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Telomere Length in Interstitial Lung Diseases

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BACKGROUND: Interstitial lung disease (ILD) is a heterogeneous group of rare diseases that primarily affect the pulmonary interstitium. Studies have implicated a role for telomere length (TL) maintenance in ILD, particularly in idiopathic interstitial pneumonia (IIP). Here, we measure TL in a wide spectrum of sporadic and familial cohorts of ILD and compare TL between patient cohorts and control subjects.

METHODS: A multiplex quantitative polymerase chain reaction method was used to measure TL in 173 healthy subjects and 359 patients with various ILDs, including familial interstitial pneumonia (FIP). The FIP cohort was divided into patients carrying *TERT* mutations, patients carrying *SFTPA2* or *SFTPC* mutations, and patients without a proven mutation (FIP-no mutation).

RESULTS: TL in all cases of ILD was significantly shorter compared with those of control subjects (P range: .038 to $<$.0001). Furthermore, TL in patients with idiopathic pulmonary fibrosis (IPF) was significantly shorter than in patients with other IIPs ($P = .002$) and in patients with sarcoidosis ($P < .0001$). Within the FIP cohort, patients in the FIP-telomerase reverse transcriptase (TERT) group had the shortest telomeres ($P < .0001$), and those in the FIP-no mutation group had TL comparable to that of patients with IPF ($P = .049$). Remarkably, TL of patients with FIP-surfactant protein (SFTP) was significantly longer than in patients with IPF, but similar to that observed in patients with other sporadic IIPs.

CONCLUSIONS: The results show telomere shortening across all ILD diagnoses. The difference in TL between the FIP-TERT and FIP-SFTP groups indicates the distinction between acquired and innate telomere shortening. Short TL in the IPF and FIP-no mutation groups is indicative of an innate telomere-biology defect, while a stress-induced, acquired telomere shortening might be the underlying process for the other ILD diagnoses. CHEST 2015; 148(4):1011-1018

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ABBREVIATIONS: COP = cryptogenic organizing pneumonia; CTD-ILD = connective tissue disease-associated interstitial lung disease; FIP = familial interstitial pneumonia; HP = hypersensitivity pneumonitis; IIP = idiopathic interstitial pneumonia; ILD = interstitial lung disease; iNSIP = idiopathic nonspecific interstitial pneumonia; IPF = idiopathic pulmonary fibrosis; PCR = polymerase chain reaction; SFTP = surfactant protein; SR-ILD = smoking-related interstitial lung disease; TERT = telomerase reverse transcriptase; TL = telomere length; T/S = telomere/single-copy gene

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Interstitial lung diseases (ILDs) are a group of diseases that primarily affect the pulmonary interstitium. Although ILD is a heterogeneous group of diagnoses, they are classified together based on similar clinical, radiologic, physiologic, or pathologic features.[1] Four groups are distinguished within ILD: diseases with a known cause, idiopathic interstitial pneumonias (IIPs), granulomatous diseases, and a miscellaneous group.[2] The etiology of a number of ILDs is unknown, which presents limitations to classification and, hence, to treatment.[3] Therefore, it is important to investigate which features are common and which are unique in ILD.

Multiple ILDs have been associated with short telomere length (TL).[4-6] Telomeres protect genetic information by acting as a buffer against the chromosomal shortening that is inherent to cell growth. Critical shortening of the telomeres leads to cell-cycle arrest. Maintaining TL is necessary, therefore, for ongoing cell proliferation.[7] Loss in TL can be restored by the ribonucleoprotein telomerase. The relevance of telomere biology in ILD was first discovered in IIP. Patients with familial disease were found to carry mutations in the telomere maintenance genes *TERT* and *TERC*.[8,9] These patients also had distinctly short telomeres in their blood cells. Next, a cohort of patients with IIP who did not carry these mutations, both familial and sporadic, was also shown to have shorter telomeres, compared with control subjects. A significant portion of these patients who did not carry the mutations had TL below that of the 10th and even below the first percentile of control subjects.[4,6] Telomere biology-related genetic factors are suggested to underlie telomere shortening in these patients. This suggestion is underlined by discoveries of familial disease causing mutations in other telomere biology-related genes beside *TERT* and *TERC*, such as *DKC1* in the telomerase complex, *TINF2* in the shelterin complex, and *RTEL1*, which interacts with the shelterin complex.[10-12] Associations with short TL have also been described in other diseases, such as asthma, COPD,[13,14] cardiovascular disease,[15] and cancer.[16] Therefore, it is important to distinguish between genetically predisposed, innately telomere-related diseases and diseases in which short telomeres are acquired due to increased oxidative stress, inflammation, or accelerated cell turnover.[17] It has been suggested, therefore, that the degree of difference in TL between healthy control subjects and patients determines if the short telomeres reflect acquired stress states or innate, telomere-driven, degenerative changes.[18]

We hypothesized that measuring TL in a broad selection of ILD diagnoses would allow us to identify ILD diagnoses with an innate telomere-related pathobiology. Therefore, we measured TL of peripheral blood cells in healthy control subjects and in seven different ILD diagnoses. Subsequently, we determined the degree of difference between healthy control subjects and ILD. We also assessed differences in TL among ILDs and, in particular, among the different forms of IIP.

In familial IIP, also called familial interstitial pneumonia (FIP), it has been found that a diagnosis of idiopathic pulmonary fibrosis (IPF) is most frequent, but all subtypes of IIP can be present.[19] In this group, two classes of disease-causing mutations can be distinguished. Besides the telomerase-related mutations, there are mutations in surfactant proteins (SFTPs) that are known to cause FIP.[20,21] The surfactant mutations cause endoplasmic reticulum stress, which can lead to pulmonary fibrosis.[22] An effect of these mutations on TL, however, has never been investigated. To further explore TL in ILD, we subdivided the FIP cohort into three groups—those with the SFTP mutation (FIP-SFTP), those with telomerase reverse transcriptase (TERT)-related mutations (FIP-TERT), and those who did not carry a mutation (FIP-no mutation)—and compared these to the nonfamilial ILD data.

Materials and Methods

Patients and Control Subjects

A total of 359 patients diagnosed with ILD at the Department of Pulmonology of the St. Antonius Hospital in Nieuwegein were retrospectively included in this study. The patients were diagnosed with IPF, idiopathic nonspecific interstitial pneumonia (iNSIP), cryptogenic organizing pneumonia (COP), smoking-related ILD (SR-ILD), hypersensitivity pneumonitis (HP), sarcoidosis, connective tissue disease-associated ILD (CTD-ILD), or FIP. Diagnosis was made in accordance with international guidelines.[2,23-25] For IIP cases with coexisting patterns, multidisciplinary discussion determined the clinical significance of the individual patterns.[25] FIP was defined as two or more first-degree family members with IIP and was documented in 67 patients in 49 different families, and 18 affected family members. Upon first visit to the outpatient clinic, patients are asked to fill out a questionnaire regarding familial disease status. In case of a positive anamnesis for familial disease, the possibility of FIP and retrieval of further medical information were discussed by the respective physician. Fourteen of the 49 families have been described by van Moorsel et al[21] as "[familial pulmonary fibrosis] FPF 1-10, 15, and 17-19." A histopathologic pattern of usual interstitial pneumonia was present in these patients, sometimes with coexistence or superimposition of other patterns, as is also known from other FIP reports.[8,19] Patients with FIP had been screened for mutations on all exons of the genes *TERT*, *TERC*, and *SFTPC*, and on exon 6 of *SFTPA2*. Mutation-carrying familial patients were classified as a separate group and subdivided in SFTPC-C or -A2 carriers (FIP-SFTP) and telomerase mutation carriers (FIP-TERT). No mutations were found in *TERC*. The remaining FIP cohort, therefore, consisted only of patients without an identified mutation (FIP-no mutation). The control subjects