primary analysis? Columbia University

PART II: Brief Analysis Description

Introduction:
Lung function declines markedly with age even in individuals without clinical pulmonary disease.\(^1\) Lung function is also one of the most robust predictors of physical function and mortality. If the mechanisms responsible for lung aging were better understood, especially how they interact with exogenous exposures, it may be possible to optimize lung function throughout the lifespan. This may have an outsized benefit to improving overall human health with aging.

Telomere shortening promotes cellular senescence and may integrate other processes which contribute to aging.\(^2\) Telomeres are associated with lung diseases, though the relationship remains poorly defined. Genetic syndromes which result in markedly short telomeres, such as dyskeratosis congenita, commonly exhibit a phenotype of pulmonary fibrosis.\(^3\) Familial and sporadic pulmonary fibrosis cases are enriched for mutations in telomerase genes.\(^4\) In pooled analyses of general population studies, individuals with shorter telomeres have a markedly increased risk of developing pulmonary fibrosis.\(^5\) General population cohorts have also shown significant associations between shorter telomere length and worse lung function.\(^6\) The association between telomeres and pulmonary function may be partly due to telomere dysfunction causing alveolar stem cell failure.\(^7\) Additionally, short telomeres markedly increase pulmonary pathology that develops in the presence of a respiratory insult such as smoking.\(^8\)

There are no well-adjusted, well-powered studies of the association of telomere length (TL) with longitudinal change in lung function in a multi-ethnic population, despite GLI equations highlighting the importance of accounting for age and ethnicity with respect to change in pulmonary function.\(^1\) Thus, defining the association of TL to pulmonary function respecting age and ethnicity is vital to advance TL as a candidate marker of pulmonary aging. Because TL is most often measured in epidemiologic studies from peripheral blood leukocytes, which may reflect immune aging more specifically, further clarification of the role of TL would be provided by determining the association of TL to incident respiratory infection.

We therefore propose to examine associations between leukocyte telomere length (LTL), decline in lung function, and incident respiratory infections in the NHLBI Pooled Cohorts Study, which provides a large sample of highly phenotyped adults of many ethnicities with longitudinal follow up for validated lung function measures and cause-specific hospitalization and mortality data. This data will furthermore provide an opportunity for stratification by established risk factors for accelerated lung function decline, including clinical lung diseases and cigarette smoking. The proposed work will thereby elucidate the absolute and relative contribution of telomere length to pulmonary aging in the general population.

Research Hypotheses:

Hypothesis 1: Shorter telomere length will be cross-sectionally associated with poorer pulmonary function.
Hypothesis 2: Shorter telomere length will be associated with more rapid decline in longitudinal pulmonary function, incident airflow limitation, and incident restriction. These associations will be modified by smoking status.

Hypothesis 3: Shorter telomere length will be associated with increased rate of respiratory infection.

Data

We propose to use data from nine cohorts that have been harmonized and pooled as part of the NHLBI Pooled Cohorts Study:

1. Atherosclerosis Risk in Communities (ARIC) Study
2. Coronary Artery Risk Development in Young Adults (CARDIA) Study
3. Cardiovascular Health Study (CHS)
4. Framingham Heart Study (FHS)
5. Health Aging and Body Composition (Health ABC) Study
6. Hispanic Community Health Study/Study of Latinos (HCHS/SOL)
7. Jackson Heart Study (JHS)
8. Multiethnic Study of Atherosclerosis (MESA)
9. Strong Heart Study (SHS)

Inclusion/exclusion criteria: Participants will be included if they have complete data for LTL, at least one of the primary outcomes, age, height, and smoking. Based on published reports below, approximately 14,137 participants have had LTL measured at one visit point. Due to some variability in measuring LTL to proximate spirometry in CHS, MESA, and SHS, the actual number may be slightly lower, including roughly 8,700 participants with PCR measurements and 5,300 by Southern blot. Longitudinal analysis of change in pulmonary function can be accomplished in roughly 6,965 participants (4,087 by PCR and 2,878 by Southern blot), with actual numbers being lower due to attrition.

<table>
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<tr>
<th>Study</th>
<th>LTL N</th>
<th>Method</th>
<th>LTL Year</th>
<th>Spiro Years</th>
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<td>SB</td>
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<td>Exam 3, 4, 5</td>
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<td>14137</td>
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**PCR N** 8741
**Southern N** 5396

*SB = Southern blot

**Primary exposure:**
Leukocyte telomere length.

**Primary outcomes:**

*Hypothesis 1*
FEV1, FVC, and FEV1/FVC, using spirometry data that has been validated, harmonized, and pooled by the NHLBI Pooled Cohorts Study. This will allow us to examine both obstructive and restrictive lung disease.

*Hypothesis 2*
Change in FEV1, FVC, and FEV1/FVC as continuous variables; incident airflow limitation; incident restriction; incident ILD diagnosis. Additional testing of smoking as an effect modifier.

*Hypothesis 3*
Incidence of hospitalization or mortality due to respiratory infection. Hospitalizations and mortality due to respiratory infection will be defined as previously in CHS and ARIC by Yende et al: We used previously validated International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes 480 through 487 in the first 5 discharge diagnoses fields to identify pneumonia hospitalizations.

**Potential confounders and/or effect modifiers:**
Block 1: Age, gender, race/ethnicity, height, BMI
Block 2: + Smoking (current/former, pack years, and ever/never)
Block 3: + Educational attainment
Block 4: + Comorbidities (CVD, diabetes, hypertension)

**Analysis Plan**
Cohorts included in the analysis have measured telomere length using two methods, PCR and Southern blot. There has been much debate regarding the accuracy and precision of these methods, especially as they have been refined over the years during which these cohorts have measured telomere length. To reduce potential confounding and misclassification from combining data that used different measurement methods, we will conduct all analyses stratified in two groups: cohorts using PCR, and cohorts using Southern blot. If the pattern and magnitude of associations appear similar in both groups, we may choose to combine them to additionally show overall results, though this may also be limited by systematic bias which generally over or under estimates LTL by each method, depending on which DNA regions adjacent to the telomere are included in the assay as part of the telomere.

Initially, we will display characteristics of the samples using descriptive statistics.
**Hypotheses 1**: Multivariable-adjusted regression will be used to model the association of LTL (continuous predictor) with baseline pulmonary function, adjusting for potential confounders – as measured at baseline – sequentially by block.

**Hypothesis 2**: Generalized linear mixed models will be used to model the association of baseline LTL (continuous predictor) with longitudinal pulmonary function, adjusting for potential confounders, including time-varying confounders, sequentially by block. In the pooled data, we will use a cohort-specific unstructured covariance matrix, akin to a “stratified MANOVA” approach. *A priori*, we will also test for possible effect measure modification between LTL and smoking (current/former, ever/never, and pack years) via three-way multiplicative interaction terms and fully stratified analyses. Cox proportional hazards models will be used to model the association of baseline LTL (continuous predictor) with incident airflow limitation, incident restriction, and incident ILD diagnosis, with time-to-event defined as biological age at event, and left-truncation for age at study entry. Models will be adjusted for potential confounders sequentially by block. Cohort will be treated as a stratum term. Pulmonary function will also be included as a potential confounder. *A priori*, we will also test for possible effect measure modification between LTL and smoking (current/former, ever/never, and pack years) via multiplicative interaction terms and fully stratified analyses.

**Hypothesis 3**: Cox proportional hazards models will be used to model the association of baseline LTL (continuous predictor) with hospitalization and mortality due to incident respiratory infection, with time-to-event defined as biological age at event, and left-truncation for age at study entry. Models will be adjusted for potential confounders sequentially by block. Cohort will be treated as a stratum term. Pulmonary function will also be included as a potential confounder. *A priori*, we will also test for possible effect measure modification between LTL and smoking (current/former, ever/never, and pack years) via multiplicative interaction terms and fully stratified analyses.

**Sensitivity analyses**: In addition to complete case analyses, alternative strategies to address missing data, such as inverse-probability weighting and multiple imputation, will be explored. Competing risk regression will be explored as an alternative to Cox proportional hazards models. Results from random effects meta-analysis will be compared to those from pooled analyses. Additional sensitivity analyses include restricting the sample to those without chronic lung disease; restricting the sample to non-smokers; and adjusting for baseline FEV1, FVC, or FEV1/FVC in the analysis for Hypothesis 2.

**References**


