Peripheral Blood Telomere Attrition in Persons at Risk for Familial Pulmonary Fibrosis

To the Editor:

Shorter than expected telomeres are common among persons with pulmonary fibrosis (PF), even in the absence of a genetic variant impacting telomere maintenance (1–4). Telomere shortening is pathologically implicated in PF through cellular aging and/or senescence mechanisms in lung epithelium and other cell types (1, 5, 6). Most research on telomere length in PF has been cross-sectional. A key unanswered question is whether short telomeres observed later in life are primarily inherited or result from accelerated attrition.

Some of the results of these studies have been previously reported in the form of an abstract (7).

Methods

Unaffected first-degree relatives of patients with familial PF and no significant pulmonary symptoms were enrolled in an ongoing, prospective cohort study (NCT03437486), with methods and interim results recently published (6, 8). Briefly, participants serially donated blood and were screened for preclinical PF using prone series high-resolution chest computed tomography scans (HRCT) to detect interstitial lung abnormalities (ILAs) (8, 9). Participants with more than one sampling of peripheral blood mononuclear cells (PBMCs) at least 1 year apart (collected between 2008 and 2020) were included in this analysis. The mean PBMC telomere terminal restriction fragment length (MTL) was measured via flow cytometry with fluorescent *in-situ* hybridization (flow-FISH) by RepeatDx, Inc. (10). Expected MTL (eMTL) was calculated as $7900 - (43 \times [\text{age at sample} - 18]) / 1000$ (10), percent of expected MTL (%-eMTL) as MTL / eMTL, and annualized change as (last – first MTL) / (intermeasurement yr).

Demographics and *MUC5B* (Mucin 5B) polymorphism rs35705950 status were measured at enrollment (6, 8, 11). The ILA status on each HRCT was determined by a study radiologist, as previously described (6, 8). Pathogenic or likely pathogenic rare variants (RVs) (minor allele frequency < 0.001) (12) in telomerase pathway genes (*TERT* [Telomerase Reverse Transcriptase], *TERC* [Telomerase RNA Component], *PARN* [Polyadenylate-Specific Ribonuclease], and *RTEL1* [Regulator of Telomere Elongation Helicase 1]) were identified by whole exome sequencing and/or Sanger candidate gene sequencing and subsequently designated as disease-relevant RVs (5, 13). Patient characteristics were summarized as median (q1–q3) or number (percentage). Linear regression identified characteristics associated with MTL at enrollment (using age-adjusted %-eMTL) or the annualized change in MTL (in kb).

Results

Among 110 participants with more than 1 PBMC sample available, MTL measurement was successful in 218/230 samples, leaving 101 participants from 68 families with longitudinally-measured MTL. Participants were 51.2 (44.3–55.8) years old at enrollment, 32 (31.7%) were male, 73 (72.3%) were never-smokers, and 89/92 (96.7%) were White (Table 1). A *MUC5B* risk allele was identified in 33 participants (33.7%). A disease-relevant telomerase RV was identified in 17 participants (16.8%), and 12 (11.8%) were noncarriers in a family with a disease-relevant telomerase RV. The enrollment HRCT revealed early/mild ILA in 17 participants (16.8%); during a median (q1–q3) time for ILA observation of 6.1 (4.9–8.3) years, 80 participants (79.2%) had at least 1 follow-up HRCT and nonresolving ILA were observed during total follow-up in 23 participants (22.8%), including 14 (13.9%) with ILA progression and 2 (2.0%) diagnosed with PF.

The median first MTL was 5.7 kb (5.1–6.5) or 88.1% eMTL (79.1–101.4), with 31 participants (30.7%) at or below the 10th percentile for age (Table 1) (10). As expected, older individuals had shorter MTL at baseline (estimate, −0.025 kb per +1 year; SE, 0.012; $P = 0.04$). Individuals from families without a disease-relevant telomerase RV had shorter than expected MTL (median, 92% eMTL) (Figure 1A). Telomerase RV carriers had shorter telomeres than all noncarriers combined (−14.1%; SE, 3.83; $P < 0.001$), and an ordered trend was suggested whereby individuals from families without a disease-relevant telomerase RV were the longest, noncarrier telomerase family members were intermediate (but approached carriers), and telomerase RV carriers were shortest (ordered ordinal linear regression estimate, −7.3%; SE, 1.86; $P = 0.0002$) (Figure 1A). Subjects with nonresolving ILAs on HRCT also had modestly shorter telomeres at enrollment (−6.6%; SE, 3.58; $P = 0.069$). *MUC5B* genotype was not associated with telomere length.

In the telomere attrition analysis, the median time between samples was 6.1 (5.0–7.8) years. The median annual change in MTL was −0.063 kb/year (−0.116, 0) (Table 1), and is greater than expected in healthy adults (−0.043 kb/year) (10). The first-sample MTL was associated with annualized MTL change, with longer telomeres experiencing greater attrition (−0.059 kb/year per +1kb first-measured MTL; SE, 0.013; $P < 0.001$) (Table 1). Telomerase RV carrier status was not associated with telomere attrition rate (Table 1 and Figure 1B).

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Table 1. Cohort Characteristics and Their Estimated Effect on Mean Telomere Length at Enrollment or Annual Rate of Telomere Attrition During Follow-up

Characteristic (*N* = 101)	Summary Median (q1 to q3) or *n* (%)	Effect of Characteristics on MTL Estimate (SE), *P* Value	
		First %-eMTL*	Annual MTL Change†
Age at first MTL, yr	51.2 (44.3 to 55.8)	n/a	n/a
Male Sex	32 (31.7)	−2.09 (3.28), 0.52	−0.011 (0.030), 0.71
White race (*n* = 92)	89 (96.7)	n/a	n/a
Cigarette Smoking			
Ever-smoker	28 (27.7)	0.58 (3.41), 0.87	0.038 (0.032), 0.24
Pack-yr smoked	0 (0 to 4)	0.12 (0.15), 0.42	0.0007 (0.001), 0.60
MUC5B GT or TT	33 (32.7)	−1.93 (3.25), 0.55	−0.017 (0.030), 0.57
Telomerase mutation status‡			
Individual carrier	17 (16.8)	−14.1§ (3.83), <0.001	0.012§ (0.038), 0.74
Gene in family, noncarrier	12 (11.9)	n/a	n/a
ILA status‖			
Early/mild ILA on first HRCT	17 (16.8)	0.82 (4.08), 0.84	0.023 (0.038), 0.56
ILA on any HRCT	23 (22.8)	−6.59 (3.58), 0.069	0.0005 (0.034), 0.99
Have >1 HRCT	80 (79.2)	n/a	n/a
ILA observation time, yr¶	6.1 (4.9 to 8.3)	n/a	n/a
First MTL measurement	—	n/a	—
Actual, kb	5.7 (5.1 to 6.5)	—	−0.059 (0.013), <0.001
%-eMTL	88.1 (79.1 to 101.4)	—	−0.004 (0.001), <0.001
Below 10th percentile	31 (30.7)	—	0.0998 (0.029), 0.001
Below first percentile	6 (5.9)	—	0.211 (0.057), <0.001
Annualized MTL change	—	n/a	n/a
Actual, kb	−0.063 (−0.116 to 0)	—	—
%-eMTL	−0.35 (−1.18 to 0.69)	—	—
Time between MTL, yr**	6.1 (5.0 to 7.8)	—	—

Definition of abbreviations: %-eMTL = percent of expected mean telomere length; HRCT = high-resolution chest computed tomography; ILA = interstitial lung abnormalities; MTL = mean telomere length; SE = standard error.
*Gives the unadjusted effect (via linear regression) of the characteristic (the named group for categorical or per +1 increase for continuous variables) on the first MTL measurement, expressed as the age-adjusted %-eMTL. Data include effect (SE) and *P* value.
†Gives the unadjusted effect (via linear regression) of the characteristic (the named group for categorical or per +1 increase for continuous variables) on the annualized change in MTL expressed as actual value (in kb). Data are presented as effect (SE), *P* value.
‡The individual carriers had a pathogenic or likely pathogenic rare variant in *PARN* (*n* = 2), *RTEL1* (*n* = 5), or *TERT* (*n* = 10). The individual noncarriers of a family gene came from families with a disease-associated rare variant in *RTEL1* (*n* = 6) or *TERT* (*n* = 6).
§Reference group is all noncarriers of telomerase rare variant.
‖The ILA status was recorded by a dedicated thoracic radiologist as none, early/mild, or extensive as previously described (8). Of note, no participant in this analysis had extensive ILA on the first HRCT. The "ILA on any HRCT" status was on the basis of all HRCTs completed through January 31, 2022; if ILA was present but known to have resolved completely with subsequent imaging, the subject was categorized as "no ILA on any HRCT".
¶Time for ILA observation is from the first HRCT to the first occurrence of pulmonary fibrosis diagnosis, the most recent HRCT in the study, or February 7, 2022 (date of data extraction, applicable to those with one HRCT who are not known to have pulmonary fibrosis).
**Minimum value was 1.6 yr.

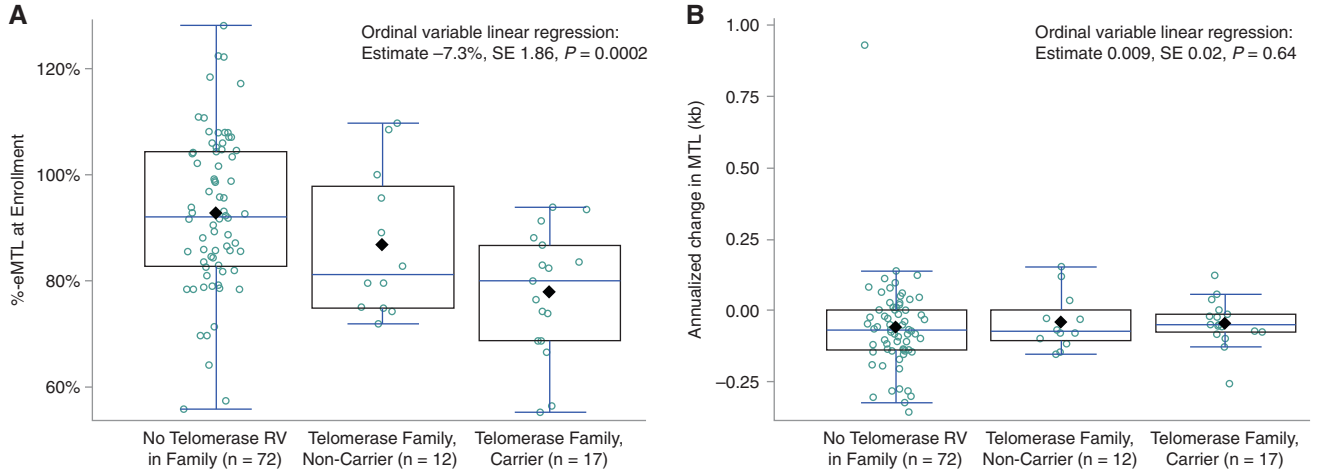

Figure 1. The relationship between personal and familial telomerase pathway rare variant (RV) carrier status and peripheral blood telomere length at baseline and during follow-up. (*A*) The data distribution for %-eMTL at enrollment, stratified by individual and family telomerase RV carrier status. (*B*) The data distribution for the annualized change in MTL (in kb) stratified by individual and family telomerase RV carrier status. The data are shown as mean (diamond), range (whiskers), first and third quartiles (lower and upper box lines, respectively), and median (center box line). The individual data points are green circles. %-eMTL = percent of expected mean telomere length; MTL = mean telomere length.

Discussion

Short telomeres in PBMCs, defined by telomere length at or below the 10th percentile for age, are present in up to 25% of sporadic and 40% of familial PF (4). In this cohort of individuals at risk for familial PF, we found shorter than expected telomeres, regardless of family or personal telomerase RV carrier status. In addition, the point estimate for annualized MTL attrition (−0.063 kb) in the cohort exceeded expected annualized telomere attrition during adulthood (−0.043 kb) (10), suggesting that increased telomere attrition contributes to the reduction in MTL in persons at risk for familial PF. This conclusion should be interpreted with caution, as a direct comparison of findings in this cohort to those in healthy adults with comparably collected samples for longitudinally measured MTL is not currently available. In addition, the strong association between initial MTL and subsequent annualized change in MTL could represent regression to the mean (14, 15). Subjects with ILAs on HRCT had shorter telomeres compared with at-risk subjects without ILAs, further supporting the idea that telomere shortening contributes to the development of PF.

In families with telomeropathy, short telomeres have been attributed to the inheritance of shorter telomeres and more rapid attrition (1). We found that telomerase RV carriers had shorter PBMC telomeres than the rest of the cohort; however, telomerase family members who were noncarriers also demonstrated a reduced MTL that approximated carriers. Despite the differences in initial MTL, telomerase RV carriers did not experience a greater rate of telomere attrition than noncarriers. As telomerase RV carriers already had short telomeres, our results may reflect a threshold effect or regression to the mean. In addition, telomerase pathway genes or variants may have a heterogeneous functional impact, and our study is underpowered to test the impact of each individual gene (Table 1) on the telomere attrition rate. Together, these data support the importance of inheritance as a mechanism of short telomeres in telomerase families.

Our cohort of individuals at risk for familial PF offers a unique opportunity to understand the pathobiology that precedes PF, but with several limitations. First, we do not have information on several variables that may affect cross-sectional or longitudinal MTL, including MTL at birth or recent history of infection, or other stressors (14, 16). Second, the PBMC telomere length or its dynamics may not represent telomere length in cells from target tissues (6, 17). Third, longitudinally measured MTL data from other comparator cohorts, including persons with pulmonary fibrosis and healthy individuals, are not currently available but would be useful in future studies. Fourth, our finding that the %-eMTL in noncarriers of a familial telomerase RV approaches that of carriers is contrary to several publications (3, 4), although the occurrence of PF and short telomeres among noncarriers of a familial RV has been described (18).

This analysis supports inherited short telomere length as an important factor contributing to short telomeres observed in telomerase families and may support a role in accelerated attrition in some individuals. ■

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Progressive Interstitial Lung Disease in Relatives of Patients with Pulmonary Fibrosis

To the Editor:

First-degree relatives of patients with sporadic and familial pulmonary fibrosis have been demonstrated to have high rates of interstitial lung abnormalities (ILA) and interstitial lung disease (ILD) (1). However, less is known about the rates of progression in these relatives (2).

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Methods

Relatives enrolled as described previously in the CGS-PF (Clinical Genetics and Screening for Pulmonary Fibrosis) study (1) had baseline pulmonary function tests and chest computed tomography (CT) scans that were repeated 2 years after enrollment. Relatives underwent prone volumetric chest CT scans at full inspiration, and CTs were assessed for the presence of ILA defined by Fleischner Society recommendations (3) and subtyped as previously described (4, 5). All relatives with ILA on either baseline or 2-year CT had both sets of images simultaneously compared in order to determine imaging progression as previously defined (6). For comparison, relatives were divided into two groups: *1*) those with ILA at either baseline or 2-year follow-up; and *2*) those without ILA (no ILA or indeterminate) at both time points. Progression was assessed using thresholds of lung function decline alone (5% and 10%) or in combination with radiologic changes, including an adaptation of criteria used by the INBUILD trial of either an FVC loss of greater than 10% or 5–10% with progression on CT (7). Continuous variables were compared with Wilcoxon rank-sum and categorical variables with Fisher exact tests. Multivariable models were adjusted for age, sex, and history of ever smoking. Two-sided *P* values less than 0.05 were considered statistically significant. All analyses were performed using Statistical Analysis Software version 9.4 (SAS Institute).

Results

Of the 107 relatives in the original CGS-PF study, 73 had 2-year follow-up CTs, of which 20 had ILA at baseline and 53 did not. There were no statistically significant differences in baseline characteristics between relatives who did and did not participate in the 2-year follow-up. At 2 years, 21 had ILA, including 19 who had ILA at baseline and 2 additional cases with incident ILA, 51 relatives were without ILA at both baseline and 2-year follow-up, and 1 participant with ILA at baseline that was not present at the 2-year follow-up. Table 1 presents baseline characteristics for those with ILA at either time point and those without ILA at both time points. Compared with those without ILA, relatives with ILA were more likely to be male and, at baseline, had higher absolute monocyte counts and lower FEV_1/FVC ratio and percent predicted measures of FVC, TLC, and D_{LCO}.

At the 2-year follow-up, the majority (13 [65%]) of those with ILA at baseline had radiologic progression, and 2 (4%) of those without ILA at baseline developed ILA (examples shown in Figure 1). Of the 15 total relatives with radiologic progression, 4 (27%) were from families with familial pulmonary fibrosis; the remaining 11 (73%) had a single first-degree relative with IPF. Of the 20 relatives with baseline ILA, 6 (30%) had definite fibrosis, of which 5 (83%) had 2-year radiologic progression, whereas 8 of the 14 (57%) without baseline fibrosis progressed. At 2 years, FEV_1, FVC, and D_{LCO} remained reduced in those with ILA compared to without ILA. Although there were no statistically significant differences in the loss of FVC and D_{LCO} from baseline to 2 years between relatives with and without ILA, those with ILA had greater loss of FEV_1 in both unadjusted analyses (Table 1) and after adjusting for covariates (−145 ml; 95% confidence interval, −249 ml to −40 ml; $P = 0.007$) when compared with those without ILA. Almost half of the relatives with ILA (10 of the 22 [45%] with ILA at either time point; 9 of the 20 [45%] with ILA at