Subclinical Lung Disease, Macrocytosis, and Premature Graying in Kindreds With Telomerase (*TERT*) Mutations

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Background: Mutations in the human gene encoding the protein component of telomerase (*TERT*) are the most common genetic defect in patients with familial idiopathic pulmonary fibrosis (IPF). The subclinical phenotypes of asymptomatic members of these families have not been evaluated with respect to *TERT* mutation status or telomere length.

Methods: We measured a variety of pulmonary, blood, skin, and bone parameters for 20 subjects with heterozygous *TERT* mutations (carriers) and 20 family members who had not inherited a *TERT* mutation (noncarriers) to identify the spectrum of phenotypes associated with mutations in this gene. The two groups were matched for sex, age, and cigarette smoking. Three *TERT* mutation carriers had IPF (IPF carriers). The rest of the carriers were apparently healthy (asymptomatic carriers) and were compared with the noncarriers.

Results: Asymptomatic carriers exhibited significantly lower diffusing capacity of lung for carbon monoxide (DLCO), impaired recruitment of DLCO with exercise, radiographic signs of lung fibrosis, and increased fractional lung tissue volume quantified by high-resolution chest CT scan than noncarriers. RBC and platelet counts were significantly lower, and the mean corpuscular volume and mean corpuscular hemoglobin concentration were significantly higher in carriers than in noncarriers. Carriers reported significantly earlier graying of hair than noncarriers. *TERT* mutation status is more accurately predicted by short telomere lengths than any of these measured phenotypes.

Conclusions: *TERT* mutation carriers exhibit early preclinical signs of lung fibrosis, bone marrow dysfunction, and premature graying. These clinical features and short telomere lengths characterize patients with germline *TERT* mutations.

CHEST 2011; 140(3):753–763

Abbreviations: DLCO = diffusing capacity of lung for carbon monoxide; DMCO = diffusing capacity of membrane for carbon monoxide; FTV = fractional tissue volume; HRCT = high-resolution CT; IPF = idiopathic pulmonary fibrosis; LN(T/S) = circulating leukocyte and telomere length; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; *TERT* = human gene encoding the protein component of telomerase; UIP = usual interstitial pneumonia; Vc = pulmonary capillary blood volume

Idiopathic pulmonary fibrosis (IPF) is a progressive lung disease characterized by lung scarring and worsening gas exchange. The disease increases in prevalence with advanced age¹ and is fatal within ~3 years after diagnosis.² Despite many well-designed multicenter trials, no medical therapies have been found to be definitively effective for IPF. Better understanding of its pathogenesis should provide a basis to design future therapies.

The familial form of IPF is rare.³ Mutations in four genes (those encoding the protein and RNA

components of telomerase^{4,7} and surfactant proteins A2⁸ and C^{9,10}) are found in ~20% of kindreds; the rest are unexplained at the molecular level. Earlier clinical studies of familial IPF have described cellular, radiographic, physiologic, and pathologic changes in related family members.^{11–13} These studies were performed irrespective of molecular or genetic analysis. Since then, the clinical features of early lung disease in a kindred with a surfactant protein C mutation has been described.¹⁴ Heterozygous loss-of-function mutations in the gene encoding the protein component of

FIGURE 1. Telomere lengths of family members from *TERT* kindreds. Mean telomere lengths as measured by a quantitative polymerase chain reaction assay is shown for *TERT* mutation carriers who are healthy and asymptomatic (asymptomatic carriers, ●), *TERT* mutation carriers with IPF (IPF carriers, ■), and subjects without *TERT* mutations (noncarriers, ○). A, The telomere lengths of subjects are shown relative to the 50th percentile (center line) as well as the 10th and 90th percentiles for a previously described reference cohort of 195 unrelated healthy individuals aged 19 to 89 years (shaded region).⁷ B, Mean O-E age-adjusted telomere length for the control reference cohort,⁷ 20 subjects without *TERT* mutations [–], and 20 related subjects with *TERT* mutations [+]. **P* = .035. ***P* = 1.4×10^{-22} . IPF = idiopathic pulmonary fibrosis; LN(T/S) = circulating leukocytes and telomere lengths; O-E = observed minus expected; *TERT* = human gene encoding the protein component of telomerase.

telomerase (*TERT*) are found in up to 18% of kindreds with familial pulmonary fibrosis and 3% of sporadic cases.⁵⁻⁷ Although each *TERT* mutation is rare and generally private for the family in which it is found, mutations in this gene are collectively the most common genetic defect found in familial pulmonary fibrosis. Because 50% of first-degree relatives carry the same inborn *TERT* mutations as the proband, these families provide an opportunity to study the natural progression of this disease.

There is reduced penetrance of lung disease in *TERT* mutation carriers, especially for those aged < 50 years.⁷ Some individuals with these mutations demonstrate extrapulmonary disease, including aplastic anemia and liver cirrhosis.⁷ The spectrum of

subclinical phenotypes of asymptomatic *TERT* mutation carriers is unknown. It is also unknown whether any of the phenotypes correlate with telomere length. To examine these issues, we report a prospective study of 40 subjects matched for the presence or absence of a *TERT* mutation and find that pulmonary and hematologic abnormalities as well as premature graying of hair are specifically associated with *TERT* mutations.

MATERIALS AND METHODS

Subjects

Forty subjects from 11 kindreds with familial pulmonary fibrosis were enrolled in the Clinical Translational Research Center at the University of Texas Southwestern Medical Center in Dallas, Texas. The protocol was approved by the Institutional Review Board (FWA5087, Protocol #102004-029). Written informed consent was obtained from all subjects.

Subject Recruitment

Subjects were recruited from 21 previously characterized kindreds and one newly identified kindred (e-Table 1). Each subject with a *TERT* mutation (carrier, *n* = 20) was matched to a family member from the same kindred of a similar age (mean difference, 3 years) who did not inherit the *TERT* mutation (noncarriers, *n* = 20). Between one and six pairs of individuals were enrolled from 11 families (e-Table 1). Because there is no known genotype-phenotype correlation for *TERT* mutations and familial pulmonary fibrosis, the pairs were recruited regardless of their specific mutation. In contrast, because this disease is strongly associated with age, sex, and smoking history,¹² the two groups were carefully matched for these variables. We strove for a mean age of 50 years

Manuscript received November 5, 2010; revision accepted February 1, 2011.

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Funding/Support: This work was supported by the National Institutes of Health [grants HL093096 (C. K. G.), HL097010 [(C. Y.) and DK063242 (C. C. W. H.)], the Doris Duke Charitable Foundation (A. D. and C. K. G.), and the American Heart Association (C. K. G.).

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DOI: 10.1378/chest.10-2865

Table 1—Demographics, Examination Findings, and Telomere Lengths of Subjects With and Without a TERT Mutation (Carriers and Noncarriers)

Characteristics	Noncarriers (n = 20)	Carriers (n = 20)
Demographic		
Age, y	50.6 ± 11.7	50.5 ± 12.7
Male sex, %	55	55
Smokers, %	50	50
Smoking, pack-y	9.5 ± 17.8	7.8 ± 11.0
Alcohol consumption, drinks/wk	5.6 ± 11.0	6.2 ± 15.6
Examination findings		
Height, in	67.2 ± 4.6	67.6 ± 4.3
Weight, kg	79.3 ± 49.7	84.5 ± 43.0
Dry inspiratory crackles on chest examination, No.	2	10 ^a
Telomere lengths		
LN(T/S) ratio	1.49 ± 0.20	1.11 ± 0.17 ^b
O-E LN(T/S)	−0.09 ± 0.19	−0.47 ± 0.17 ^b
O-E range	−0.58 to 0.14	−0.87 to −0.18

Data are presented as mean ± SD, unless otherwise indicated. LN(T/S) = circulating leukocyte and telomere length; O-E = observed minus expected; TERT = human gene encoding the protein component of telomerase.

^a*P* = .014 by Fisher exact test.

^b*P* < 1.0 × 10^{−6}, carriers vs noncarriers in a two-tailed Student *t* test.

because few TERT mutation carriers develop pulmonary fibrosis before this age.⁷ Three of 20 TERT mutation carriers had been given a diagnosis of IPF, as defined using the American Thoracic Society/European Respiratory Society Guidelines,² prior to recruitment. One subject declined radiographic evaluations. One subject with IPF declined exercise testing after enrollment, and another was unable to perform the exercise tests because of hypoxemia. Thus, there were three TERT mutation carriers with overt IPF, 17 asymptomatic TERT mutation carriers, and 20 family members without a TERT mutation (noncarriers).

Table 2—Pulmonary Function Tests and Physiologic Parameters of Subjects With and Without a TERT Mutation (Carriers and Noncarriers)

Characteristics	Noncarriers	Carriers	IPF Carriers	Asymptomatic Carriers
Pulmonary function test, No.	20	20	3	17
FEV ₁ , % predicted	105.4 ± 14.3	100.1 ± 17.6	80 ± 13.5 ^a	103.6 ± 16.0
FVC, % predicted	111.2 ± 14.8	105.8 ± 22.1	77 ± 18.5 ^b	110.9 ± 18.8
FEV ₁ /FVC	77.7 ± 4.4	78.2 ± 7.1	85 ± 6.6 ^a	76.9 ± 6.6
TLC, % predicted	100.1 ± 12.4	92.1 ± 18.3	64.3 ± 11.0 ^c	97.0 ± 14.6
RV, % predicted	76.5 ± 23.6	62.3 ± 19.7 ^a	35 ± 5.6 ^a	67.1 ± 17.1
VC, % predicted	111.2 ± 14.8	106.2 ± 22.1	77.7 ± 17.6 ^b	111.2 ± 19.0
DLCO, % predicted	87.5 ± 17.1	71.5 ± 17.0 ^c	38.7 ± 5.5 ^c	77.3 ± 10.0 ^a
Physiologic parameter	20	19	2	17
Heart rate at rest, beats/min	82.5 ± 11.5	92.7 ± 13.1 ^a	98.2 ± 7.6	92.0 ± 13.6 ^a
Respiratory rate, breaths/min	14.44 ± 3.90	14.49 ± 4.03	17.33 ± 1.18	14.15 ± 4.12
Tidal volume, L/m ²	1.03 ± 0.31	1.09 ± 0.41	1.08 ± 0.21	1.09 ± 0.43
Minute ventilation, L/min	14.18 ± 4.22	14.88 ± 4.73	18.28 ± 2.31	14.48 ± 4.82

Data are presented as mean ± SD. DLCO = diffusing capacity of lung for carbon monoxide; IPF = idiopathic pulmonary fibrosis; RV = residual volume; TLC = total lung capacity; VC = vital capacity. See Table 1 legend for expansion of other abbreviation.

^a*P* ≤ .05, carriers vs noncarriers in a two-tailed Student *t* test.

^b*P* ≤ .005, carriers vs noncarriers in a two-tailed Student *t* test.

^c*P* ≤ .0005, carriers vs noncarriers in a two-tailed Student *t* test.

Blood Chemistries

A nonfasted venous blood sample was collected on day 1 for CBC count, reticulocyte count, total iron level, total iron binding capacity, and comprehensive metabolic panel. Another venous blood sample was collected on day 2 for a repeat CBC count. Aliquots of frozen serum were analyzed for thyroid stimulating hormone, folate, and vitamin B12 levels.

Pulmonary Function Testing and Cardiopulmonary Exercise Testing

Spirometry, lung volumes, and diffusing capacity of lung for carbon monoxide (DLCO) were measured (Jaeger MasterScreen; CareFusion; San Diego, California) according to American Thoracic Society recommendations.^{15,16} Maximal oxygen uptake was determined by continuous incremental exercise on a bicycle ergometer. Lung volume, DLCO, and pulmonary blood flow were measured simultaneously at two alveolar oxygen tensions at rest and at 30%, 60%, and 90% of the subject's maximal workload by a rebreathing technique.¹⁷ Diffusing capacity of membrane for carbon monoxide (DMCO) and pulmonary capillary blood volume (Vc) were calculated and used to express DLCO at standard conditions (DLCO standard [hemoglobin], 14.6 g/dL; PAO₂, 120 mm Hg)¹⁸ and as % predicted reference values.^{17,19} The slopes of the relationships of DLCO, DMCO, and Vc with respect to pulmonary blood flow were compared among subject groups as indices of functional microvascular recruitment.¹⁷

High-Resolution CT Scanning

Noncontrasted three-phase chest high-resolution CT (HRCT) scanning was performed at prone and supine end inspiration and supine end expiration (GE Lightspeed 16; GE Healthcare; Waukesha, Wisconsin), and 1.25-mm thickness images were obtained at 10-mm intervals from apex to costophrenic angle. The images were classified in a blinded fashion by a chest radiologist (C. B.) into the following four categories:

- (1) No reticular changes, including micronodules, discrete cysts, mosaic perfusion, or consolidation;

- (2) Minimal radiographic densities, including apical, single-level, or scattered peripheral or peribronchial reticulations, parenchymal bands, irregular linear opacities, and mild ground glass opacities;
- (3) Increased radiographic densities, including multilobe peripheral or peribronchial reticulations, subpleural lines, and mild ground glass opacities, but no honeycombing or any findings inconsistent with usual interstitial pneumonia (UIP); and
- (4) Compatible with UIP, including peripheral and basal-predominant reticulations with or without honeycombing.²

A semi-automated image analysis program (Microsoft Visual C++ 6.0), developed and validated by our group,²⁰ was used to quantitatively analyze these CT images. Fractional tissue volume (FTV) was calculated voxelwise as the volume ratio of tissue/(tissue + air) and shown in three-dimensional color maps. The HRCT scan-derived tissue volume includes the volume of alveolar tissue, extraseptal structures (airways and blood vessels) < 1 to 2 mm in diameter, and the blood within these small vessels.

Bone Mineral Density and Skin Biopsy Specimens

Dual-energy radiographic absorptiometry scans of the L1 to L4 lumbar vertebrae, the left proximal femur, and the left distal radius were performed. Under sterile conditions, a punch skin biopsy specimen was obtained in 38 subjects from the dorsal hand and the hip, formalin fixed, paraffin embedded, sectioned, and stained with hematoxylin and eosin for measuring the thickness of the epidermis and dermis.

Telomere Length

Genomic DNA was isolated from circulating leukocyte and telomere length [LN(T/S)] measured using a multiplexed quantitative polymerase chain reaction assay.⁷ The control population used in Figures 1A and 1B has been previously described.⁷

Statistical Analysis

Results are expressed as mean $\pm$ SD and compared between groups by Student *t* test. Linear trend tests were determined using Prism (GraphPad Software, Inc; La Jolla, California) statistical software. The least absolute shrinkage and selection operator technique²¹ was used to construct a parsimonious model to predict *TERT* mutation status using R package glmnet (R Foundation for Statistical Computing; Vienna, Austria).²² Data for the 38 subjects who completed the entire study (18 carriers, 20 noncarriers) were used. The tuning parameter ($\lambda = 0.114$) was chosen to minimize the misclassification error rate by a 10-fold cross-validation.

RESULTS

The *TERT* mutation carrier and noncarrier groups were matched for age, sex, and smoking history (Table 1). There was no difference between the subjects' mean heights and weights. There was also no statistically significant difference between the asymptomatic *TERT* mutation carriers and noncarriers with respect to age, sex, and smoking history.

Telomere lengths were significantly shorter for carriers than for noncarriers. The telomere lengths of all carriers were $\leq$ 50th percentile; 80% of carriers had lengths $\leq$ 10th percentile (Fig 1). The telomere lengths of the noncarriers were significantly shorter

FIGURE 2. Diffusion capacity measurements of subjects at rest and with exercise. A, % Predicted single-breath DLCO for subjects without *TERT* mutations (noncarriers, ○), *TERT* mutation carriers who are asymptomatic (asymptomatic carriers, ●), and *TERT* mutation carriers with IPF (IPF carriers, ■). The results are graphed for all subjects. *Two-tailed Student *t* test $P = .037$ for asymptomatic carriers vs noncarriers. ***Two-tailed Student *t* test $P = 9.1 \times 10^{-5}$ for IPF carriers vs noncarriers. B-D, Recruitment of DLCO (B), DMCO (C), and Vc (D) with respect to cardiac output in noncarriers, asymptomatic carriers, and IPF carriers. The DLCO is expressed under standardized conditions of hemoglobin (14.6 mg/dL) and PAO_2 (120 mm Hg); Qc is the calculated cardiac output. The Vc was estimated by the Roughton-Forster method.²³ The best-fit lines are as follow: DLCO std, $1.71 Qc + 10.40$ (asymptomatic carriers, $R^2 = 0.68$); DLCO std, $1.17 Qc + 13.04$ (noncarriers, $R^2 = 0.69$; $P = .001$); DLCO std, $1.24 Qc + 2.43$ (IPF carriers, $R^2 = 0.88$); DMCO, $2.75 Qc + 28.85$ (asymptomatic carriers, $R^2 = 0.22$); DMCO, $1.32 Qc + 34.64$ (noncarriers, $R^2 = 0.14$; $P = .05$); DMCO, $1.80 Qc + 5.39$ (IPF carriers, $R^2 = 0.74$); Vc, $5.75 Qc + 20.23$ (asymptomatic carriers, $R^2 = 0.65$); Vc, $5.14 Qc + 23.07$ (noncarriers, $R^2 = 0.75$; $P > .5$); and Vc, $3.70 Qc + 18.01$ (IPF carriers, $R^2 = 0.34$). *P* values indicate a comparison of the slopes of individual regression lines between asymptomatic carriers and noncarriers. DLCO = diffusing capacity of lung for carbon monoxide; DLCO std = diffusing capacity of lung for carbon monoxide at standard conditions; DMCO = diffusing capacity of membrane for carbon monoxide; Vc = pulmonary capillary blood volume. See Figure 1 legend for expansion of other abbreviations.

than the reference control subjects but not to the same degree as the mutation carriers.

The three *TERT* mutations carriers with IPF (IPF carriers) had smaller lung volumes and a markedly decreased DLCO at rest than the noncarriers (Table 2). An intermediate decrease in the DLCO also was seen when the asymptomatic *TERT* mutation carriers were compared with the noncarriers ($77.3\% \pm 10.0\%$

vs $87.5\% \pm 17\%$, $P = .038$) (Fig 2). No difference was found between the groups in respiratory rate, tidal volume, or minute ventilation, although resting heart rates were significantly higher for the carriers than for the noncarriers (92.7 ± 13.1 beats/min vs 82.5 ± 11.5 beats/min, $P = .017$).

Upon exercise, IPF carriers showed a marked reduction in DLCO recruitment with respect to cardiac output (e-Table 2). DLCO recruitment was moderately reduced in asymptomatic carriers compared with noncarriers (Fig 2B) ($P = .001$) due to a reduction of

DMCO (Fig 2C) ($P = .05$) rather than to Vc (Fig 2D) ($P > .5$).

Representative HRCT images are shown in Figure 3. Sixty-five percent (13 of 20) of the noncarriers were classified as having no reticulations (group 1) compared with 21% (four of 19) of the asymptomatic carriers. Four of the mutation carriers had findings compatible with UIP (group 4), including three with known IPF and a 55-year-old male asymptomatic carrier. None of the noncarriers had evidence of UIP. More asymptomatic carriers showed group 2 and

FIGURE 3. High-resolution CT (HRCT) scans of subjects without *TERT* mutations (noncarriers) and those with *TERT* mutations (asymptomatic carriers and IPF carriers). Inspiratory HRCT scans were binned into four different categories based on radiographic evidence of reticular changes. The categories are indicated from left to right as follows: group 1, no reticular changes; group 2, minimal radiographic densities (scans included in this category had evidence of apical, single-level, or scattered peripheral or peribronchial reticulations; parenchymal bands; irregular linear opacities; or mild ground glass opacities); group 3, increased radiographic densities (scans included in this category had evidence of multilobe peripheral or peribronchial reticulations, subpleural lines, or mild ground glass opacities; they did not display honeycombing or any findings inconsistent with UIP); and group 4, consistent with UIP (scans in this category had features typical of IPF with peripheral and basal-predominant reticulations with or without honeycombing).² The total number of subjects in each category is indicated beneath a representative HRCT scan radiograph and in graphical form. UIP = usual interstitial pneumonia. See Figure 1 legend for expansion of other abbreviations.

3 abnormalities than the noncarriers. Seven asymptomatic carriers had mild reticulations, and four had multilobar radiographic densities; five and two noncarriers had evidence of these findings, respectively.

The FTV was calculated from the HRCT scans of the chest (Fig 4). The *TERT* mutation carriers had higher FTVs in all lobes than did the noncarriers (Table 3), and were especially apparent in carriers who had smoked (Fig 5A). Significant associations were found between FTV and both the FVC and DLCO (Figs 5B, 5C, respectively).

TERT mutation carriers had a significantly lower RBC count but not hemoglobin or hematocrit levels (Fig 6, Table 4). The mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were significantly higher in carriers than in noncarriers (MCV, 97.19 ± 4.54 fL vs 90.66 ± 5.14 fL; $P = 6.6 \times 10^{-8}$; MCHC, 33.24 ± 1.51 g/dL vs 30.85 ± 2.06 g/dL; $P = 1.05 \times 10^{-7}$). There was no history of excessive alcohol consumption or evidence of folate or vitamin B12 deficiency or thyroid dysfunction in the carriers to explain the elevated MCV (Tables 1, 4). In addition, the carriers had significantly lower platelet counts than the noncarriers in the absence of overt thrombocytopenia (Fig 6, Table 4).

The amount of gray hair was estimated from visual inspection or by self-reports for those subjects who dye their hair. The carriers had a younger age of graying than the noncarriers (33 ± 11 years vs 39 ± 7 years, $P = .041$) (Table 5). Forty percent of the carriers compared with 5% of the noncarriers reported graying of hair at or before age 30 years.

Telomere length was significantly associated with RBC count (Fig 6D) ($P = .012$) and DLCO (Fig 6F) ($P = .008$). An inverse relationship was found between the telomere length and MCV (Fig 6E) ($P = .0003$) and expiratory total lung FTV (Fig 6G) ($P = .002$).

Comprehensive metabolic profiles showed no evidence of liver dysfunction in the carriers (e-Table 3), although aspartate aminotransferase and alanine aminotransferase levels were above normal in one *TERT* mutation carrier. Similarly, there was no evidence of decreased bone mineral density or thinning of the skin in the *TERT* mutation carriers compared with family member control subjects (e-Tables 4, 5).

Among the 26 quantitative variables associated with *TERT* mutation status at a significance level of .05 by logistic regression, four variables were retained in a model to predict mutation status as follows:

$P(\text{mutation carrier})$

$$= \frac{\exp(-2.41 - 3.57 \times \text{TL} + 0.047 \times \text{MCHC} + 0.12 \times \text{Gray} - 0.27 \times \text{DLCO})}{1 + \exp(-2.41 - 3.57 \times \text{TL} + 0.047 \times \text{MCHC} + 0.12 \times \text{Gray} - 0.27 \times \text{DLCO})}$$

where TL is the observed minus the expected LN(T/S), Gray indicates whether the subject noted

FIGURE 4. Fractional tissue volume (FTV) was calculated from HRCT scans of the chest as described in the “Materials and Methods” section. A, Axial HRCT images obtained at supine end inspiration from a subject without a *TERT* mutation (noncarrier, left), an asymptomatic subject with a *TERT* mutation (asymptomatic carrier, middle), and a *TERT* mutation carrier with IPF (IPF carrier, right). Corresponding color maps show the distribution of FTV in the same images. B, Three-dimensional color maps show the topographical surface distribution of FTV in three orientations. The three coordinate axes are x, subject’s right to left; y, posterior to anterior; and z, cephalad to caudal. The highest FTV is seen in the posterior, peripheral regions of the lower lobes. See Figure 1 and 3 legends for expansion of abbreviations.

graying of hair at or before age 30 (yes = 1, no = 0), and DLCO is the predicted DLCO at rest corrected for alveolar volume and sex. The mean misclassification error and the SD of error equal 0.13 and 0.044, respectively. We tested the marginal association of each variable with *TERT* mutation status and found telomere length [observed minus expected LN(T/S)] to be the most significant predictor of *TERT* mutation status ($P = .00048$). The other independent predictive parameters were the MCHC ($P = .0027$), the predicted DLCO at rest ($P = .010$), and whether the subject demonstrated graying of hair at or before age 30 ($P = .016$).

DISCUSSION

We report the significant subclinical abnormalities in asymptomatic subjects at risk for developing

Table 3—FTV of Subjects With and Without a TERT Mutation (Carriers and Noncarriers)

Characteristics	Noncarriers	Carriers	IPF Carriers	Asymptomatic Carriers
HRCT scan FTV, No.	20	19	3	16
Inspiratory, total lung	0.104 ± 0.030	0.121 ± 0.032	0.156 ± 0.040 ^a	0.114 ± 0.0280
Expiratory, total lung	0.193 ± 0.043	0.273 ± 0.073 ^b	0.321 ± 0.096 ^c	0.232 ± 0.056 ^a
Left-side lung	0.207 ± 0.051	0.267 ± 0.084 ^a	0.358 ± 0.127 ^c	0.249 ± 0.065 ^a
Left upper lobe	0.173 ± 0.049	0.231 ± 0.084 ^a	0.314 ± 0.118 ^c	0.215 ± 0.070 ^a
Left lower lobe	0.226 ± 0.241	0.296 ± 0.302 ^a	0.403 ± 0.138 ^c	0.284 ± 0.067
Right-side lung	0.184 ± 0.040	0.232 ± 0.061 ^c	0.295 ± 0.076 ^c	0.221 ± 0.052 ^a
Right upper lobe	0.167 ± 0.035	0.214 ± 0.064 ^a	0.272 ± 0.067 ^b	0.204 ± 0.059 ^a
Right middle lobe	0.144 ± 0.033	0.176 ± 0.051 ^a	0.224 ± 0.088 ^c	0.168 ± 0.039
Right lower lobe	0.240 ± 0.061	0.306 ± 0.074 ^c	0.390 ± 0.076 ^c	0.290 ± 0.065 ^a

Data are presented as mean ± SD. FTV = fractional tissue volume; HRCT = high-resolution CT. See Table 1 legend for expansion of other abbreviation.

^a $P \leq .05$, carriers vs noncarriers in a two-tailed Student t test.

^b $P \leq .0005$, carriers vs noncarriers in a two-tailed Student t test.

^c $P \leq .005$, carriers vs noncarriers in a two-tailed Student t test.

IPF due to inherited *TERT* mutations. This group showed evidence of early lung disease compared with age-similar family members who did not inherit a *TERT* mutation. In addition, the *TERT* mutation carriers exhibited lower RBC and platelet counts,

increased red cell MCV and MCHC, and early graying of hair.

Others have shown radiographically apparent fibrosis and lower DLCO in asymptomatic members of familial IPF kindreds without defining their genetic status.^{12,13}

FIGURE 5. Supine expiratory total lung FTV for subjects without *TERT* mutations (noncarriers, ○) and those with *TERT* mutations with or without IPF (IPF carriers [■] and asymptomatic carriers [●], respectively). A, The results are graphed for all subjects (left), those who had never smoked (middle), and those who were either current or past smokers (right). *Two-tailed Student t test $P = .024$. ***Two-tailed Student t test $P = 7.4 \times 10^{-4}$. The one-tailed Student t test P values analyzing the subgroups are 0.10 (n.s.), 0.034 (†), and 2.9×10^{-3} (†††). B, Expiratory total lung FTV vs FVC % predicted. The best-fit line is drawn ($R^2 = 0.256$, $P = .001$). C, Expiratory total lung FTV vs DLco % predicted. The best-fit line is drawn ($R^2 = 0.227$, $P = .002$). See Figure 1, 2, and 4 legends for expansion of abbreviations.

FIGURE 6. Blood counts and quantitative phenotypes of subjects without *TERT* mutations (noncarriers, ○) and those with *TERT* mutations with or without IPF (IPF carriers [■] and asymptomatic carriers [●], respectively). A, RBC counts (millions/mL) for carriers are significantly lower than for family member control subjects. $**P = 2.9 \times 10^{-3}$. B, MCV (fL) is significantly higher for carriers than for noncarriers. $***P = 6.6 \times 10^{-8}$. C, Platelet counts (thousands/ μ L) for carriers are lower than for noncarriers. $*P = .032$. D, RBC count (millions/ μ L) vs observed minus expected age-adjusted telomere length for all subjects. The best-fit line is drawn ($R^2 = 0.153$, $P = .012$). E, MCV (fL) vs observed minus expected age-adjusted telomere length for all subjects. The best-fit line is drawn ($R^2 = 0.294$, $P = .0003$). F, DLco % predicted vs observed minus expected age-adjusted telomere length for all subjects. The best-fit line is drawn ($R^2 = 0.170$, $P = .008$). G, Expiratory total lung FTV vs observed minus expected age-adjusted telomere length for all subjects. The best-fit line is drawn ($R^2 = 0.230$, $P = .002$). MCV = mean corpuscular volume. See Figure 1, 2, and 4 legends for expansion of other abbreviations.

We confirm these findings in the present cohort of *TERT* mutation carriers. Additionally, to our knowledge, we demonstrate for the first time a significant reduction in functional alveolar microvascular recruitment with exercise and a quantitative increase in HRCT scan-derived FTV in asymptomatic carriers. Consistent with the diffuse pathophysiology, FTV is elevated in multiple lobes in asymptomatic carriers and inversely correlated with FVC and DLCO. There is overlap between the asymptomatic carriers and noncarriers with regard to DLCO and radiographically apparent reticulations. Neither of these two clinical parameters predicts *TERT* mutations with complete

accuracy, and longitudinal studies will be needed to determine whether they are useful endophenotypes.

Several extrapulmonary phenotypes are significantly associated with *TERT* mutations. The reduction in RBC and platelet counts is not surprising because patients with bone marrow disorders, including aplastic anemia, myelodysplastic syndrome, and acute myeloid leukemia, have been shown to carry germline *TERT* mutations.²⁴⁻²⁶ A novel finding from the present study suggests that macrocytosis without anemia or reticulocytosis is an early preclinical finding. Macrocytosis is relatively common, with prevalence estimates of 1.7% to 3.6% of the general population;

Table 4—Blood Test Results of Subjects With and Without a TERT Mutation (Carriers and Noncarriers)

Characteristics	Noncarriers (n = 20)	Carriers (n = 20)
WBC, thousand/ μ L	7.14 $\pm$ 1.82	7.03 $\pm$ 1.65
RBC, million/ μ L	4.46 $\pm$ 0.33	4.20 $\pm$ 0.40 ^a
Hemoglobin, g/dL	13.67 $\pm$ 1.37	13.95 $\pm$ 1.12
Hematocrit, %	40.34 $\pm$ 3.69	40.83 $\pm$ 3.26
MCV, fL	90.66 $\pm$ 5.14	97.19 $\pm$ 4.54 ^b
MCH, pg	30.85 $\pm$ 2.06	33.24 $\pm$ 1.51 ^b
MCHC, g/dL	33.85 $\pm$ 0.84	34.17 $\pm$ 0.48
RDW, %	13.72 $\pm$ 0.84	13.65 $\pm$ 0.78
Platelet, thousand/ μ L	237.65 $\pm$ 56.87	209.92 $\pm$ 56.16 ^c
Reticulocyte count, %	1.20 $\pm$ 0.33	1.34 $\pm$ 0.40
Reticulocyte count, cells/ μ L	54,550 $\pm$ 15,953	56,209 $\pm$ 15,693
Total Fe, μ g/dL	83.2 $\pm$ 35.8	91.2 $\pm$ 52.1
TIBC, μ g/dL	355.15 $\pm$ 84.52	325.85 $\pm$ 39.37
Folate, serum, ng/mL	15.58 $\pm$ 5.53	16.98 $\pm$ 5.62
Vitamin B12, serum, pg/mL	463.5 $\pm$ 200.3	391.5 $\pm$ 135.0
TSH, milli-IU/L	1.62 $\pm$ 1.07	1.29 $\pm$ 0.70

Data are presented as mean $\pm$ SD. Fe = iron; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; RDW = RBC distribution width; TIBC = total iron-binding capacity; TSH = thyroid stimulating hormone. See Table 1 legend for expansion of other abbreviation.

^a $P < .005$, carriers vs noncarriers in a two-tailed Student *t* test.

^b $P \leq 1 \times 10^{-5}$, carriers vs noncarriers in a two-tailed Student *t* test.

^c $P < .05$, carriers vs noncarriers in a two-tailed Student *t* test.

about 60% of patients do not have associated anemia.^{27,28} In the absence of thyroid dysfunction, alcoholism, and vitamin B12 or folate deficiency, *TERT* haploinsufficiency is a likely cause of the macrocytosis in this cohort.

TERT mutations can cause dyskeratosis congenita,^{29,30} which has been associated with progeroid features, including early graying of hair. We noted that many *TERT* mutation carriers have an earlier onset of graying or more rapid graying over time in the absence of other progeroid features, such as short stature, osteoporosis, and decreased body fat and skin thickness. It is unclear whether the higher resting heart rate in *TERT* mutation carriers indicates an underlying cardiovascular phenotype.

Table 5—Aging Characteristics of Subjects With and Without a TERT Mutation (Carriers and Noncarriers)

Characteristics	Noncarriers (n = 20)	Carriers (n = 20)
Age at start of graying, y	39 $\pm$ 7	33 $\pm$ 11 ^a
Subjects with gray hair before age 30	5	40 ^b
Estimated % gray hair	37	61
Uses hair dye	35	25
Estimated % baldness	6	8

Data are presented as mean $\pm$ SD or %. See Table 1 legend for expansion of abbreviation.

^a $P < .05$, carriers vs noncarriers in a two-tailed Student *t* test.

^b $P = .02$ by Fisher exact test.

Our data indicate that short telomere length is the most powerful predictor of a *TERT* mutation. Short telomere lengths of peripheral blood cells have been shown to identify pediatric diseases of bone marrow failure, including dyskeratosis congenita.^{31,32} Of all the phenotypes we studied, the degree of shortening is most strongly associated with RBC macrocytosis (elevated MCV and MCHC). The source of genomic DNA for all these studies is circulating leukocytes, so this measurement may most accurately reflect telomerase activity within the bone marrow.

We found that 50% (eight of 16) of asymptomatic *TERT* mutation carriers had a DLCO $< 80\%$ predicted and at least HRCT scan category 2 abnormalities. However, by the same measure, 20% (four of 20) of noncarrier family members also had a DLCO $< 80\%$ predicted and at least minimal HRCT scan findings. It is surprising that a relatively high number of family member control subjects had detectable pulmonary abnormalities. Past smoking histories (14-60 cumulative pack-years) for two subjects may partly account for these findings. We speculate that the phenotypes for at least one of the control subjects may be related to telomere shortening. He was a 61-year-old never smoker whose telomere length was at the first percentile of normal with crackles on examination, 95% gray hair, low DLCO (62% predicted), HRCT scan category 3 findings, reduced RBC counts (4.03 million/ μ L), and borderline anemia (38.3%). Mouse models of telomerase dysfunction have elegantly demonstrated stable epigenetic inheritance of shortened telomere lengths in the absence of a germline mutation.^{33,34} Hematopoietic defects have been attributed to these shortened telomere lengths in mice.³⁵ The support in favor of the epigenetic inheritance of telomere lengths in humans includes observations of shorter telomere lengths in related family members as well as progressive telomere shortening over successive generations.^{7,36}

The finding of occult genetic disease in patients, due to inheriting short telomeres from parents when the mutation is not inherited, has profound implications for the complexity of genetic counseling in this era of personalized genomic medicine. Clinical tests for telomerase mutations are currently available. The penetrance of pulmonary fibrosis in *TERT* mutation carriers is not 100% but is related to age, sex, and environmental exposure.⁷ In addition, those individuals within *TERT* kindreds who test negative for the mutation may still exhibit mild pulmonary or blood phenotypes. None of the noncarriers had evidence of IPF, but 20% of the noncarriers had some reduction in DLCO and evidence of HRCT scan abnormalities (groups 2 and 3 changes). Their risk of disease is not zero but may be related to telomere shortening. Additional clinical evaluation of populations would

need to be performed to quantify the risk of disease related to telomere length.

There are several limitations to the present study. The two groups of carriers and noncarriers were collectively matched for sex and smoking status. Because of the number of *TERT* kindreds, the structure of the individual pedigrees, and the pool of available subjects, we were not able to match each individual pair for age, sex, and smoking history. The assessment of graying hair is subjective but, nonetheless, relevant to telomerase dysfunction. There is also a lack of longitudinal follow-up. Since completion of the study, one subject, a previously asymptomatic 55-year-old *TERT* mutation carrier, developed worsening dyspnea and cough after an acute exposure to moldy corn and chronic exposure to cigarette smoke and cockatiels (all known fibrogenic agents). His subsequent clinical work-up led to a diagnosis of IPF, with pulmonary function test results worsening over 7 months (FVC decline from 102%-66%, DLCO decline from 72%-46%), increased peripheral reticulations on chest CT scan (classified as group 4 in this study), and surgical lung biopsy specimen findings consistent with UIP. At least for this subject, a picture of his preclinical disease was seen by HRCT scan 7 months before the diagnosis of IPF was clinically established, and his decline occurred relatively rapidly. It is unknown whether there has been any clinical progression for the other subjects.

In conclusion, we describe a coherent set of subclinical findings in families at risk for IPF in relation to shortened telomeres and inherited *TERT* mutations. These results have implications for the complexity of genetic counseling in today's era of personalized genomic medicine and available genetic testing. For the *TERT* mutation carrier, knowledge of an inherited genetic risk may lead to environmental modification and avoidance of fibrogenic exposures. It is currently unknown whether genetic diagnosis or detection of preclinical phenotypes will predict prognosis or response to pharmaceutical interventions.

ACKNOWLEDGMENTS

Author contributions: Dr Garcia had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Dr Diaz de Leon: contributed to the data analyses and interpretation and read the manuscript and approved its final version.

Dr Cronkhite: measured the telomere lengths, contributed to the analysis of the results, and read the manuscript and approved its final version.

Dr Yilmaz: measured the FTV from the radiographs, contributed to the analysis of the results, and read the manuscript and approved its final version.

Dr Brewington: contributed to the analysis of the radiographs and read the manuscript and approved its final version.

Dr Wang: performed the skin biopsies, contributed to the analysis of the results, and read the manuscript and approved its final version.

Dr Xing: contributed to the statistical analyses, revision of the manuscript, and approval of the manuscript in its final version.

Dr Hsia: contributed to the design of the study and analysis of the microvascular recruitment and FTV data, made substantial edits to the manuscript, and approved the manuscript in its final version.

Dr Garcia: conceived the study; wrote the protocol; and contributed to the data analysis, writing and editing of the manuscript, and approval of the manuscript in its final version.

Financial/nonfinancial disclosures: The authors have reported to *CHEST* the following conflicts of interest: Dr Wang has received grant money from the University of Texas Southwestern Medical Center at Dallas in the form of salary support provided to the Department of Dermatology. He also has received support from Galderma Laboratories, LP, in the form of unrestricted research grants for pilot projects. Dr Garcia has received other grant monies. Drs Diaz de Leon, Cronkhite, Yilmaz, Brewington, Xing, and Hsia have reported that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article.

Role of sponsors: Funds provided were unrestricted. All scientific decisions were made independently by the authors. The sponsors had no role in the design of the study, the collection and analysis of the data, or in the preparation of the manuscript.

Other contributions: We thank the subjects for their participation in this research study; Jennifer Fehmel, BA; Madhuri Poduri, MSc; Tyron Lewis, BS, MBA; and Arhaanth Reddy, BA, for excellent technical assistance; Helen Hobbs, MD, and Craig Glazer, MD, for helpful discussions; and Omar Jaffer, MD, for his assistance in analyzing the bone mineral density data.

Additional information: The e-Tables can be found in the Online Supplement at <http://chestjournal.chestpubs.org/content/140/3/753/suppl/DC1>.

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