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Telomere-related lung fibrosis is diagnostically heterogeneous but uniformly progressive

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ABSTRACT Heterozygous mutations in four telomere-related genes have been linked to pulmonary fibrosis, but little is known about similarities or differences of affected individuals.

115 patients with mutations in telomerase reverse transcriptase (*TERT*) (n=75), telomerase RNA component (*TERC*) (n=7), regulator of telomere elongation helicase 1 (*RTEL1*) (n=14) and poly(A)-specific ribonuclease (*PARN*) (n=19) were identified and clinical data were analysed.

Approximately one-half (46%) had a multidisciplinary diagnosis of idiopathic pulmonary fibrosis (IPF); others had unclassifiable lung fibrosis (20%), chronic hypersensitivity pneumonitis (12%), pleuroparenchymal fibroelastosis (10%), interstitial pneumonia with autoimmune features (7%), an idiopathic interstitial pneumonia (4%) and connective tissue disease-related interstitial fibrosis (3%). Discordant interstitial lung disease diagnoses were found in affected individuals from 80% of families. Patients with *TERC* mutations were diagnosed at an earlier age than those with *PARN* mutations (51±11 years *versus* 64±8 years; p=0.03) and had a higher incidence of haematological comorbidities. The mean rate of forced vital capacity decline was 300 mL·year^{-1} and the median time to death or transplant was 2.87 years. There was no significant difference in time to death or transplant for patients across gene mutation groups or for patients with a diagnosis of IPF *versus* a non-IPF diagnosis.

Genetic mutations in telomere related genes lead to a variety of interstitial lung disease (ILD) diagnoses that are universally progressive.

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Mutations in four telomere-related genes lead to progressive pulmonary fibrosis, regardless of ILD diagnosis http://ow.ly/TH2B300Yjvt

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Introduction

The interstitial lung diseases are a heterogeneous collection of diseases characterised by deposition of extracellular matrix within the pulmonary interstitium leading to cough, shortness of breath and hypoxia. The prototype, idiopathic pulmonary fibrosis (IPF), is a distinct type of chronic lung fibrosis that demonstrates a devastating progressive decline in lung function usually resulting in respiratory failure [1, 2]. The cause of IPF remains unknown, but recent investigations have implicated telomere shortening as a key contributor to its pathogenesis and survival characteristics [3–5].

Telomerase is a reverse transcriptase enzyme that adds TTAGGG repeats to chromosomal ends during cell replication. Mutations in the protein component of telomerase (*TERT*) and the RNA component of the enzyme (*TERC*) lead to short telomere lengths and familial pulmonary fibrosis [6, 7]. *TERT* mutations are the most common mutation found in individuals with familial pulmonary fibrosis as they are found in ~15% of affected kindreds [8]. Mutations in dyskerin (*DKC1*) and TRF1-interacting protein 2 (*TINF2*) have also been found in patients with familial interstitial pneumonia [9, 10]. Recently, two other genes, *RTEL1* and *PARN*, have been associated with shortened telomere lengths and familial pulmonary fibrosis [11–13]; thus, six telomere-related genes have been linked to this disease. *RTEL1* encodes for protein that contains an *N*-terminal helicase that unwinds the telomere T-loop structures during DNA replication. *PARN* contributes to the poly (A)-specific maturation of *TERC* RNA and maintenance of telomere length [14]. Evidence for a gene dosage exists as two copies of deleterious *TERT* [15], *RTEL1* [16–18] and *PARN* [14, 19, 20] mutations have been found in young children with dyskeratosis congenita and multiorgan disease that includes pulmonary fibrosis.

Clinically, a diagnosis of IPF predicts more rapid disease progression and a shorter life expectancy compared with the non-IPF interstitial lung diseases [21]. Telomere length has also been shown to be associated with a shorter life expectancy in IPF [5, 22] and prior descriptions of pulmonary fibrosis patients with *TERT* mutations have shown that many have a clinical diagnosis of IPF [6–8]. We sought to test the hypothesis that patients with pulmonary fibrosis due to heterozygous genetic mutations in *TERT*, *TERC*, *RTEL1* or *PARN* share a common clinical phenotype (IPF) and a progressive clinical course.

Methods

Study design and participants

In this observational cohort study, patients with familial pulmonary fibrosis were identified at the University of Texas Southwestern Medical Centre (UTSW; Dallas, TX, USA) or were referred from different academic medical centres for participation. This study was approved by the UTSW institutional review board and written informed consent was obtained from all living subjects. 38 (59%) of 64 kindreds were previously reported; clinical data of each case has been independently reviewed in this study. For 28 cases, the presence of a genetic mutation in an affected individual could be inferred from pedigree structure and genotyping of living family members. In all these cases, the presence of a mutation is imputed only for individuals who are obligate carriers based upon their position in the pedigree between subjects with confirmed variants. Clinical information on subjects including demographics, laboratory results, pulmonary function testing, radiographic studies and pulmonary histopathological specimens were collected. Ethnicity was self-reported or family-reported. Genomic DNA samples from healthy control participants aged 19–89 years [3, 5, 8] were obtained from a cohort of unrelated, multiethnic individuals from Dallas, TX, USA.

Procedures

Genomic DNA was isolated from circulating leukocytes using an Autopure LS (Qiagen, Valencia, CA, USA). Telomere lengths of genomic DNA from circulating leukocytes were measured using a quantitative PCR assay as previously described [3, 5, 8] and are represented as a logarithm-transformed relative ratio of telomeres to a single copy gene (T/S) or an age-adjusted telomere length. Sequencing the *TERT, TERC, RTEL1* and *PARN* genes was performed as previously described [7, 11]. No mutations in *DKC1* [9] or *TINF2* [10] were found in this cohort. Clinical workup and management was directed by local physicians. To account for variation in practices, all data were independently reviewed using current clinical guidelines to establish a multidisciplinary diagnosis [2, 23, 24].

Available chest computed tomography (CT) images (n=73) were reviewed by a chest radiologist (K. Batra) who was blinded to clinical history, mutation analysis, telomere length and histopathology. The scans were graded for 31 distinct radiographic features and were classified into one of three radiographic patterns: definite usual interstitial pneumonia (UIP), possible UIP and inconsistent with UIP [2].

Available lung histopathological specimens (n=42) were analysed by a pulmonary pathologist (J. Torrealba) who was blinded to clinical history, mutation analysis, telomere length and radiographic characteristics. Available specimens, including surgical lung biopsies and lung explants, were graded for 15 different histopathological features. Each specimen was given a histopathologic diagnosis according to established guidelines [23].

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Statistical analysis

Characteristics of patient groups were compared using one-way ANOVA (for continuous variables) or Fisher's exact test (for categorical variables). The differences in the age at diagnosis were tested using linear regression, including gene and generation as predictors (or by a t-test, when there were only two generations). Telomere length relative T/S ratios were logarithm-transformed to ensure that residuals were normally distributed and had constant variance. We estimated the relation between telomere length and age using linear regression of control participants, as previously described [5]. The estimated regression coefficients were used to calculate the observed minus expected, or age-adjusted, telomere length of each subject.

Changes in pulmonary function test (PFT) characteristics were analysed using linear mixed-effects models for patients with at least two separate PFT measurements. We included age and sex as covariates to account for differences in age and the time of the first PFT measurement and in sex proportions for the various genes. To test whether the rate of change in PFT depended on age and sex, we initially included the age×time and sex×time interaction terms in the model. In separate models, we tested whether the average PFT characteristics or the rate of change in PFT depended on telomere length or diagnosis, by including the terms for the fixed effects of telomere length (>10th percentile or <10th percentile) or diagnosis (IPF or non-IPF) and time-dependent interaction terms in the model. Terms that were not statistically significant (p-value>0.05) were excluded from the final model. The parameters were estimated using the restricted maximum likelihood procedure. The need for the random effects was assessed using likelihood ratio tests.

Median transplant-free survival time (time from diagnosis to transplant or death) and overall survival was calculated using the Kaplan–Meier curves. Cox proportional hazards models were used to assess the effect of age, sex, clinical parameters (forced vital capacity (FVC) % predicted and diffusing capacity of the lung for carbon monoxide (D_{LCO}) % predicted), gene mutation carrier group, telomere length, and diagnosis (IPF *versus* non-IPF) on transplant-free survival time. All analyses were performed using R version 3.2.2 statistical analysis software (www.R-project.org).

Results

Patient characteristics

We identified 115 individuals with pulmonary fibrosis from 64 families with mutations in one of four genes linked to telomere shortening: *TERT*, *TERC*, *RTEL1* and *PARN* (n=75, 7, 14 and 19, respectively). Each genetic mutation was rare and unique to the family in which it was discovered. For 38 (60%) families only one affected individual was included (supplemental table S1). Characteristics of subjects with monogenic pulmonary fibrosis are described in tables 1 and 2. Most affected individuals were Caucasian. More affected females were found with *TERC* (86%) or *PARN* (53%) mutations, although this result did not reach statistical significance in the comparison across all gene groups. While the mean age at diagnosis for all mutation carriers was 58 years old, individuals with *TERC* mutations were diagnosed at a younger mean age (51±11 years) and *PARN* mutation carriers were diagnosed at a later mean age (64±8 years, p=0.03). We find no evidence for an effect of smoking on the age of diagnosis. Compared with subjects with *TERT* mutations, the age-adjusted telomere lengths of the *PARN* mutation carriers were longer (−0.36±0.14 *versus* −0.58±0.27; p=0.013). Similarly, a higher percentage of *PARN* mutation carriers had blood leukocyte telomere length >10th percentile than the entire cohort (40% *versus* 13%; p=0.049).

Anaemia and macrocytosis were the most common haematological abnormalities, found in 28% and 24% of the total cohort, respectively. *TERC* mutation carriers with pulmonary fibrosis had a higher incidence of leukopenia, thrombocytopenia, aplastic anaemia or myelodysplastic syndrome (p<0.05) (table 2). Two subjects with *RTEL1* mutations (14%) had lung cancer, which is statistically significant when compared with the other groups (p=0.017). The overall incidence of cancer was 9.7%. Additional comordid conditions are presented in supplemental table S2.

Clinical ILD diagnoses

Of the 77 subjects for whom there was sufficient data to make a multidisciplinary diagnosis, 35 (46%) had a diagnosis of IPF (table 1). The most common non-IPF diagnoses included unclassifiable lung fibrosis (20%), chronic hypersensitivity pneumonitis (12%) and pleuroparenchymal fibroelastosis (10%) (figure 1a–f). Two patients with connective tissue disease-associated ILD had a rheumatological diagnosis of scleroderma. There was no statistically significant difference in the distribution of ILD diagnoses across gene groups.

Affected members of the same family often have discordant diagnoses, despite their inheritance of the same germline genetic mutation. Of the 15 different families for whom a multidisciplinary ILD diagnosis was made for ⩾2 affected family members (table 3), a discordant diagnosis was seen across affected individuals in 12 (80%) families and a concordant diagnosis of IPF was found for three (20%) families. Figure 1i–l displays lung histopathology of individuals with discordant diagnoses from two families.

TABLE 1 Characteristics of interstitial lung disease subjects with heterozygous *TERT*, *TERC*, *RTEL1* or *PARN* mutations

	Total	*TERT*	*TERC*	*RTEL1*	*PARN*	p-value
Subjects n	115	75	7	14	19	
Age at diagnosis mean±SD years	58±10	58±10	51±11	60±11	64±8	**0.03**
Male	58 (50.4)	40 (53.3)	1 (14.3)	8 (57.1)	9 (47.4)	0.25
Ethnicity						
Caucasian	102 (88.7)	63 (84)	6 (85.7)	14 (100)	19 (100)	0.10
Black	2 (1.7)	2 (2.7)	0 (0)	0 (0)	0 (0)	1
Hispanic	8 (7)	8 (10.7)	0 (0)	0 (0)	0 (0)	0.35
Asian	1 (0.9)	0 (0)	1 (14.3)	0 (0)	0 (0)	0.061
Other	2 (1.7)	2 (2.7)	0 (0)	0 (0)	0 (0)	1
Dyspnea	99 (86.1)	64 (85.3)	7 (100)	13 (92.9)	15 (78.9)	0.58
Cough	77 (67)	51 (68)	5 (71.4)	11 (78.6)	10 (52.6)	0.47
Crackles	75 (65.2)	47 (62.7)	5 (71.4)	10 (71.4)	13 (68.4)	0.93
Clubbing	25 (21.7)	16 (21.3)	0 (0)	4 (28.6)	5 (26.3)	0.48
Smoking status						
Current/past	46 (40.7)	30 (40)	1 (14.3)	7 (50)	8 (47.1)	0.45
Never	67 (59.3)	45 (60)	6 (85.7)	7 (50)	9 (52.9)	0.45
Unknown	2 (10.5)				2 (10.5)	
Median (interquartile range) pack-years (n=41)	12 (20–30)	19 (12–29)	35 (35–35)	34 (16–77)	20 (19–29)	0.36
Baseline PFTs						
FVC mean±SD % predicted (n)	72±18 (62)	71±17 (44)	80±13 (4)	66±18 (9)	88±22 (5)	0.16
*D*LCO mean±SD % predicted (n)	53±15 (55)	52±15 (40)	55±22 (4)	48±15 (6)	64±8 (5)	0.52
Diagnosis n	77	54	5	10	8	
IPF	35 (45.5)	27 (50)	1 (20)	3 (30)	4 (50)	0.46
NSIP	2 (2.6)	2 (3.7)	0 (0)	0 (0)	0 (0)	1
DIP	1 (1.3)	1 (1.9)	0 (0)	0 (0)	0 (0)	1
PPFE	8 (10.4)	5 (9.3)	1 (20)	2 (20)	0 (0)	0.30
Unclassifiable	15 (19.5)	9 (16.7)	1 (20)	3 (30)	2 (25)	0.68
Chronic hypersensitivity pneumonitis	9 (11.7)	6 (11.1)	1 (20)	1 (10)	1 (12.5)	0.85
CTD-ILD	2 (2.6)	1 (1.9)	1 (20)	0 (0)	0 (0)	0.18
IPAF	5 (6.5)	3 (5.6)	0 (0)	1 (10)	1 (12.5)	0.55
Telomere length						
Observed–expected mean±SD	−0.56±0.26	−0.58±0.27	−0.7±0.25	−0.51±0.13	−0.36±0.14[#]	0.058
<10th percentile	74 (87.1)	55 (90.2)	7 (100)	6 (85.7)	6 (60)	
>10th percentile	11 (12.9)	6 (9.8)	0 (0)	1 (14.3)	4 (40)	**0.049**

Data are presented as n (%), unless otherwise stated. [#]: different from TERT group with a p-value of 0.013. p-values <0.05 are shown in bold. PFTs: pulmonary function tests; FVC: forced vital capacity; *D*LCO: diffusion capacity of the lung for carbon monoxide; IPF: idiopathic pulmonary fibrosis; NSIP: nonspecific interstitial pneumonia; DIP: desquamative interstitial pneumonia; PPFE: pleuroparenchymal fibroelastosis; CTD-ILD: connective tissue disease-associated interstitial lung disease; IPAF: idiopathic pneumonia with autoimmune features.

Chest CT and histopathological correlations

73 cases had chest CT scans available for review. These were classified into one of three radiographic patterns: definite UIP, possible UIP and inconsistent with UIP (supplemental table S3). Nearly one-half (47%) of all CT scans represented a definite UIP pattern. Overall, one-third of the scans were consistent with possible UIP and 20% of the scans were found to be inconsistent with a UIP pattern.

We found a significant association between emphysema and gene group (p=0.032). Compared with other gene groups, a higher proportion of *RTEL1* mutation carriers (40%) and *TERT* mutation carriers (40%) had radiographic evidence of emphysema (p=0.026 and p=0.028, respectively; supplemental table S4). We also found an association between emphysema and smoking (p=0.015).

42 surgical lung specimens were available for review (supplemental table S5). A histopathological diagnosis of UIP was found in 21 (50%) of subjects. Microscopic honeycombing was found in 33 (78%) subjects and fibroblastic foci were seen in 34 (80.9%) subjects. There was no statistically significant difference in histopathological patterns across subjects with different genetic mutations.

We assessed the correlation between the radiographic and histopathological diagnoses of 34 subjects for whom both chest CT and histopathologic specimens were available. A pathologic diagnosis of UIP was seen in 61% and 38% of cases with a radiographic diagnosis of definite UIP and possible UIP, respectively (supplemental table S6). One-half of cases with a chest CT scan that was found to be inconsistent with UIP had a histopathological diagnosis of UIP.

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TABLE 2 Selected comorbid conditions of interstitial lung disease subjects with heterozygous *TERT, TERC, RTEL1* or *PARN* mutations

	Total	*TERT*	*TERC*	*RTEL1*	*PARN*	p-value
Subjects n	115	75	7	14	19	
Pulmonary						
Pulmonary Fibrosis	115 (100)	75 (100)	7 (100)	14 (100)	19 (100)	
OSA	11 (9.6)	7 (9.3)	0	3 (21.4)	1 (5.3)	0.44
Asthma	3 (2.6)	1 (1.3)	0	1 (7.1)	1 (5.3)	0.36
COPD	11 (9.6)	7(9.3)	0	4 (28.6)	0	0.057
Haematology						
Anaemia	32 (27.8)	20 (26.7)	5 (71.4)	3 (21.4)	4 (21.1)	0.088
Macrocytosis	28 (24.3)	19 (25.3)	2 (28.6)	4 (28.6)	3 (15.8)	0.78
Leukopenia	9 (7.8)	6 (8.0)	3 (42.9)	0	0	**0.015**
Thrombocytopenia	10 (8.7)	7 (9.3)	3 (42.9)	0	0	**0.013**
AA/MDS	4 (3.5)	2 (2.7)	2 (28.6)	0	0	**0.040**
Any	54 (47)	36 (48)	5 (71.4)	7 (50.0)	6 (31.6)	0.34
Gastrointestinal						
GORD	49 (42.6)	31 (41.3)	4 (57.1)	7 (50.0)	7 (36.8)	0.73
Gastritis/PUD	6 (5.2)	4 (5.3)	0	2 (14.3)	0	0.25
Elevated LFTs[#]	5 (4.3)	3 (4.0)	0	1 (7.1)	1 (5.3)	0.85
Cirrhosis	0	0	0	0	0	
Cancer						
Lung	2 (1.7)	0	0	2 (14.3)	0	**0.017**
Breast	4 (3.0)	4 (5.3)	0	0	0	0.81
Lymphoma	2 (2.0)	2 (2.7)	0	0	0	1
Other[¶]	3 (3.0)	3 (4.0)	0	0	0	1

Data are presented as n (%), unless otherwise stated. The prevalence of comorbid conditions is compared using Fisher's exact test. p-values <0.05 are shown in bold. OSA: obstructive sleep apnoea; COPD: chronic obstructive pulmonary disease; AA: aplastic anaemia; MDS, myelodysplastic syndrome; GORD: gastro-oesophageal reflux disease; PUD: peptic ulcer disease; LFT: liver function tests. [#]: LFTs were transiently elevated in three of the five individuals; it was attributed to a drug (dapsone)-induced liver injury in one *RTEL1* mutation carrier. The aetiology of the transient elevation was unknown for the other cases. The two *TERT* mutation carriers with a persistent elevation of LFTs were infected with hepatitis C and both received interferon/ribavirin therapy. All five of these individuals had abdominal imaging and none were found to have cirrhosis. [¶]: non-melanoma skin cancers were excluded. Others include oral squamous cell, endometrial and ovarian cancer.

Genetic anticipation

In this cohort, there were nine families (four with *TERT*, two with *RTEL1* and three with *PARN* mutations) with individuals from more than one generation. We found evidence for genetic anticipation in carriers of *TERT* and *RTEL1* mutations (p=0.003 and p=0.018, respectively), but not *PARN* mutations (figure 2a).

Disease progression and survival

The mean decline in FVC was 300 mL·year^{-1} (range 260–350 mL·year^{-1} across gene groups) (table 4). The mean decline in D_{LCO} was 1.7 mL·min^{-1} per mmHg·year^{-1}. There was no significant difference in the rate of FVC or D_{LCO} decline across genes or across diagnoses. Notably, the mean rate of decline in FVC was 299 mL·year^{-1} and 296 mL·year^{-1} for those with a diagnosis of IPF or a non-IPF diagnosis, respectively.

59 (51%) patients died and 34 (30%) underwent lung transplantation over the follow-up period (table 5). Overall, the median time to death or transplantation was 2.87 years (95% CI 2.40–3.80) for all patients. The median time to death or transplantation is not significantly different across gene groups or between those with IPF or a non-IPF diagnosis. The transplant-free survival of ILD patients across the four gene groups was not significantly different (p=0.63) and is shown in figure 2b. Overall survival was also not significantly different across the four groups (p=0.86, data not shown). The transplant-free survival of patients with a mutation in one of these four genes was not significantly different for those with a diagnosis of IPF or a non-IPF diagnosis (p=0.25) and is shown in figure 2c.

In an unadjusted Cox analysis, baseline FVC % predicted was strongly associated with transplant-free survival for patients (HR 0.65 (95% CI 0.53–0.79) for each 10 percentage point FVC difference, p<0.0001) (table 6). Other unadjusted predictors of transplant-free survival included age at the time of diagnosis (HR 1.02 (1–1.04), p=0.046) and male sex (HR 1.6 (1.04–2.44), p=0.031). The various gene mutations, telomere length and

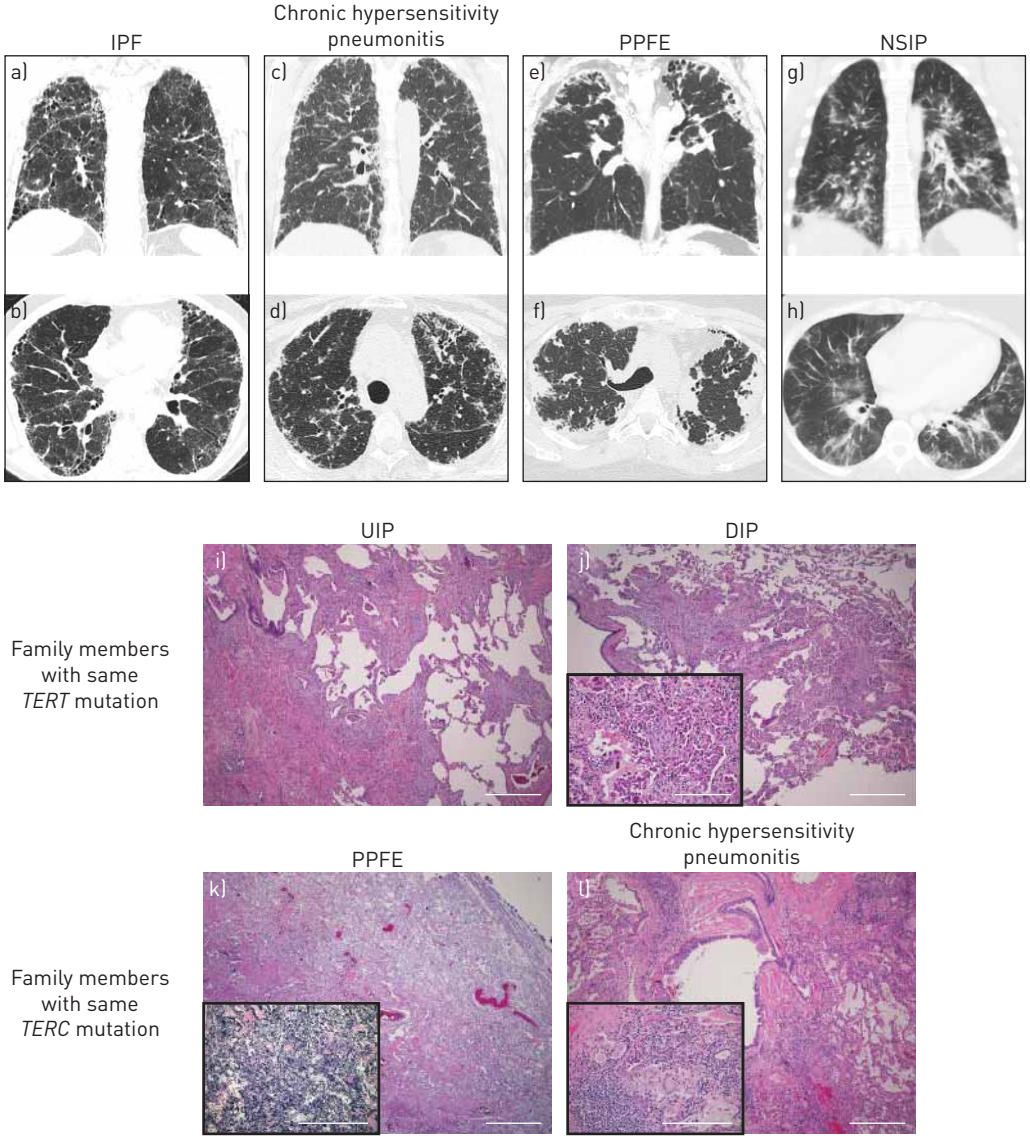

FIGURE 1 Spectrum of interstitial lung disease diagnoses in subjects with heterozygous *TERT, TERC, RTEL1* or *PARN* mutations. Representative coronal (a, c, e, g) and axial (b, d, f, h) images of chest computed tomography scans from patients with diagnoses of a and b) idiopathic pulmonary fibrosis (IPF), c and d) chronic hypersensitivity pneumonitis, e and f) pleuroparenchymal fibroelastosis (PPFE) and g and h) nonspecific interstitial pneumonia (NSIP). Representative haematoxylin and eosin stained lung sections from related ILD subjects with i and j) the same *TERT* c.2594G>A (R865H) mutation and k and l) the same *TERC* r.182g>c mutation. i) Usual interstitial pneumonia (UIP) pattern with temporal and spatial heterogeneity and fibroblastic foci at the interface between normal and fibrotic lung in a 53-year-old man who underwent lung transplantation. j) Desquamative interstitial pneumonia (DIP) with organising pneumonia, thickened interstitial septi and presence of intra-alveolar pigmented macrophages (inset) in a 68-year-old never-smoker (scale bar=500 µm; inset scale bar=200 µm). k) Pleuroparenchymal fibroelastosis (PPFE) with diffuse elastic fibre deposition (inset) near the pleural edge in a lung explant from a 60-year-old female at the time of lung transplantation (scale bar=500 µm; inset scale bar=500 µm). l) Features of chronic hypersensitivity pneumonitis with bronchiolocentric fibrosis, patchy lymphoid aggregates, bridging fibrosis and loosely formed granulomas (inset) in a lung explant from her 57-year-old sister (scale bar=500 µm; inset scale bar=500 µm).

diagnosis subtype (IPF *versus* non-IPF) were not significantly associated with survival in unadjusted analysis. After adjustment for relevant individual covariates, FVC % predicted remained an independent predictor of transplant-free survival time (HR 0.93 (0.89–0.98), p=0.004) but telomere length did not.

Discussion

Genetic mutations in *TERT, TERC, RTEL1* and *PARN* are associated with development of a short telomere syndrome, or "telomeropathy" that can be characterised by systemic abnormalities including pulmonary

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TABLE 3 Multidisciplinary diagnoses of interstitial lung disease (ILD) subjects with heterozygous *TERT*, *TERC*, *RTEL1* or *PARN* mutations from the same kindred

Gene	Mutation	Multidisciplinary diagnoses of family members with ILD
Concordant		
TERT	p.Thr874Arg	IPF, IPF
TERT	p.His925Gln	IPF, IPF
TERT	p.Gly1063Ser	IPF, IPF
Discordant		
TERT	p.Val144Met	PPFE, chronic hypersensitivity pneumonitis, unclassifiable
TERT	p.Arg486Cys	IPF, IPAF
TERT	p.Arg631Gln	IPF, PPFE
TERT	p.Pro702Leu	IPF, IPF, unclassifiable
TERT	p.Arg865His	IPF, IPF, IPF, DIP, unclassifiable
TERT	p.Arg951Trp	PPFE, IPF
TERT	p.Leu1019Phe	Chronic hypersensitivity pneumonitis, IPAF
TERC	r.182g>c	Chronic hypersensitivity pneumonitis, PPFE
RTEL1	p.Gly201GlufsX15	IPF, IPAF
RTEL1	p.Pro484Leu	Unclassifiable, IPF
RTEL1	p.Gln669X	Chronic hypersensitivity pneumonitis, PPFE
PARN	c.246–2A>G	Unclassifiable, IPAF

IPF: idiopathic pulmonary fibrosis; IPAF: interstitial pneumonia with autoimmune features; PPFE: pleuroparenchymal fibroelastosis; DIP: desquamative interstitial pneumonia.

fibrosis, bone marrow dysfunction, liver cirrhosis and early greying. Until now, phenotypic descriptions of individuals with inherited mutations in these genes have been limited to reports of paediatric patients with dyskeratosis congenita and adult patients with various manifestations of this disease spectrum. Overall, pulmonary fibrosis appears to be the most common phenotype associated with telomerase mutations [25]. Here, we studied a large collection of older adult patients with rare heterozygous telomere-related mutations who were collected based upon the familial pulmonary fibrosis phenotype. We found that this cohort exhibited similarities and differences in the clinical expressivity of haematological, gastrointestinal and emphysema phenotypes that have been noted in other cohorts [26–28]. For example, we found that severe haematological diseases were found more often in those with *TERC* mutations. The hepatic

TABLE 4 Forced vital capacity and diffusion capacity change over time for interstitial lung disease subjects with heterozygous *TERT*, *TERC*, *RTEL1* or *PARN* mutations

PFT parameter	Group	N	Mean number of measurements	Mean follow-up time years	Mean change per year	p-value
FVC L	All	71	3.89	2.42	−0.30	
	TERT	46	3.76	2.16	−0.30	NS
	TERC	6	4.67	2.23	−0.26	
	RTEL1	10	3.70	2.94	−0.35	
	PARN	9	4.22	3.30	−0.27	
FVC % predicted	All	71	3.83	2.40	−7.40	
	TERT	46	3.67	2.13	−7.48	NS
	TERC	6	4.67	2.23	−6.65	
	RTEL1	10	3.70	2.94	−7.59	
	PARN	9	4.22	3.30	−7.87	
D_{LCO} mL·min^{-1}·mmHg^{-1}	All	67	3.39	2.30	−1.73	
	TERT	44	3.25	2.04	−1.73	NS
	TERC	5	4.00	2.26	−0.81	
	RTEL1	9	3.11	2.62	−2.11	
	PARN	9	4.00	3.30	−1.80	
D_{LCO} % predicted	All	69	3.33	2.23	−6.26	
	TERT	46	3.15	1.94	−6.35	NS
	TERC	5	4.00	2.26	−2.53	
	RTEL1	9	3.11	2.62	−5.71	
	PARN	9	4.11	3.30	−7.53	

NS: nonsignificant; PFT: pulmonary function test; FVC: forced vital capacity; D_{LCO}: diffusion capacity of the lung for carbon monoxide.

TABLE 5 Cumulative events and time to death or lung transplantation for interstitial lung disease subjects with *TERT*, *TERC*, *RTEL1* or *PARN* mutations

	Subjects n	Death	Transplant	Death or transplant	Time to death or transplant years		
					n	Events n	Median (95% CI)
Total	115	59 (51)	34 (30)	93 (81)	106	88	2.87 (2.40–3.80)
TERT	75	36 (48)	27 (36)	63 (84)	70	61	2.71 (2.36–3.6)
TERC	7	3 (43)	2 (29)	5 (71)	7	5	2.54 (1.92–NA)
RTEL1	14	8 (57)	2 (14)	10 (71)	13	9	2.87 (2.30–NA)
PARN	19	12 (63)	3 (16)	15 (79)	16	13	5.73 (2.55–NA)
Multidisciplinary diagnosis	77						
IPF	35	15 (43)	14 (40)	29 (83)	35	29	2.75 (1.64–4.61)
Non-IPF	42	18 (43)	15 (36)	33 (79)	42	33	3.11 (2.56–4.82)

Data are presented as n (%), unless otherwise stated. IPF: idiopathic pulmonary fibrosis.

phenotype we observed in this cohort appears to be milder than has been described by others [27, 29]. And the radiographic emphysema phenotype was not only significantly associated with smoking and the *TERT* mutation group as has been recently described [28], but also associated with the *RTEL1* gene.

Of the 77 subjects in this cohort for whom a multidisciplinary diagnosis was made, we found that 46% met diagnostic criteria for IPF. The other diagnoses included other idiopathic interstitial pneumonias of unknown cause (nonspecific interstitial pneumonia, desquamative interstitial pneumonia, pleuroparenchymal fibroelastosis) as well as ILD attributed to known causes (chronic hypersensitivity pneumonitis and connective tissue disease-associated ILD). Over 50% of affected individuals had a diagnosis other than IPF. Other studies of ILD patients with short telomere lengths have also found high percentages of non-IPF diagnoses, including hypersensitivity pneumonitis, unclassified interstitial pneumonia and combined pulmonary fibrosis and emphysema [30, 31]. We found discordant diagnoses among affected individuals with the same inherited rare mutation in 80% of kindreds. This degree of phenotypic heterogeneity in the same family was higher than originally found by Steele *et al.* [32] in a familial interstitial pneumonia cohort (45%).

There is poor genotype–ILD phenotype correlation across patients with different telomere-related genetic mutations. The location of the lung fibrosis (lower lobe, upper lobe, peripheral, airway-centric), the pattern of lung destruction (microcystic *versus* macrocystic honeycombing), the nature of extracellular matrix deposition in the lung (collagen, elastin, homogeneity of haematoxylin-stained proteins) and the temporal extent of remodelling (homogeneous *versus* heterogeneous) are likely to be controlled by other factors, both genetic and environmental. For example, the common *MUC5B* risk polymorphism (rs35705950) is associated with a radiographic and histopathological UIP pattern [33]. Sex may be another contributing

TABLE 6 Analysis of transplant-free survival for interstitial lung disease subjects with *TERT*, *TERC*, *RTEL1* or *PARN* mutations

	N	Univariable analysis		Multivariable analysis (n=36)	
		Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
Age at diagnosis	106	1.02 (1–1.04)	0.048	1.05 (0.99–1.12)	0.096
Male	106	1.6 (1.04–2.44)	0.031	0.95 (0.28–3.23)	0.93
FVC % predicted[#,¶]	62	0.65 (0.53–0.79)	<0.0001	0.49 (0.3–0.79)	0.0035
***D*LCO % predicted**[#,¶]	55	0.81 (0.64–1.02)	0.071	1.32 (0.79–2.21)	0.28
Gene					
TERT		Ref		Ref	
TERC	106	0.79 (0.32–1.98)	0.61	0.67 (0.06–7.56)	0.74
RTEL1	106	0.75 (0.37–1.51)	0.42	0.58 (0.11–2.96)	0.51
PARN	106	0.72 (0.39–1.31)	0.28	0.26 (0.06–1.19)	0.083
Log (T/S) telomere length	80	0.76 (0.3–1.93)	0.57	6.18 (0.91–42.13)	0.063
IPF *versus* non-IPF	77	1.34 (0.81–2.23)	0.25	0.46 (0.18–1.22)	0.12

FVC: forced vital capacity; *D*LCO: diffusion capacity of the lung for carbon monoxide; IPF: idiopathic pulmonary fibrosis. [#]: hazard ratios are given per 10 percentage-points difference in % predicted FVC and *D*LCO. [¶]: analysis restricted to patients with pulmonary function test within 1 year of diagnosis.

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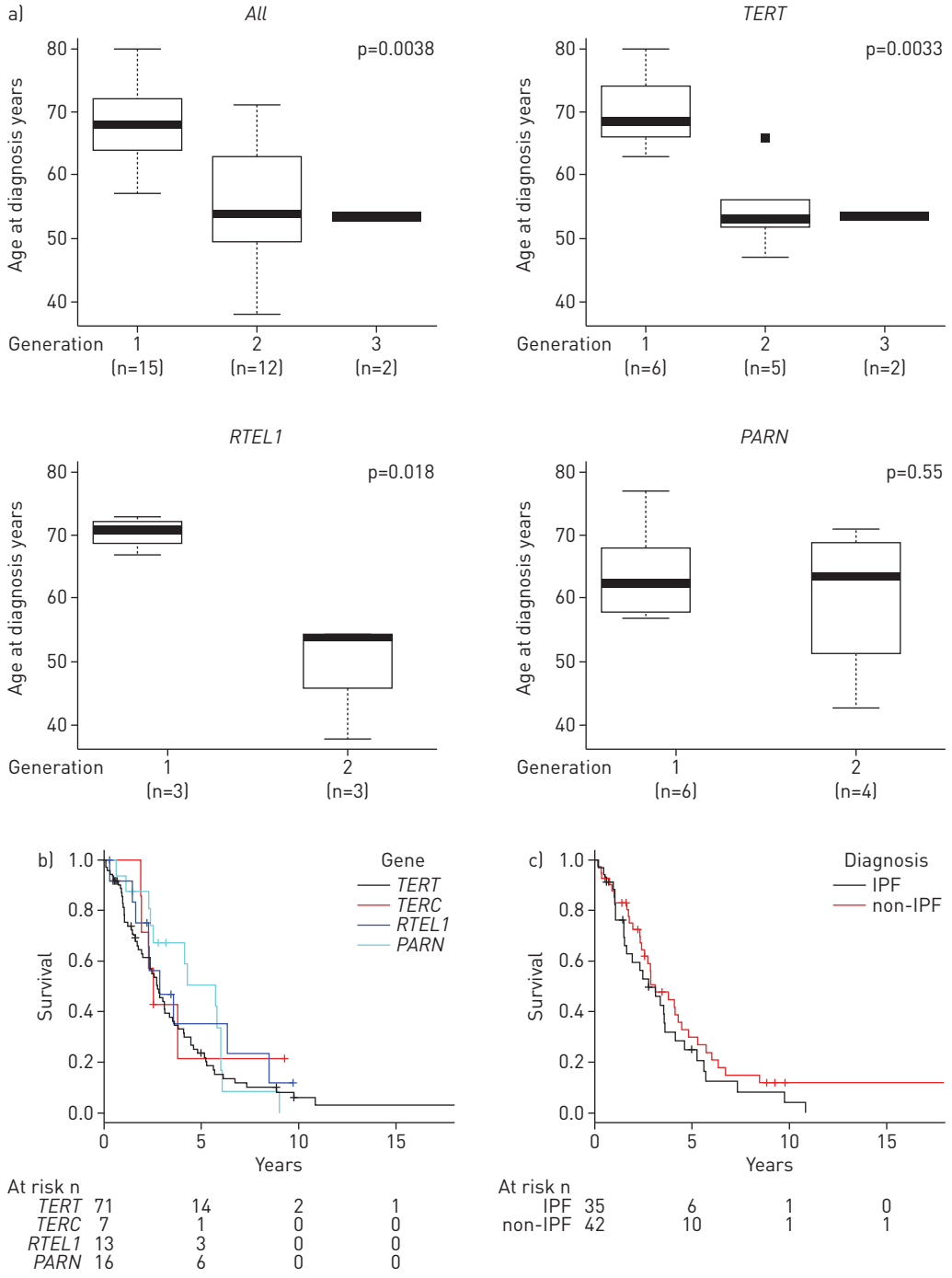

FIGURE 2 Clinical characteristics of interstitial lung disease (ILD) subjects with heterozygous *TERT*, *TERC*, *RTEL1* and *PARN* mutations. a) Evidence of genetic anticipation with an earlier age of ILD diagnosis for patients in subsequent generations of nine kindreds, including four with *TERT* mutations, two with *RTEL1* mutations and three with *PARN* mutations. The number of individuals included in the analysis is listed on the x-axis. b) Transplant-free survival of ILD patients with *TERT*, *TERC*, *RTEL1* and *PARN* mutations as depicted in a Kaplan–Meier survival plot. There is no significant difference between survival characteristics across different gene groups. c) Transplant-free survival of patients with *TERT*, *TERC*, *RTEL1* and *PARN* mutations and a diagnosis of idiopathic pulmonary fibrosis (IPF) or a non-IPF diagnosis as depicted in a Kaplan–Meier survival plot. There was no significant difference between survival characteristics across the two diagnosis groups.

factor as we find that the subgroup of patients with pleuroparenchymal fibroelastosis is predominantly female (88%). Other factors may include the duration and extent of fibrogenic environmental exposures as well as epigenetic or stochastic effects of telomere shortening in the certain lung cells. For example, there is evidence of airway-centric fibrosis and air trapping in individuals diagnosed with chronic hypersensitivity

pneumonitis; in these cases, the inhaled fibrogenic environmental agent may have led to more rounds of cell division and more extreme telomere shortening of lung airway epithelia surrounding the small airways.

However, the one consistent phenotype seen across all genetic mutations is progressive deterioration. The mean rate of PFT decline seen in these monogenic ILD patients is more rapid than what is typically seen. We find a mean change in FVC of −300 mL·year^{-1}, −7.4 FVC % predicted per year and −5.8 D_{LCO} % predicted per year for subjects with heterozygous telomere-related gene mutations. In comparison, patients in the placebo arm of multiple IPF clinical trials demonstrated a median rate of FVC decline of 130–210 mL·year^{-1} [34] and subjects in the placebo arm of the Lung Scleroderma Study had a rate of fall of 2.6±0.9 FVC % predicted per year and 3.5±1.0 D_{LCO} % predicted per year [35]. Patients with these mutations represent an extreme phenotype, similar to those that have been classified with rapidly progressive disease [36, 37].

We found that there was no difference in survival of patients categorised by diagnosis, as those with a diagnosis of IPF have a median survival of 2.75 years (95% CI 1.64–4.61) and those with a non-IPF diagnosis have a nearly equivalent survival of 3.11 years (2.56–4.82). This finding is different from historic studies demonstrating a worse survival for IPF patients with a UIP histopathology [21] and suggests that the effect of the inherited telomere-related mutation is more predictive of accelerated progression than a particular clinical diagnosis or histopathological pattern. Given the lack of prognostic significance of histopathology and the morbidity associated with surgical lung biopsy, the risk and benefits of a surgical lung biopsy should be carefully considered for patients with a mutation in one of these telomere related genes. Increased vigilance and early referral for lung transplantation evaluation is needed, even for those with a non-IPF diagnosis.

Of the four gene mutation groups, we found the longest leukocyte telomere lengths in ILD subjects with *PARN* mutations which is consistent with prior reports [11]. The mean telomere lengths of patients in the different gene mutation groups fall in the following order: *TERC*<*TERT*<*RTEL1*<*PARN*. The mean age of diagnosis for ILD patients in these different gene mutation groups was statistically significantly different and also follows this same order, with *TERC* mutation carriers diagnosed at the earliest age (51 years), followed by *TERT* mutation carriers (58 years), *RTEL1* mutation carriers (60 years) and *PARN* mutation carriers (64 years). Similarly, there was a statistically significant higher prevalence of severe haematological comorbid conditions such as leukopenia, thrombocytopenia and aplastic anaemia/myelodysplastic syndrome in the *TERC* mutation group and a low prevalence of these haematological conditions in the *RTEL1* and *PARN* mutation groups. Although not statistically significant, the mean time to death or transplant follows the same trend.

This study was an observational cohort and, as such, has been limited by ascertainment bias based upon the identification of an adult-onset pulmonary fibrosis phenotype. Patients received care at different locations and the practice patterns of their physicians varied widely. Despite our best efforts to provide a consistent multidisciplinary diagnostic approach by reviewing all primary clinical data, we were unable to establish a diagnosis for 38 patients because of missing or inaccessible chest CTs, surgical lung biopsies or lung explants. The study is also limited by a lack of comparison cohort controlling for regional and temporal clinical practices as well as genetic characteristics. Given the small numbers of patients with certain non-IPF diagnoses, such as unclassifiable pulmonary fibrosis and chronic hypersensitivity pneumonitis, we were unable to directly evaluate the rate of PFT progression or transplant-free survival for these individual groups. The large number of patients undergoing lung transplant may have influenced findings related to survival. However, sensitivity analyses censoring for transplant or treating it as a competing risk demonstrated qualitatively similar results (data not shown). Because of variable practices, we could not readily assess disease progression for IPF patients on FDA-approved medications or for patients with chronic hypersensitivity pneumonitis, connective tissue disease-associated ILD or IPAF on immunosuppression medications.

In summary, mutations in the telomere maintenance machinery genes *TERT, TERC, RTEL1* and *PARN* lead to variable interstitial lung disease phenotypes and diagnoses. Although a specific ILD diagnosis may be important in terms of therapeutic options and prognosis for patients with sporadic disease, this study suggests that the effect of mutations in four different telomere-related genes predicts a similar pathogenic mechanism that manifests as progressive pulmonary fibrosis and reduced survival. Additional studies will be needed to determine the relative utility of telomere length and genetic mutations as biomarkers for predicting therapeutic treatments and outcomes in patients with familial pulmonary fibrosis.

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