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Telomeres revisited: *RTEL1* variants in pulmonary fibrosis

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For decades, it has been recognized that cases of pulmonary fibrosis cluster in families. While previous studies have implicated mutations in several surfactant-related proteins[1-3] and telomere related genes[4-12], in most families the genetic basis of their disease has remained uncertain[13]. In this issue of the *European Respiratory Journal*, Kannengiesser and colleagues report that rare genetic variants in regulator of telomere elongation helicase (*RTEL1*) are associated with familial pulmonary fibrosis (Familial Interstitial Pneumonia, FIP) [14]. Coupled with two other recent reports[7, 10], this work suggests that mutations in *RTEL1* represent an important genetic cause of pulmonary fibrosis, responsible for disease in approximately 6% of families.

The authors selected 35 families from their registry of more than 170 kindreds without known telomerase reverse transcriptase (*TERT)* or telomerase RNA component (*TERC)* mutations and performed whole exome sequencing of genomic DNA from one or two affected individuals. Given the small size of families and limited number of subjects sequenced, standard statistical association testing was not feasible, thus the authors prioritized rare (minor allele frequency, MAF <0.01) genetic variants in genes related to telomere biology. As anticipated, no variants in *TERT* or *TERC* were identified; in addition, the authors did not identify rare variants in other FIP-associated telomere genes TERF-interacting factor 2 (*TINF2)*[5, 8, 12] or dyskerin (*DKC1)*[4, 9]. In four families, rare variants in *RTEL1* were identified, including three missense and one nonsense mutation. The identified *RTEL1* variants segregated with disease within each family and were associated with short peripheral blood telomeres. The authors then used structural modeling techniques which suggest two of the missense variants in the helicase domain likely disrupt DNA-binding (R213W) or ATP-hydrolysis (T49M); the impact of F964L, which lies in a harmonin-N domain was less certain but may alter protein folding.

As indicated by the authors, the small size of each kindred suggests that there are likely many other rare variants shared between affected individuals in each family. In addition, functional testing of these *RTEL1* variants will be required to strengthen evidence of pathogenicity. Nonetheless, in light of the strong relationship between telomere biology and lung fibrosis, genetic variation in *RTEL1* appears to be another important contributor to risk for pulmonary fibrosis.

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The biology of *RTEL1* is only beginning to be elucidated, however it is apparent that RTEL1 plays a role in a variety of fundamental cellular mechanisms related to genome stability, replication and repair[15]. Common genetic variants in *RTEL1* have been associated with peripheral blood telomere length [16] and risk for glioma [17] by genome-wide association studies. Homozygous and/or compound heterozygous *RTEL1* mutations were first identified in Hoyeraal-Hreidarsson Syndrome[18-22], including F964L. FIP-associated *RTEL1* variants localize to both the helicase domain, critical for T-loop disassembly, as well as the C-terminus where a harmonin-N domain and PCNA interacting region are found.

The fundamental mechanism underlying the pathogenesis of lung fibrosis caused by telomere-pathway mutations has so far remained elusive. It has been commonly suggested that progressive telomere shortening in type 2 alveolar epithelial cells (AEC2's) induces premature senescence and loss of regenerative capacity, however direct evidence for this hypothesis is limited. The lifespan and turnover of AEC2s in humans is not known; lineage tracing models in mice suggest turnover of the alveolar epithelium occurs slowly[23] (in comparison to the skin or gut epithelium), thus gradual telomere shortening through rounds of successive cell division in AECs seems insufficient to explain the profound telomere shortening found in IPF lungs, nor why a pulmonary phenotype is frequently the first manifestation of telomere pathway mutations. Modeling telomerase mutations in animals has proven challenging, as even late generation *Tert* and *Terc* null mice fail to recapitulate the lung phenotype associated with human telomere pathway mutations[24, 25]. Activation of a DNA-damage response in the alveolar epithelium by inducible deletion of a shelterin complex component (telomere-repeat binding factor-2, *Trf2*) led to impaired AEC proliferation, differentiation, and increased sensitivity to bleomycin[26]. While intriguing, the direct relevance of this model to heterozygous human telomere pathway mutations is not entirely clear. Nonetheless, it is apparent that there is an important relationship between telomere biology and lung fibrosis; further study of *RTEL1* and other FIP-associated telomere pathway genes may shed new light on this question.

The authors report numerous extrapulmonary phenotypes, including skeletal abnormalities, liver disease, and hematopoietic abnormalities. Liver disease was also identified in another FIP kindred carrying an *RTEL1* rare variant[10]. Bone marrow dyscrasias, skeletal abnormalities and cirrhosis have previously been observed in families with dyskeratosis congenita caused by to other telomere pathway mutations[27, 28]. Interestingly, a common genetic variant in *RTEL1* was also recently identified as a susceptibility locus for osteoporosis[29].

With a growing understanding of the genetic basis of FIP, numerous critical questions are emerging that we propose will require broad collaboration within the pulmonary fibrosis community to answer:

- Do patients with *RTEL1* variants have similar clinical courses to other FIP patients? Kannengiesser and colleagues report indicates disease onset may be earlier that in other FIP patients, although other reports suggest a pattern similar to FIP in general[7, 10].

- Do patients with *RTEL1* and/or telomerase pathway variants respond to IPF treatments including pirfenidone and nintedanib? The role of genetic predictors of therapeutic response had been virtually unexplored to date.
- Is lung transplantation safe in patients with *RTEL1* mutations? Several reports suggested *TERT* mutation carriers are at increased risk for a variety of transplant-related complications including myelosuppresion and renal failure[30, 31]. Similar challenges may be encountered in patients with *RTEL1* variants.
- With evidence of pleiotropic pulmonary and extrapulmonary presentations of FIP-associated genetic variants, what are the critical determinants of the clinical phenotype in an individual? It seems likely that additional genetic or environmental “second hits” are likely required. Answering this question will require thoughtful epidemiologic study in addition to *in vitro* and *in vivo* modeling.
- Should peripheral blood telomere length testing be performed routinely in patients with FIP? PBMC telomere length may carry prognostic significance[32], and could influence decisions regarding genetic testing.
- Should genetic testing for FIP-associated variants become routine? As it becomes clearer as to whether specific genetic variants confer prognostic or therapeutically relevant importance, we suggest this should be performed in coordination with genetic counselors[13].
- Should family members of patients with known FIP-associated genetic variants undergo routine screening for lung disease? In our experience, 15-25% of asymptomatic family members aged 50 or greater have evidence of interstitial changes on high resolution CT scan[33]. With the emergence of effective treatments for disease, a strong case for early disease detection can be made.

The evolving genetic evidence continues to implicate telomere biology as central to risk of pulmonary fibrosis. While many more questions lie ahead, identification of *RTEL1* as a pulmonary fibrosis gene provides another important piece of the puzzle of FIP genetics.

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