Telomeres in Interstitial Lung Disease: The Short and the Long of It

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Abstract

Telomeres are repetitive nucleotide sequences that cap linear chromosomes, thereby limiting progressive chromosomal shortening during cell replication. In conjunction with environmental factors, common single-nucleotide polymorphisms and rare and ultra-rare telomere-related mutations are associated with accelerated telomere shortening resulting in organ dysfunction, including interstitial lung disease (ILD). The most common telomere-related mutation-associated ILD is idiopathic pulmonary fibrosis (IPF). Up to one-third of individuals with familial IPF have shortened telomeres and/or carry a telomere-related mutation, and 1 in 10 individuals with sporadic IPF have telomere-related mutations. Regardless of ILD phenotype, individuals with short telomeres and/or known telomere-related

mutations have more rapid disease progression and shorter lung transplant-free survival. Management should include initiation of antifibrotic agents for those with an IPF phenotype and early referral to a transplant center. Patients with ILD being considered for transplant should be screened for short telomeres if there is a significant family history of pulmonary fibrosis or evidence of extrapulmonary organ dysfunction associated with a short telomere syndrome. Post-transplant management of recipients with telomere-related mutations should include careful adjustment of immunosuppression regimens on the basis of bone marrow reserve. Data on the impact of shortened telomeres on post-transplant outcomes, however, remain mixed.

Keywords: interstitial lung disease; idiopathic pulmonary fibrosis; lung transplantation; telomeres; telomere length

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Interstitial lung diseases (ILDs) are a heterogeneous group of pulmonary disorders that affect tissue in and around alveoli. State-of-the-art genetic research has implicated a number of common, rare, and ultra-rare genetic variants in ILD. Among these are telomere-related gene mutations, which are associated with prematurely shortened telomere length in somatic cells, resulting in organ dysfunction. Here we provide an overview of the relationship between telomere-related mutations, telomere shortening, and ILD. In addition to genetic and epidemiologic associations, we focus on the treatment of patients with ILD

and telomere-related mutations, including considerations before and after lung transplantation.

Telomere Function and Telomere-related Gene Mutations

Telomeres are repetitive nucleotide sequences that cap linear chromosomes. Telomeres play an essential role in stabilizing chromosome ends to prevent progressive shortening during cell replication. Telomere maintenance relies on

a complex interaction between the shelterin complex (end protection), the telomerase complex (elongation), the CST complex (capping), and various trafficking elements (1). Rare and ultra-rare variants in the genes in the telomere maintenance pathway, including those impacting telomerase enzyme activity (*TERT*, *TERC*), telomerase RNA biogenesis (*NAF1*), maturation (*PARN*), and trafficking (*DKC1*, *TCAB1*), DNA helicase activity (*RTEL1*), and shelterin function (*TINF2*), result in prematurely shortened telomere lengths in somatic cells (2). Common single-nucleotide polymorphisms (SNPs),

Telomere Length Measurement

There are several different methods for measurement of telomere length, an essential surrogate marker of telomere function (Table 1). Terminal restriction fragmentation (TRF) uses restriction enzymes to excise the telomeric region, which can then be measured using gel electrophoresis and compared with known fragment sizes. Because TRF requires relatively large quantities of nondegraded DNA, various polymerase chain reaction (PCR)-based methods have been developed. Among these, quantitative PCR is the most common and involves determining the ratio between telomeric repeat copy number (T) and a single copy reference gene (S). The relative T/S ratio is calculated by subtracting the T/S ratio of a reference sample, consisting of a pooled genomic DNA sample, from an individual's T/S ratio. Relative T/S is thus defined in relation to a population reference curve.

Quantitative fluorescence *in situ* hybridization (FISH) is a commercially available approach that uses quantification

of fluorescence intensity after hybridization with a nucleic acid telomeric repeat. An individual's fluorescence intensity is then compared with a reference population, generating a comparative assessment of telomere length. Quantitative FISH does not require fresh cells and can be used on fixed tissues. A further refinement, flow-FISH, uses antibodies for specific cell markers to sort cells, allowing measurement in unique cellular subpopulations such as type 2 alveolar cells. Because telomere shortening does not happen uniformly across chromosomes and the shortest telomeres—not average telomere length—can trigger cellular senescence, TRF, FISH, and traditional PCR-based measurements may not be as relevant as single telomere length analysis (4). Single telomere length analysis uses targeted primers to determine whether critical telomere shortening has occurred at specific chromosomes. Refinements, including Telomere Shortest Length Assay, allow for the determination of whether telomere shortening has occurred on one or more specific chromosomes (5).

In the clinical setting, most commercially available, Clinical Laboratory

particularly in *TERT* and *TERC*, have also been associated with shortened telomeres (3). Regardless of whether the telomere-related mutation is a common SNP or a rare or ultra-rare variant, once a critical threshold of telomere shortening is reached—in conjunction with related genomic instability and defects in DNA repair—the cell enters senescence and eventually dies. Cells in systems with higher lifetime replicative demands—lungs, bone marrow, gastrointestinal tract, and liver—may reach this threshold sooner, resulting in organ dysfunction.

Table 1. Telomere length measurement techniques

Test	Specimen Required	Advantages	Additional Considerations
TRF	DNA (microgram range)	Reference standard in large-scale population-based studies Good replicability	Measures average telomere length Not commercially available Labor intensive
Q-PCR	DNA (nanogram range)	Commercially available Can be used on archived samples from large epidemiological genetic databases Multiple published studies available for comparison	Measures average telomere length on the basis of standard single-copy gene ratio Inconsistent replicability
Q-FISH	Actively dividing cells	Commercially available Can be used on fixed tissues Can be used to assess specific cellular subtypes	Measures average telomere length Unless comparing telomere length between experimental groups, requires reference standards from TRF to interpret fluorescence units
Flow-FISH	Fresh or frozen cells (although freezing may impact reliability)	Commercially available Can be used for multiple different cell types	Labor intensive Measures average telomere length Additional preparation required to assess nonperipheral blood cells
STELA	DNA (picogram range)	Direct telomere measurement per specific chromosome, including shortest telomeres	Labor intensive Research purposes only
TeSLA	DNA (nanogram range)	Measurement of telomere length on all chromosomes, including shortest telomeres	Labor intensive Research purposes only Cannot measure the longest telomeres

Definition of abbreviations: FISH = fluorescence *in situ* hybridization; flow-FISH = flow cytometry–fluorescence *in situ* hybridization; Q-FISH = quantitative fluorescence *in situ* hybridization; Q-PCR = quantitative polymerase chain reaction; STELA = single telomere length analysis; TeSLA = telomere shortest length assay; TRF = telomere restriction fragment.

Improvement Amendments—certified companies offer PCR- or flow-FISH–based telomere measurement. Although there appears to be more interlaboratory variability in quantitative PCR assays (up to 20% in one study), a number of quality control interventions can improve testing consistency (6, 7). The lack of a common reference standard and agreed-on test method, however, may limit comparison between studies. In particular, data are needed to assess how many individuals with “normal” telomere length would be classified as having short telomeres and vice versa on the basis of different methods. Such information is an essential part of establishing an international standard for telomere length testing.

Interstitial Lung Disease and Short Telomeres

The phenotypic manifestations of telomere-related mutations are variable. Children and adolescents with biallelic and x-linked mutations may develop severe multisystem disease, termed the “short telomere syndrome,” such as dyskeratosis congenita or Hoyeraal-Hreidarsson syndrome (8, 9). These conditions are characterized by bone marrow failure, premature greying of the hair, mucosal leukoplakia, cutaneous reticulated hypopigmentation, nail dystrophy, increased risk for head and neck squamous cell carcinoma and skin cancer, and, less commonly, cirrhosis. In contrast, individuals with monoallelic telomere-related mutations are at risk for later-in-life manifestations of shortened telomeres, including ILD.

Telomere-related mutations and short telomeres are implicated in the pathogenesis of an array of ILDs (Table 2). The best characterized of these is idiopathic pulmonary fibrosis (IPF). Drawing on the observation that IPF can cluster in familial cohorts and that individuals with dyskeratosis congenita are at unusually high risk for IPF, Armanios and colleagues found *TERT* and *TERC* mutations in 8% of probands with familial IPF (10). Similar mutations were found in patients with sporadic IPF (11). Subsequent studies have found up to 30% of patients with familial IPF have shortened telomeres and/or carry a telomere-related mutation (depending on the population, telomere length measurement assay used, and cell type; e.g., most commonly peripheral blood mononuclear cells [PBMC]) (12, 13). In larger cohorts of sporadic IPF, approximately 25% of patients have telomere lengths below the 10th percentile and around 10% have telomere-related mutations, most commonly in *TERT* (12, 14). Telomere-related mutations have also been found in 12% of patients with rheumatoid arthritis ILD and appear to be more common in pleuroparenchymal fibroelastosis (PPFE) (15, 16).

The discovery of a link between telomere-related mutations and ILD has allowed for longitudinal cohort studies of mutation carriers, yielding several observations. First, although genetic anticipation can be seen in adults from families with telomere-related gene mutations, it is unusual for clinically significant disease to develop earlier than 40 years old (17, 18). Onset of ILD appears

to be related to degree of telomere shortening, with earlier occurrence in *TERC* mutation carriers (early 50s) compared with *TERT* and *PARN* (mid to late 60s) (19). Second, radiographic abnormalities on computed tomography scan and isolated reductions in diffusion capacity may precede development of clinically significant ILD by years or even decades (20, 21). Although a typical usual interstitial pneumonia (UIP) radiographic pattern is most commonly observed, atypical features, including PPFE-like changes, centrilobular fibrosis, and upper lung fibrosis, may be found in up to 20% of patients (19, 22).

Consistent with this finding, not all patients with telomere-related mutations who develop ILD have IPF. Chronic hypersensitivity pneumonitis, unclassifiable pulmonary fibrosis, nonspecific interstitial pneumonia, and PPFE have all been described in this population (19, 23). Although the focus of this review is on ILD, telomere length also appears to be related to emphysema susceptibility, and there are several case reports of combined pulmonary fibrosis and emphysema in families with telomere-related mutations (24–26). The true prevalence of individuals with telomere-related mutations and/or shortened telomeres among combined pulmonary fibrosis and emphysema cohorts, however, remains underdescribed (27).

Telomere-related mutations exhibit incomplete penetrance, and not all individuals with relevant mutations have short telomeres or develop ILD (28). It may be that some individuals who have telomere-related mutations would have developed ILD had they been studied later in life or that inheritance of longer telomere length abrogated the impact of the telomere gene mutation (20). Regardless, it is likely that other exposures, including cigarette smoking or pulmonary toxicants such as silica, contribute to the development of ILD (24). The lack of replicative reserve may also lead to increased susceptibility to alveolar epithelial cell injury and subsequent fibrosis after viral infection and other insults (21, 29). Varying environmental exposures may be one reason telomere length in type 2 alveolar cells does not correlate well with PBMC telomere length in individuals with IPF and short telomeres (21). In addition, individuals with telomere-related mutations who carry other gene mutations or SNPs associated with ILD, such as in

Table 2. Associations between interstitial lung disease and specific telomere-related mutations

Interstitial Lung Disease	Telomere-related Mutation	References
Idiopathic pulmonary fibrosis	<i>TERT</i> , <i>TERC</i> , <i>NAF1</i> , <i>PARN</i> , <i>DKC1</i> , <i>RTEL1</i> , <i>TINF2</i>	10, 19, 22, 62, 63
Nonspecific interstitial pneumonia	<i>TERT</i> , <i>TERC</i> , <i>PARN</i> , <i>RTEL1</i> , <i>DKC1</i>	31, 37, 38, 64, 65
Acute interstitial pneumonia	<i>TERT</i>	22
Cryptogenic organizing pneumonia	<i>TERT</i>	22
Smoking-related interstitial lung disease	<i>TERT</i>	19
Unclassifiable pulmonary fibrosis	<i>TERT</i> , <i>TERC</i> , <i>RTEL1</i> , <i>PARN</i>	19, 31, 46
Pleuroparenchymal fibroelastosis	<i>TERT</i> , <i>TERC</i> , <i>RTEL1</i>	16, 19
Lymphoid interstitial pneumonia	No reports	
Hypersensitivity pneumonitis	<i>TERT</i> , <i>TERC</i> , <i>RTEL1</i> , <i>PARN</i>	19, 22, 31, 46
Combined pulmonary fibrosis and emphysema	<i>TERT</i> , <i>TERC</i> , <i>NAF1</i>	24, 25, 26

MUC5B, may be at higher risk for developing IPF (30).

Notably, individuals with short telomeres and/or telomere-related mutations have more rapid disease progression and decreased transplant-free survival (19, 31). This appears to be true regardless of a UIP, chronic hypersensitivity pneumonitis, nonspecific interstitial pneumonia, or unclassifiable pulmonary fibrosis phenotype (32, 33). Again stressing the importance of the approach to telomere length measurement, other studies have found that the number of chromosomes with shortened telomeres—as measured by Telomere Shortest Length Assay—as well as the overall mean telomere length predict pulmonary fibrosis progression (5).

Management of ILD Associated with Telomere-related Mutations

Given the potential for more rapid progression, patients with telomere-related mutations and significant ILD should be referred to a lung transplant center at the time of diagnosis to undergo appropriate testing to determine transplant candidacy (19). For individuals who are too early for transplant, who are not transplant candidates, or who are anticipated to have a lengthy wait time, consideration should be given to use of agents that may limit disease progression.

First-line therapy for individuals with short telomeres and a UIP/IPF phenotype should include the antifibrotic agents nintedanib or pirfenidone, which have been shown to limit forced vital capacity decline and improve progression-free survival (34). These individuals, particularly *TERT/TERC* mutation carriers, may not be as likely to respond to pirfenidone as those without short telomeres, but additional subgroup analyses on the basis of telomere length are ongoing (35, 36). Gastrointestinal side effects of pirfenidone do not appear to be more frequent or to impact adherence in patients with telomere-related mutations compared with those without (35). Finally, although there is some suggestion that polymorphisms in non-telomere IPF-associated genes (such as *TOLLIP*) predict response to *N*-acetylcysteine, there are no specific studies to suggest that antioxidant or immunosuppressive therapy may offer

benefit to patients with telomere mutation-related ILD (37).

Danazol, a synthetic androgenic sex hormone, has been shown to activate *TERT* transcription and telomerase enzymatic activity in hematopoietic cells. A small study showed that danazol can reduce telomere length attrition in patients with short telomeres (less than first percentile) with and without known telomere-related mutations (38). Among the seven enrolled patients with pulmonary function tests available before danazol administration, the rate of decline in diffusing capacity slowed. Studies in dyskeratosis congenita, however, have not found a benefit of androgen therapy on telomere length, suggesting that the effect of androgens on hematologic outcomes may be independent of telomere effect (39, 40). In addition, the long-term administration of these agents may be associated with hepatic toxicity, and there are case reports of worsening pulmonary fibrosis after danazol initiation and withdrawal (41, 42). We recommend that, in the absence of more conclusive evidence through ongoing clinical trials regarding the effects of danazol on lung function, use be restricted to those patients with aplastic anemia (43).

Ongoing research in the management of ILD associated with telomere-related mutations includes gene therapy to insert functional *TERT* (44); the use of agents such as dasatinib and quercetin that may induce apoptosis in senescent fibroblasts, which are implicated in pulmonary fibrosis; and regulators of the wnt pathway, such as lithium, which have been associated with increased telomere length (45).

Lung Transplantation and ILD Associated with Telomere-related Mutations

Given the relationship between telomere-related mutations and ILD and the potential for extrapulmonary organ dysfunction, including immune dysregulation, there is growing interest in the lung transplant community in the assessment and management of these patients (46). For example, George and colleagues found that among 13 patients with short telomeres and ILD referred for lung transplantation, 53% had bone marrow abnormalities, most commonly hypocellular marrow with macrocytosis and relatively normal

peripheral counts (47). Data on the impact of shortened telomeres on post-transplant outcomes, however, are mixed.

Early case series suggested that transplant recipients with known telomere-related mutations and short telomeres were more likely to have hematologic complications and acute kidney injury (48–50). There were also case reports of graft-versus-host disease in recipients with telomere-related mutations (51). In a single-center cohort study, Newton and colleagues found that ILD recipients with telomeres below the 10th percentile had higher rates of severe primary graft dysfunction, shorter time to chronic lung allograft dysfunction (CLAD), and worse survival, but no increased risk for acute or chronic renal or hepatic disease or hematologic events (52). These results, however, have yet to be validated in larger cohort studies, and average mortality in the short-telomere ILD group was similar to national benchmarks. Another study found no difference in post-transplant outcomes between recipients with and without short telomeres, with the exception of reduced rates of acute cellular rejection in the latter group (53). In addition, there are conflicting data on the impact of donor telomere length on post-transplant allograft function (53, 54). Pathologic review of lung tissue from recipients with CLAD show shorter telomere lengths than age-adjusted controls (unused donor lungs), but it is unknown whether this reflects a predisposition to CLAD related to underlying donor telomere length or if tissue-specific telomere shortening occurs in CLAD (55).

Differences in tissue tested (lung versus PBMC), telomere measurement assay used (flow-FISH versus PCR), follow-up time, pretransplant immunosuppression and post-transplant management protocols, and study populations (known telomere-related mutations vs. short telomeres regardless of mutation status) likely explain some of the discrepancies between these studies. Without clear evidence linking telomere length to post-transplant outcomes, we recommend that pretransplant screening for short telomeres among ILD candidates be limited to those whose evaluation suggests other manifestations of shortened telomeres. For example, testing should be considered in individuals with a personal history of early graying (before 30 years old), cytopenias or macrocytosis, and/or abnormal liver function tests or imaging

suggestive of hepatic impairment without other explanation; or with a family history of one or more first-degree relatives with ILD (50). Identification of short telomeres (less than 10th percentile) in PBMC should prompt screening for telomere-related mutations, particularly as individuals with these mutations and short telomeres appear to be at the highest risk for post-transplant bone marrow complications (48–50). The absence of a known telomere-related mutation, however, does not abrogate the potential impact shortened telomeres may have on the pre- and post-transplant course. Given the implications that telomere-related mutations can have for other members of a patient's family, close collaboration with medical genetics and genetic counselors is important before initiating a screening program (56).

In our opinion, the post-transplant management of candidates with telomere-related mutations and/or shortened telomeres, particularly with pretransplant hematologic manifestations, should involve careful selection of induction immunosuppression. This includes avoiding T cell-depleting agents such as antithymocyte globulin, when possible, which have been associated with increased telomere shortening and decreased telomerase activity in kidney transplant recipients (57). Close observation for leukopenia post-transplant as well as renal and hepatic dysfunction is warranted, as is reduction in the dose of cell cycle inhibitors in the setting of persistent lymphopenia. This may be particularly relevant for recipients with *TERC* mutations, who appear to be at higher risk for bone marrow dysfunction (19). There are also *in vitro* data that calcineurin inhibitors,

particularly cyclosporine, may shorten telomeres more significantly than rapamycin (58, 59). Although the impact of this potential accelerated shortening on bone marrow reserve is unknown, a trial of mammalian target of rapamycin inhibitors could be considered in recipients with persistent cytopenias to allow for lower doses of calcineurin inhibitors. Finally, there are insufficient data to recommend a trial of danazol for lung transplant recipients with telomere-related mutations and refractory bone marrow suppression, particularly given the potential increased risk of hepatic toxicity and venous thromboembolism.

One study found that rates of cytomegalovirus viremia and cytomegalovirus-related infectious complications were higher in transplant recipients with short telomeres (60). This has not, however, been found in other cohorts (52–54). The decision to extend the prophylactic course of antivirals such as valganciclovir, however, must be balanced against the potential marrow suppressive effects of these agents (53). Intermittent monitoring for Epstein-Barr virus viremia should be considered, particularly in Epstein-Barr virus mismatch recipients who display other signs of bone marrow suppression. Finally, screening for post-transplant skin cancers should be strongly encouraged in transplant recipients with short telomeres and ILD, and posaconazole or isavuconazonium should be preferentially used over voriconazole—an agent associated with increased risk of skin cancers—in situations where antifungal prophylaxis or therapy is needed (47, 61).

Conclusions

The advent of precision genomic medicine has led to an unprecedented ability to link clinical phenotype to aberrant cellular pathway and, in turn, to genetic mutation. In pulmonology, the connection between ILD, short telomeres, and telomere-related mutations has identified a group of patients at risk for more rapid disease progression and extrapulmonary manifestations of their condition. The ability to identify patients with telomere-related ILD has, however, outstripped our ability to offer specific treatments to this population. Ongoing work is needed in bringing the most sophisticated telomere measurement techniques into clinical practice and in identifying telomere lengthening or stabilizing interventions and their impact on disease development and progression. As more phenotypically asymptomatic carriers are identified because their relatives have undergone genetic testing, there will be an increasing need for longitudinal cohort data on appropriate ILD screening tests (serial chest radiographs, computed tomography scans, and/or pulmonary function tests) and intervals. Research on timing and effect of early initiation of antifibrotic therapy will also be important in guiding these conversations. Finally, a more comprehensive understanding of the impact of telomere-mutation related ILD on lung transplant management and outcomes will help refine specific post-transplant care pathways for this population. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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