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Genetic Aspects of Pulmonary Fibrosis

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Abstract

Pulmonary fibrosis is a broad term for a group of lung diseases, idiopathic or familial, that results in fibroblasts and excess tissue in the lungs. Because it is generally seen in multiple generations of families, it is referred to as familial pulmonary fibrosis. The genetic aspects underlying the disease is unclear; however, there are a plethora of proposed genetic mechanisms that have been studied. The most commonly proposed genetic mechanisms, involving telomere shortening and surfactant overproduction, will be discussed in this review.

Introduction

Pulmonary fibrosis (PF) describes a group of interstitial lung diseases that result in the accumulation of fibroblasts and tissue in the lung. PF often results from environmental exposures, such as drug toxicity and inhalation of fibrogenic dust, or systemic diseases such as connective tissue disorders, or as a sporadic disease¹. Idiopathic pulmonary fibrosis (IPF) is a progressive, fatal disease, categorized under the pulmonary fibrosis designation, resulting in scarring of the lungs due to unknown reasons^{1,2}. Prognosis of the disease is generally poor, with those diagnosed living an average of 2-5 years after diagnosis.

Mortality results from progressive, unresolved scarring of the lungs that leads to respiratory insufficiency¹. IPF typically presents for the first time in individuals over 50 years of age, and 75 percent of people with IPF are males who currently or previously smoked cigarettes². Even though the cause of the

disease is mostly unknown, up to 20 percent of diagnosed individuals have a family member with the disease and thus the disease is referred to as familial pulmonary fibrosis (FPF)^{1,2}.

Diagnosis of pulmonary fibrosis can be challenging as symptoms resemble many respiratory illnesses, and conventional testing may not show any visible abnormalities until the disease has progressed. Typical in pulmonary fibrosis patients is a “honeycomb” lung [Fig1], a distinctive pattern seen on computerized tomography (CT) scans^{1,3}. The appearance of the honeycomb lung shows empty pockets (bubbles) where solid lung tissue should be³. Not only is diagnosis of FPF difficult, so is treatment of the disease.

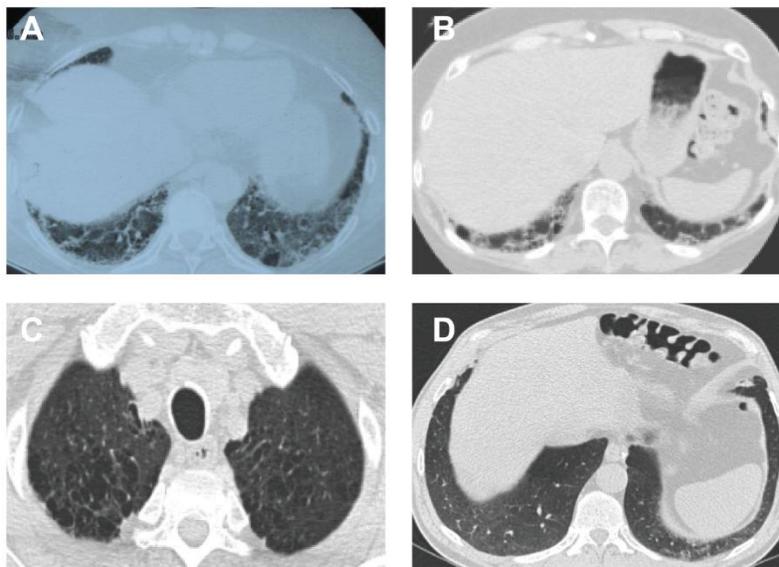


Figure 1. A,B show a typical computerized tomography (CT) scan displaying the characteristic “honeycomb” pattern observed in individuals with pulmonary fibrosis. C,D show the typical lung in a healthy adult without pulmonary fibrosis.

There are no treatments (*e.g.*, medications or procedures) that resolve or even markedly improve FPF. Current treatments are used to slow the progression of lung scarring however they typically do not lessen the major symptoms of the disease- dyspnea and chronic cough². As the scarring in the lungs worsens, symptoms increase in intensity and can have an adverse effect on day-to-day activities such as showering, eating, dressing, and walking. Unique to the disease are the abnormalities presented in pulmonary function tests, radiographs, and biopsies (*e.g.*, honeycomb pattern)³.

The inheritance pattern of the disease is most consistent with autosomal dominant with incomplete penetrance, meaning individuals with an implicated genotype may or may not show the disease^{1, 4, 5}. One common hypothesis is that telomere shortening, related to mutations in various genes, precipitates the onset of pulmonary fibrosis. Important to this effect is telomerase, a DNA polymerase that synthesizes new telomere repeats onto chromosomal ends⁴. Two important parts of this process include *TERT*, a reverse transcriptase, and *TERC*, a telomerase RNA component. *TERT* copies the template *TERC*, undergoes translocation, and then adds the successive repeats onto chromosomal ends⁵. Mutations in either *TERT* or *TERC* are the cause of about 8-15 percent of familial pulmonary fibrosis cases⁵.

A small percentage of individuals with pulmonary fibrosis that have short telomeres also suffer from premature aging syndrome designated as dyskeratosis congenita which also results in aplastic anemia—destruction of bone marrow⁴. Pulmonary fibrosis and dyskeratosis congenita both share similar pathophysiology in that several of the same telomere mutations have been identified in other genes including *TERT*, *TERC*, *DKC1*, *TINF2*, and *RTEL1*^{1,4,6-8}. The connection between the two diseases further provides evidence linking telomeropathy and FPF pathogenesis.

FPF has also been linked to mutations in another main group of genes: surfactant metabolism genes. These genes include *SFTPC* (surfactant protein C), *SFTPA2* (surfactant protein A2) and *ABCA3* (ATP-binding cassette subfamily A, member 3)^{1,9-12}. More specifically, *SFTPC* mutations have been frequently identified in children with idiopathic pneumonias. Genome sequencing and analysis revealed that half of the cases were sporadic and the other half were inherited from parents. Analysis revealed an autosomal dominant with incomplete penetrance, congruent with how FPF is inherited¹².

Another common mutation closely linked to FPF occurs in the gene encoding mucin 5B (*MUC5B*). Extensive genome-wide sequencing identified a single nucleotide polymorphism (SNP) in the promoter region of *MUC5B* in some individuals with FPF¹³. The role of mucins, glycosylated proteins in mucus, is to give mucus its viscoelastic properties¹³. Mucus is important in trapping inhaled particles and removing

them via coughing or ciliary exportation processes. It has been shown in several studies that overproduction of *MUC5B* interferes with alveolar repair and reduces lung clearance, thus potentially leading to FPF¹³.

In summary, there is substantial evidence that FPF is caused by mutations in either surfactant metabolism genes or mutations causing premature telomere shortening. The following sections will discuss evidence for each of these hypotheses by reviewing studies that specifically examine some of the major mutations identified in *RTEL1*, *TERT*, *MUC5B*, and *SFTPC*. Novel mutations in *PARN* and *ZCCHC8* will also be discussed.

***RTEL1* Mutation**

A major mutation associated with premature telomere shortening that has recently emerged is in the regulator of telomere elongation helicase 1 (*RTEL1*) gene. *RTEL1* is an ATP-dependent DNA helicase, which plays a major role in DNA replication, genome stability, DNA repair, and telomere maintenance¹. Several studies have focused on the presence of heterozygous *RTEL1* mutations in individuals diagnosed with FPF. In one particular study, that has been cited in a plethora of reviews on FPF, whole-exome sequencing revealed heterozygous *RTEL1* mutations in individuals diagnosed with FPF, thus solidifying the hypothesis that *RTEL1* mutations play a role in the onset of FPF^{1,8}.

Kannengieser, *et al.* (2015) conducted whole-exome sequencing analysis of 47 patients (35 male) from 35 separate families, whom had diagnosed FPF, and found no mutations in the typical genes associated with premature telomere shortening (*e.g.* *TERT* and *TERC*) or surfactant protein mutations (*e.g.* *SFTPC*)¹. Heterozygous *RTEL1* mutations were identified in four separate families with FPF. Patients with the *RTEL1* mutation were compared to healthy, age-matched cohorts (n=13)¹. Each individual studied was a male smoker. Missense mutations comprised three of the four *RTEL1* mutations while the other was due to a duplication mutation¹. The duplication mutation led to a frameshift and caused a premature stop codon¹. Structural modelling revealed that two of the missense mutations were likely to disrupt DNA binding or ATP hydrolysis¹. Individuals with the *RTEL1* mutation also showed decreased telomere length

compared to the healthy cohorts, suggesting that the mutation induces premature telomere shortening¹. This study suggested that heterozygous mutations in *RTEL1* are a potential genetic basis for FPF.

RTEL1 mutations were linked to telomere length in a study done by Stuart *et al.* (2015) that was similar to Kassengiesser *et al.* (2015) study. Stuart *et al.* (2015) found that probands with *RTEL1* mutations demonstrated higher percentages of short telomere lengths than the control⁸. Telomere length was also measured in this study and, similar to the previously mentioned study, it was noted that the mean telomere lengths of related individuals, who did not inherit the *RTEL1* mutation, were shorter than those of unrelated individuals. However, the mean telomere lengths of the related individuals were longer than those affected by the *RTEL1* mutation⁸. The findings from these two studies suggest that *RTEL1* gene mutations play a significant role in telomere shortening, largely by missense mutation, resulting in the onset of FPF.

***TERT* Mutation**

Mutations in *TERT* apparently underlie inheritance of FPF in 8-15% of cases⁵. Telomerase reverse transcriptase, *TERT*, is one of two essential components that makes up telomerase, a ribonucleoprotein that synthesizes telomere DNA⁵. Several studies have suggested that heterozygous mutations in *TERT* cause telomere shortening via haploinsufficiency⁵. As discussed previously, a mutation in *TERT* is also found to cause dyskeratosis congenita, a disorder in which 20% of affected individuals develop FPF⁴. The link between *TERT* and FPF has been observed in several studies.

In a study by Tsakiri *et al.* (2007), 46 individuals, with a family history of FPF were identified and their genes sequenced for *TERT* mutations and five missense mutations were identified. As expected, these mutations produced significantly decreased levels of telomerase activity⁴. Compared to healthy family members, the mean telomere length was significantly shorter for those individuals with heterozygous *TERT* mutations. When comparing heterozygous affected individuals to their asymptomatic carrier family members, telomere length was considerably shorter in the affected individuals⁴. This suggests that

individuals with *TERT* mutations show a shortened telomere length and decreased levels of telomerase, when compared to healthy individuals as well as healthy family members.

Another study showed similar results to the study discussed previously. Alder *et al.* (2011) studied two families with a history of FPF present and observed a pattern of shortened telomere length in individuals affected by *TERT* mutations compared to related, healthy individuals⁵. In six of nine individuals with the *TERT* mutation, telomere length fell below the first percentile, a range specific for the presence of telomerase maintenance defects⁵. In four related individuals, telomere length fell below the 10th percentile while two individuals fell below the first percentile, indicating *TERT* mutations were the cause of shortened telomere length⁵. Affected individuals with *TERT* mutations showed an overall decrease in telomerase activity, thus telomere shortening was expected. These results suggest that heterozygous *TERT* mutations are a potential genetic cause of the onset of FPF.

***MUC5B* Mutation**

Mucins (*e.g.* *MUC5B*) are glycosylated proteins responsible for giving mucus its viscoelastic properties. Mucus is important in removing debris and trapped particles, including bacteria, dying epithelial cells, and leukocytes¹³. *MUC5B* is present most abundantly in the distal airways of the respiratory tract, and since IPF presents with abnormalities in the distal airway it would make sense that a mutation in *MUC5B* could contribute to the development of IPF. Dual immunofluorescence analysis of *MUC5B* showed that in 80% of patients with IPF there was an overabundance of *MUC5B* cells present in the distal airways. Further evidence supporting *MUC5B* mutations is that epithelial cells that express *MUC5B* are the dominant mucin-expressing cell type in microscopic honeycomb cysts, which are filled with *MUC5B* protein¹³.

Overexpression of *MUC5B* would result in excess mucus, and this has been the proposed mechanism contributing to IPF. Specifically, excess *MUC5B* is thought to cause the development of IPF resulting from excessive lung injury and non-repair¹³. Excess mucus causes the onset of many respiratory

disease, including chronic obstructive pulmonary disease (COPD), asthma, and cystic fibrosis (CF), so it is entirely plausible that excess mucus could contribute to the onset of IPF. The mechanism of action is unclear, but several hypotheses have been proposed in multiple studies.

The first hypothesis is that because *MUC5B* reduces mucosal host defense and lung clearance of inhaled particles and debris which may lead to scar tissue formation which is characteristic of IPF¹³. A risk factor identified with IPF is cigarette smoking, so it is reasonable to assume that inhaled particles from the smoking might cause defects in mucosal host defense and may lead to formation of scar tissue. The second hypothesis is that excessive *MUC5B* interferes with alveolar repair¹³. Alveolar type II epithelial cells expand after injury to help repair damage, but with excess *MUC5B*, this process may be impaired. The proposed mechanism is that *MUC5B* potentially interferes with interactions between alveolar cells and the matrix or interferes with surfactant properties¹³.

Overall, it is suggested that excessive *MUC5B* in the distal airways enhances injury and disrupts repair of alveoli. The two proposed hypotheses could even potentially both contribute to IPF. The probable role of *MUC5B* in interfering with surfactant properties could link to the much-studied *SFTPC* mutation that is found in several cases of FPF.

***SFTPC* Mutation**

Surfactant protein C mutations (*SFTPC*) are one of the more commonly found in surfactant metabolism genes. *SFTPC* is a hydrophobic protein, exclusively produced by alveolar type II epithelial cells^{11,12}. Surfactant protein C enhances the surface tension-reducing capacities of alveolar fluid and is dependent on *ABCA3*¹¹. Surfactant protein C is synthesized by alveolar type II cells, allowed to mature, and then are released into the alveoli with other proteins and phospholipids^{11,12}. Mature surfactant protein C is stored and then secreted into the alveolar space. Once in the lung, surfactant protein C is expressed only in alveolar type II epithelial cells^{11,12}. Surfactant protein C has domains called BRICHOS, which auto-

protect the peptide from aggregation and 75% of all mutations reported in *SFTPC* are in a BRICHOS domain¹². One particular study focused on the potential link between BRICHOS and *SFTPC*.

Ono *et al.* (2011) studied three generations of a Japanese family, in which six of the individuals were diagnosed with FPF. In order to determine the potential genetic link, this group sequenced the patients' *SFTPC* and *ABCA3* genes¹². Sequencing revealed a novel heterozygous mutation in the BRICHOS domain of the *SFTPC* gene while no mutations were present in the *ABCA3* genes. Western blotting comparing the normal and mutant *SFTPC* genes showed an increased amount of surfactant C protein compared to the normal genes as well as increased expression of unfolded protein response proteins and chaperone proteins¹². These proteins were then shown to be upregulated. The results suggest that mutations in the BRICHOS domain of the *SFTPC* gene lead to apoptosis which then injured type II alveolar epithelial cells, inducing FPF¹².

Mutant *SFTPC* genes were the focus of a Dutch study as well in which 22 unrelated patients with FPF were identified from a cohort of 229 patients with idiopathic pulmonary fibrosis (IPF)¹¹. *SFTPC* was sequenced in 20 of the patients with FPF and in 20 patients with IPF¹¹. Sequencing of *ABCA3* was also performed in the patients with FPF. In 5 of the 20 unrelated patients with FPF, a deletion mutation in *SFTPC* was detected while no mutations were detected in the 20 patients with IPF¹¹. Two variant mutations, resulting in amino acid substitutions, were found in *ABCA3* genes of adult patients with FPF.

CT scans revealed the typical pattern of interstitial pneumonia and nodular septa thickening along with multiple lung cysts [Fig 2]—this is often typical of adult *SFTPC* mutation carriers¹¹. The results indicate that mutations in *SFTPC* were the major cause of FPF development in the cohort. While *RTEL1*, *TERT*, and *SFTPC* are relatively known mutations associated with FPF, more novel mutations, such as *PARN* and *ZCCHC8*, have also been investigated.

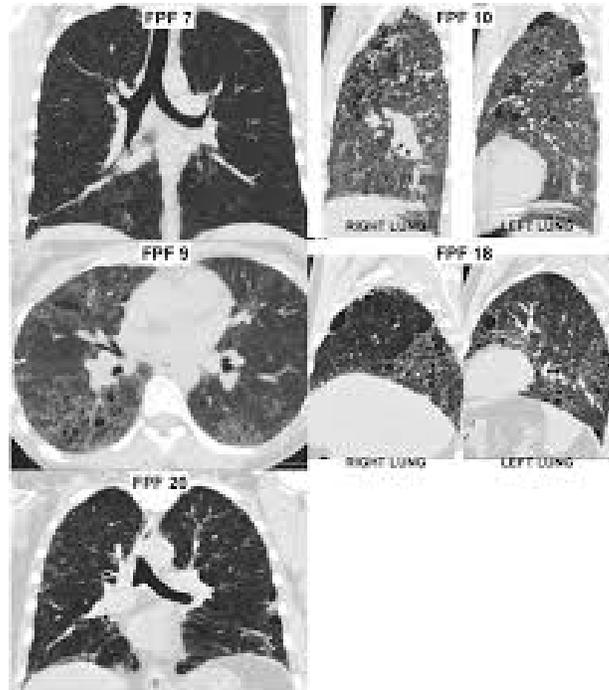


Figure 2. Computerized tomography (CT) scans of adult, SFTPC mutation carriers shown above.

***PARN* Mutation**

PARN, encoding polyadenylation-specific ribonuclease deadenylation nuclease, is a 3' exoribonuclease, not previously implicated in disease or telomere maintenance, but has recently been connected to the potential onset of FPF⁸. In a 2015 study, *PARN* was shown to have six damaging variants in individuals with FPF, but zero mutations in the controls⁸. *PARN* mutations in this same study were shown to act similar to *RTEL1* mutations in that six out of the seven individuals with either mutation showed a shortened telomere length compared to the control and their relatives⁸. The *PARN* mutation is a relatively understudied mutation, so while the results from the 2015 study show support for the *PARN* mutation in the onset of FPF, the mechanism is unclear. However, *PARN* has been shown to assist in the maturation of snoRNAs which are known to associate with four proteins, three of which have mutations in the genes encoding them, causing dyskeratosis congenita⁸.

***ZCCHC8* Mutation**

Another novel mutation recently discovered as a potential genetic cause of FPF involves a mutation in *ZCCHC8* which encodes for a zinc-knuckle containing protein. In terms of its mechanism, *ZCCHC8* is largely associated with *TERC*, another gene shown to cause an estimated 15% of FPF cases¹⁴. A study conducted by Gable *et al.* (2019) showed that the link between *ZCCHC8* and *TERC* plays a pivotal role in causing FPF. The study focused on an individual with low levels of *TERC*, telomere shortening, and a family history consistent with PF¹⁴.

Functional testing of *ZCCHC8* revealed that all three mutation carriers had 50% lower *ZCCHC8* protein levels compared to the control¹⁴. CRISPR/Cas9 editing was used to detect the effect of *ZCCHC8* on *TERC*, and the results showed that decreased levels of the mutated gene corresponded to decreased levels of *TERC*¹⁴. Using telomere repeat amplification protocol, telomerase activity was measured. The *ZCCHC8*-deficient cells showed significantly decreased activity, thus collective the data indicates that the gene is necessary for *TERC* maturation and for proper telomerase functioning¹⁴.

Conclusion

While the genetics contributing to familial pulmonary fibrosis are partially known, there are still a plethora of mutations that could underlie the onset of the disease. The importance of understanding the genetics behind the disease is vital because there are no known treatments for FPF. The onset of the disease comes on rapidly and the disease seems to progress remarkably quickly after diagnosis. Because the symptoms can resemble most respiratory illnesses, it is important to understand the characteristic patterns observed in FPF—nodular thickening, especially in the case of surfactant mutations, interstitial pneumonia, and in a majority of cases, honeycomb lung. Future research should focus on the mechanisms underlying how mutations such as *MUC5B* and *RTEL1* affect other cells causing development of FPF.

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