

Compound heterozygous mutation of RTEL1 in interstitial lung disease complicated with pneumothorax and emphysema: A case report and literature review

Man Luo^{1,2}  | Jiao-Li Wang^{1,2,3,4}

¹Department of Respiratory Medicine, Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine, Hangzhou, China

²Department of Translation Medicine Center, Key Laboratory of Clinical Cancer Pharmacology and Toxicology Research of Zhejiang Province, Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine, Hangzhou, China

³The Fourth Clinical Medical College of Zhejiang Chinese Medical University, Hangzhou, China

⁴Zhejiang University Cancer Center, Hangzhou, China

Correspondence

Man Luo, Department of Respiratory Medicine, Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine, #261 Huansha Road, Hangzhou, Zhejiang Province, China.
Email: luoman926@163.com

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Abstract

Interstitial lung diseases (ILDs) are common respiratory diseases with limited treatment options and poor prognoses. Early and accurate diagnosis of ILD is challenging and requires a multidisciplinary discussion. We report a 32-year-old patient admitted to our hospital with cough and increasing dyspnea on exertion. Computerized tomography scan of his chest demonstrated diffuse interstitial abnormalities, emphysematous changes, and a pneumothorax. Whole-exome sequencing (WES) and Sanger sequencing indicated a compound mutation of heterozygosity in RTEL1 gene c.2992C > T(p.Arg998*) and c.482T > C(p.Val161Ala). In-silicon analysis revealed the pathogenic nonsense mutation c.2992C > T, which introduced a premature stop codon in exon 30 of RTEL1. The patient is still alive with progressive dyspnea to now. We reviewed the pathophysiology of ILD patients carrying RTEL1 mutations and the roles of RTEL1 mutation in guiding treatment and prognostication in ILD.

KEYWORDS

homozygous mutation, interstitial lung disease (ILD), RTEL1, whole exome sequencing

INTRODUCTION

Interstitial lung disease (ILD) represents a large and heterogeneous group of pulmonary disorders. Idiopathic pulmonary fibrosis (IPF), the most common of the idiopathic interstitial pneumonias, is a chronic and progressive disease which is associated with high morbidity and early mortality.¹ There is accumulating evidence^{2–8} that pirfenidone and nintedanib are equally efficacious in slowing disease progression in IPF. Given these promising results, early and accurate diagnosis of ILDs could potentially improve outcomes through early initiation of appropriate management and timely referral for lung transplantation.⁹

The past decade has witnessed the emergence of rare genetic variants responsible for the genesis and development of ILD. Among rare genetic mutations, the heterozygous regulator of telomere elongation helicase 1 (RTEL1) mutations has been evidenced in 5%–9% of familial ILD by three independent research groups.^{10–12} RTEL1 is essential in telomere-length regulation, DNA repair, and the maintenance of genomic stability.¹³ Mutations of RTEL1 are related to pulmonary fibrosis including familial and idiopathic pulmonary fibrosis.¹⁴ However, the age at onset, symptoms, disease severity and prognosis of ILD with RTEL1 mutations are highly variable.

In this study, we report a novel compound heterozygous mutation in the RTEL1 gene (c.2992C > T and c.482T > C)

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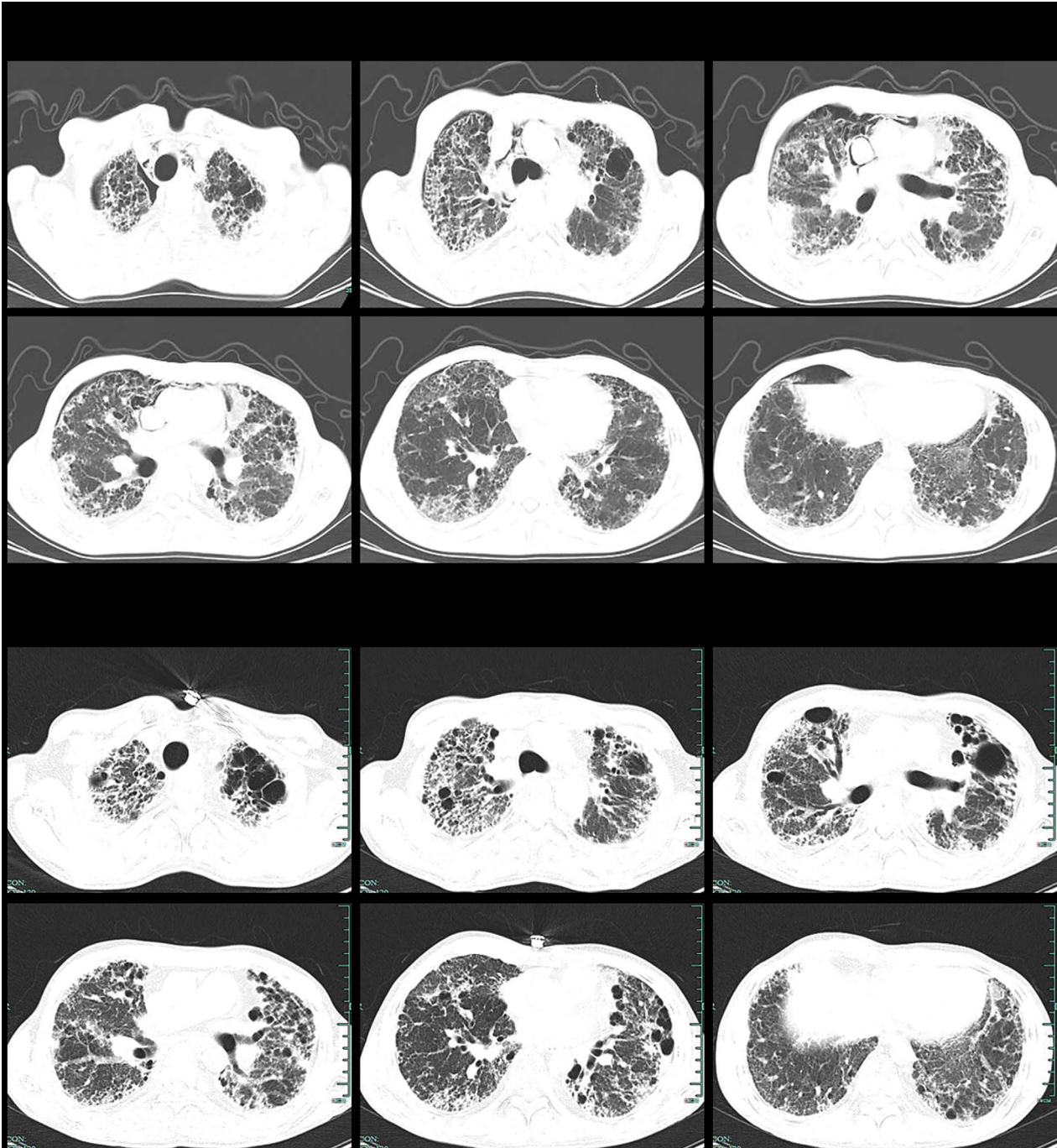


FIGURE 1 Serial chest CT images at different timings. (A) Initial chest CT scan (27 February 2021) showed diffuse bilateral reticular abnormalities, a pneumothorax in the right, bilateral pleural thickening, and mediastinal emphysema. (B) Chest CT scan three-month post-initial (4 June 2021) showed improved, ongoing diffuse interstitial inflammation tending to be chronic and fibrotic combined with pulmonary emphysema. CT, computed tomography

in the proband, which was associated with fibrotic interstitial abnormalities and emphysematous changes.

CASE REPORT

A 32-year-old man presented to the pulmonary clinic with 12 months of cough and 3 months of increasing exertional dyspnea in late February 2021. Four days before this

presentation, he noted an abrupt worsening of dyspnea and right-sided chest pains. He had no history of atopy, parasitic infection, malignancy, and immune dysregulation. He had no known drug allergies. He lived in an apartment in an urban area of Hangzhou and had not travelled outside for many years. He smoked 10 cigarettes/day for 12 years but did not drink alcohol, or use illicit drugs. His mother died of lung cancer at 41 years old. His father and two sisters were healthy.

TABLE 1 Overview and bioinformatic prediction of the compound RTEL1 mutation

Genomic position on chr.	Exon/ CDS	cDNA change	Protein change	Hom /Het	Mutation type	1000 genome	ExAC	Gnom AD	Mutation taster
chr20:6232 4564	EX30 /CDS 29	NM_032957.4: c.2992C > T	p.Arg998*	Het	Nonsense	Not found	0.000017	0.000028	Pathogenic
chr20:6229 3913	EX5/CDS 4	NM_032957.4: c.482T > C	p.Val161Ala	Het	Missense	Not found	Not found	Not found	VUS

Abbreviation: VUS, variants of uncertain significance.

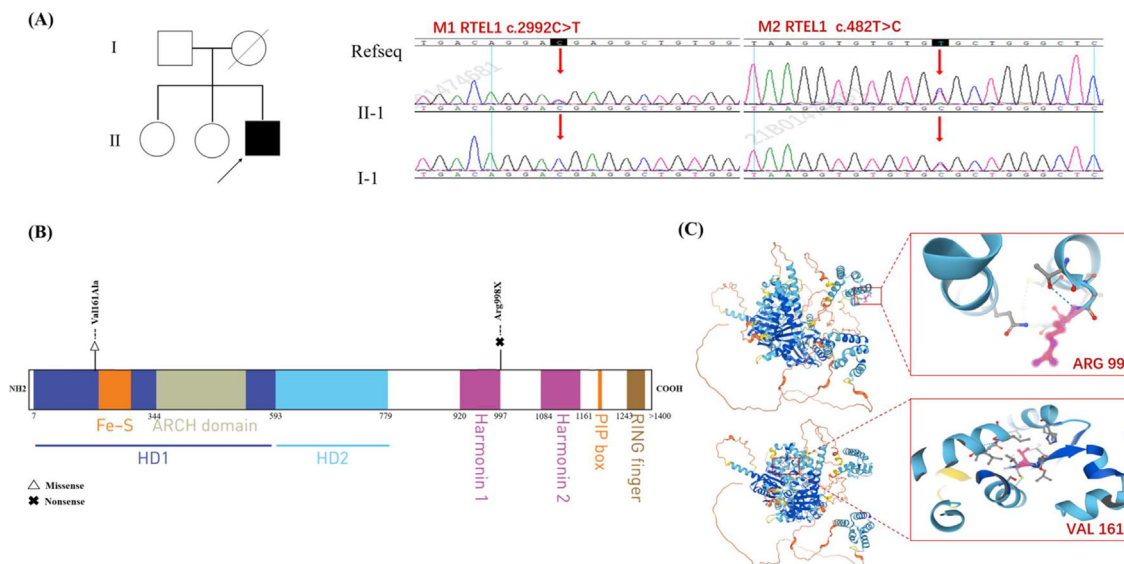


FIGURE 2 (A) Family map and sanger sequencing. The red arrow indicates variant information in sanger sequencing. (B) Schematic illustration is drawn to scale of the two variants of RTEL1. (C) Position of p.Arg998* and p.Val161Ala in pink on the three-dimensional structure of RTEL1 modelled by AlphaFold. RTEL1, regulator of telomere elongation helicase 1

On examination, his respiratory rate was 30 bpm, and oxygen saturation was 90% while breathing ambient air. Lung auscultation revealed decreased breath sounds on the right upper chest and bilateral velcro-like crackles at the lung bases. Hair greying has been remarkable since his teenage years. But there was no mucocutaneous triad of abnormal skin pigmentation, nail dystrophy and leukoplakia.

The white-cell count was 5800 (reference range 3500–9500 per μl), with a mean corpuscular volume (MCV) of 105.4 (reference range 80–100 fl) platelet 123,000 (reference range 125,000–350,000 per μl), and C-reactive protein 8.0 (reference range <8.0 mg/L). D-dimer was elevated at 1330 (reference <500 ng/ml). The serum CYFRA 21-1 level was 5.54 (reference 0–3.5 $\mu\text{g/L}$), suggesting an increased cancer risk. The ESR level was 33 (reference 0–15 mm/h). Serum total immunoglobulin E was elevated at 1925 (reference 0–87 kIU/L). The serum immunoglobulin IgA level was 4.06 (reference 0.69–3.82 g/L). Other routine laboratory examinations were normal. He was negative for HIV and multiple respiratory pathogens, including influenza A and B viruses, respiratory syncytial virus, adenovirus, mycoplasma pneumonia and chlamydia pneumonia. The cultures of sputum and blood, and the sputum acid-fast bacillus test

were negative. Hypersensitivity pneumonitis screens were negative, as were tests for autoantibody profile. The chest CT scan showed diffuse interstitial abnormalities as well as a pneumothorax and emphysematous changes in the mediastinum (Figure 1A). Transthoracic echocardiography revealed a left ventricular ejection fraction of 51% and mild tricuspid regurgitation. Abdominal and urinary B-ultrasonic and ECG were normal.

Bronchoscopy was not performed because of the presence of the pneumothorax and mediastinal emphysema. A surgical lung biopsy was recommended but rejected. The multidisciplinary consensus diagnosis was that he had a genetically related IILD. The whole exome sequence subsequently demonstrated compound heterozygosity for two mutations in the RTEL1 gene [NM_032957.4: c.2992C > T (p.Arg998*) and NM_032957.4: c.482T > C (p.Val161Ala)] as shown in Table 1. Bioinformatics analyses predicted that the variant p.Arg998* was pathogenic (Figure 2A). This nonsense truncating mutation causes early termination of protein RTEL1, resulting in the deletion of the PIP motif (Figure 2B). However, another novel variant, p.Val161Ala, located in the helicase ATP-binding domain, has never been reported (it is a variant of uncertain significance). The

three-dimensional structure of the wild-type RTEL1 protein was generated by the AlphaFold¹⁵ online server and the position of p.Arg998* and p.Val161Ala were located (Figure 2C).

Supplemental oxygen and nebulized treatment with budesonide and acetylcysteine, methylprednisolone, and intravenous fluids were initiated. Pirfenidone was started after further discussions. A referral for lung transplantation was made. His symptoms of cough and shortness of breath improved. Further chest CT scans on 4 June 2021 (Figure 1B) revealed resolution of the pneumothorax and emphysematous changes. However, the interstitial abnormalities and emphysematous changes remained unchanged. The pulmonary function test was refused by the patient at his last visit to our outpatient clinic 3 months after discharge from hospitalization. He returned to his home in Anhui and started home oxygen therapy shortly thereafter. He is still alive with progressive dyspnea to now.

DISCUSSION

RTEL1 heterozygous mutation has been reported to be presented with dyskeratosis congenita (DC), myelodysplastic syndrome (MDS) and various lung phenotypes.^{14,16–18} A series of extrapulmonary manifestations including premature hair greying, nail dystrophy, haematological abnormalities, and liver function abnormalities, suggest a diagnosis of telomeropathy and the need for more detailed testing.^{19,20} According to a retrospective study,¹⁴ the interstitial lung disease (ILD) subtype most commonly found in *RTEL1* mutation carriers is idiopathic pulmonary fibrosis (IPF), but other types of ILD, including chronic hypersensitivity pneumonitis (HP), pleuroparenchymal fibroelastosis (PPFE), unclassifiable fibrosis and connective tissue disease (CTD)–ILD were also observed. Combined pulmonary fibrosis and emphysema were reported in 24.4% of patients. In our case, the patient presented none of the three features that are characteristic of DC. He showed hair greying without any other extrapulmonary manifestations. The initial complete blood count (CBC) suggested no aplastic anaemia or bone marrow failure (BMF). Subsequent serial CBC revealed gradually reduced levels of haemoglobin and platelets. The examination of bone marrow smear and biopsy was normal. Moreover, the rapid-onset thrombocytopenia and anaemia were partially relative to the side effects of the TMP/SMX and piperacillin/tazobactam which were empirically provided before diagnosis was made. It is not uncommon for DC-associated PF to occur later in life without significant skin abnormalities and BMF. Pulmonary complications have been reported in up to 20% of patients with DC and related TBDs.²¹ According to a recent study,²² about 50% of patients showed an atypical UIP pattern with the presence of upper-or mid-lung predominance, multiple cysts, or diffuse ground glass opacity. Therefore, fibrotic interstitial lung disease due to DC should be kept in mind during the follow-up, and correct the diagnosis if necessary.

The chest image of our patient showed significant emphysematous changes, which occurred as characteristic features in α -1 antitrypsin deficiency. However, the patient presented neither liver disease nor necrotizing panniculitis, the C-ANCA was negative, and the whole exome sequence indicated no pathogenic variants in *SERPINA1*.

The pathophysiology of ILD in patients carrying RTEL1 mutations is not yet fully understood. The full-length RTEL1 is essential for the elongation of the single-stranded telomeric overhang by telomerase.²³ Vannier et al. observed that mouse cells disrupted for the RTEL1-PCNA interaction (PIP mutant) exhibited accelerated senescence, replication fork instability, reduced replication fork extension rates, and increased origin usage.²⁴ Therefore, it is reasonable to speculate that loss of function of the C-terminal helicase domain underlies the short telomeres phenotype. When telomere dysfunction causes alveolar stem cell failure, it is sufficient to provoke regenerative defects, inflammatory responses and susceptibility to injury.²⁵ Furthermore, immunohistochemistry showed that RTEL1 was expressed by bronchial and alveolar epithelial cells, suggesting that RTEL1 alteration is involved in alveolar epithelial dysfunction.¹⁴

Pirfenidone and nintedanib have been associated with a reduced decline of FVC in IPF patients with a rare telomere related gene (TRG) variant.^{26,27} Several studies confirmed the safety and effectiveness of antifibrotics in patients with a TRG mutation, and no difference was observed between pirfenidone and nintedanib in terms of efficacy.^{3,6,27,28} Moreover, the side effect profile of antifibrotic treatment in patients with TRG mutation is similar to those observed in the general IPF population.^{3,6} In our case, steroids temporarily relieved the symptoms for a few months, but dyspnea continued to worsen afterward, and antifibrotics failed to slow the progression of fibrosis. Elevated serum D-dimer is associated with an increased risk of acute exacerbation of ILD,²⁹ and significantly elevated serum total immunoglobulin E makes the immune microenvironment complicated.

TRG mutation carriers are frequently potential candidates for lung transplantation (LT) because of their younger age of onset and lower transplant-free survival.^{30,31} However, it has been shown that patients with telomerase complex mutations suffer an increased risk of severe haematological complications and poor survival after LT.^{32,33} In addition, reduced telomere length has also been associated with a shorter time to onset of chronic lung allograft dysfunction (CLAD).³⁴ However, a recent study³⁵ found no difference in the overall post-LT median survival between the groups with (3.75 years) and without (3.0 years) TRG mutation. Univariate analysis showed the diagnosis of myelodysplasia and a reduced telomere length before LT in TRG mutation carriers were the only factors significantly associated with overall survival, suggesting that LT is hazardous but might be offered to TRG mutation carriers after careful immune-haematological evaluation.

A post-hoc analysis of randomized clinical trials showed that patients with a rare variant of TERT, PARN, TERC or RTEL1 had a more rapid decline in FVC than patients

without mutations.²⁶ Justet et al.²⁸ reported a decline of FVC ($314.5 \pm 107.6 \text{ ml}\cdot\text{year}^{-1}$) in TRG mutation carriers, similar to that measured by Newton et al. in another study ($300 \text{ ml}\cdot\text{year}^{-1}$).¹⁸

In conclusion, early-onset ILD should prompt consideration of ILD genetic testing, particularly if there is a family history of lung disease. Patients with RTEL1 mutation can be associated with various types of ILD and exhibit a more rapid decline in pulmonary function. Antifibrotic treatment is of benefit in this subgroup. LT might be offered after a careful genotype–phenotype assessment.

AUTHOR CONTRIBUTION

Man Luo interpreted the data, prepared the figure and tables, and wrote and submitted the manuscript. Jiao-Li Wang contributed to the interpretation of images and figures and critically revised the manuscript.

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CONFLICT OF INTEREST

None declared.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available.

ETHICS STATEMENT

The authors declare that appropriate written informed consent was obtained for the publication of this manuscript and accompanying images.

ORCID

Man Luo  <https://orcid.org/0000-0003-2393-102X>

REFERENCES

- Cottin V, Hirani NA, Hotchkiss DL, Nambiar AM, Ogura T, Otaola M, et al. Presentation, diagnosis and clinical course of the spectrum of progressive-fibrosing interstitial lung diseases. *Eur Respir Rev*. 2018; 27(150):180076.
- Noble PW, Albera C, Bradford WZ, Costabel U, Glassberg MK, Kardatzke D, et al. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. *Lancet*. 2011;377(9779): 1760–9.
- King TE Jr, Bradford WZ, Castro-Bernardini S, Fagan EA, Glaspole I, Glassberg MK, et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. *N Engl J Med*. 2014;370(22):2083–92.
- Nathan SD, Albera C, Bradford WZ, Costabel U, Glaspole I, Glassberg MK, et al. Effect of pirfenidone on mortality: pooled analyses and meta-analyses of clinical trials in idiopathic pulmonary fibrosis. *The Lancet Respir Med*. 2017;5(1):33–41.
- Nathan SD, Costabel U, Albera C, Behr J, Wuyts WA, Kirchgaessler KU, et al. Pirfenidone in patients with idiopathic pulmonary fibrosis and more advanced lung function impairment. *Respir Med*. 2019;153:44–51.
- Richeldi L, du Bois RM, Raghu G, Azuma A, Brown KK, Costabel U, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. *N Engl J Med*. 2014;370(22):2071–82.
- Richeldi L, Kolb M, Jouneau S, Wuyts WA, Schinzel B, Stowasser S, et al. Efficacy and safety of nintedanib in patients with advanced idiopathic pulmonary fibrosis. *BMC Pulm Med*. 2020;20(1):3.
- Brown KK, Flaherty KR, Cottin V, Raghu G, Inoue Y, Azuma A, et al. Lung function outcomes in the INPULSIS(®) trials of nintedanib in idiopathic pulmonary fibrosis. *Respir Med*. 2019;146:42–8.
- Spagnolo P, Ryerson CJ, Putman R, Oldham J, Salisbury M, Sverzellati N, et al. Early diagnosis of fibrotic interstitial lung disease: challenges and opportunities. *Lancet Respir Med*. 2021;9(9):1065–76.
- Cogan JD, Kropski JA, Zhao M, Mitchell DB, Rives L, Markin C, et al. Rare variants in RTEL1 are associated with familial interstitial pneumonia. *Am J Respir Crit Care Med*. 2015;191(6):646–55.
- Kannengiesser C, Borie R, Ménard C, Réocreux M, Nitschké P, Gazal S, et al. Heterozygous RTEL1 mutations are associated with familial pulmonary fibrosis. *Eur Respir J*. 2015;46(2):474–85.
- Stuart BD, Choi J, Zaidi S, Xing C, Holohan B, Chen R, et al. Exome sequencing links mutations in PARN and RTEL1 with familial pulmonary fibrosis and telomere shortening. *Nat Genet*. 2015;47(5): 512–7.
- Barber LJ, Youds JL, Ward JD, McIlwraith MJ, O'Neil NJ, Petalcorin MI, et al. RTEL1 maintains genomic stability by suppressing homologous recombination. *Cell*. 2008;135(2):261–71.
- Borie R, Bouvry D, Cottin V, Gauvain C, Cazes A, Debray MP, et al. Regulator of telomere length 1 (RTEL1) mutations are associated with heterogeneous pulmonary and extra-pulmonary phenotypes. *Eur Respir J*. 2019;53(2):1800508.
- Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, et al. Highly accurate protein structure prediction with AlphaFold. *Nature*. 2021;596(7873):583–9.
- Cardoso SR, Ellison ACM, Walne AJ, Cassiman D, Raghavan M, Kishore B, et al. Myelodysplasia and liver disease extend the spectrum of RTEL1 related telomeropathies. *Haematologica*. 2017;102(8): e293–6.
- Cronkhite JT, Xing C, Raghu G, Chin KM, Torres F, Rosenblatt RL, et al. Telomere shortening in familial and sporadic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2008;178(7):729–37.
- Newton CA, Batra K, Torrealba J, Kozlitina J, Glazer CS, Aravena C, et al. Telomere-related lung fibrosis is diagnostically heterogeneous but uniformly progressive. *Eur Respir J*. 2016;48(6):1710–20.
- Opresko PL, Shay JW. Telomere-associated aging disorders. *Ageing Res Rev*. 2017;33:52–66.
- Molina-Molina M, Borie R. Clinical implications of telomere dysfunction in lung fibrosis. *Curr Opin Pulm Med*. 2018;24(5):440–4.
- Niewisch MR, Savage SA. An update on the biology and management of dyskeratosis congenita and related telomere biology disorders. *Expert Rev Hematol*. 2019;12:1037–52.
- Wang P, Xu Z. Pulmonary fibrosis in dyskeratosis congenita: a case report with a PRISMA-compliant systematic review. *BMC Pulm Med*. 2021;21(1):279.
- Awad A, Glouster G, Lamm N, Tawil S, Hourvitz N, Smoom R, et al. Full length RTEL1 is required for the elongation of the single-stranded telomeric overhang by telomerase. *Nucleic Acids Res*. 2020;48(13): 7239–51.
- Vannier JB, Sandhu S, Petalcorin MI, Wu X, Nabi Z, Ding H, et al. RTEL1 is a replisome-associated helicase that promotes telomere and genome-wide replication. *Science*. 2013;342(6155):239–42.
- Alder JK, Barkauskas CE, Limjunyawong N, Wu X, Nabi Z, Ding H, et al. Telomere dysfunction causes alveolar stem cell failure. *Proc Natl Acad Sci U S A*. 2015;112(16):5099–104.
- Dressen A, Abbas AR, Cabanski C, Reeder J, Ramalingam TR, Neighbors M, et al. Analysis of protein-altering variants in telomerase genes and their association with MUC5B common variant status in patients with idiopathic pulmonary fibrosis: a candidate gene sequencing study. *Lancet Respir Med*. 2018;6(8):603–14.
- Justet A, Klay D, Porcher R, Reeder J, Ramalingam TR, Neighbors M, et al. Safety and efficacy of pirfenidone and nintedanib in patients with idiopathic pulmonary fibrosis and carrying a telomere-related gene mutation. *Eur Respir J*. 2021;57(2):2003198.

28. Justet A, Thabut G, Manali E, Molina Molina M, Kannengiesser C, Cadranet J, et al. Safety and efficacy of pirfenidone in patients carrying telomerase complex mutation. *Eur Respir J*. 2018;51(3):1701875.
29. Ishikawa G, Acquah SO, Salvatore M, Padilla ML. Elevated serum D-dimer level is associated with an increased risk of acute exacerbation in interstitial lung disease. *Respir Med*. 2017;128:78–84.
30. Borie R, Tabèze L, Thabut G, Nunes H, Cottin V, Marchand-Adam S, et al. Prevalence and characteristics of TERT and TERC mutations in suspected genetic pulmonary fibrosis. *Eur Respir J*. 2016;48(6):1721–31.
31. Popescu I, Mannem H, Winters SA, Hoji A, Silveira F, McNally E, et al. Impaired cytomegalovirus immunity in idiopathic pulmonary fibrosis lung transplant recipients with short telomeres. *Am J Respir Crit Care Med*. 2019;199(3):362–76.
32. Silhan LL, Shah PD, Chambers DC, Snyder LD, Riise GC, Wagner CL, et al. Lung transplantation in telomerase mutation carriers with pulmonary fibrosis. *Eur Respir J*. 2014;44(1):178–87.
33. Borie R, Kannengiesser C, Hirschi S, Le Pavec J, Mal H, Bergot E, et al. Severe hematologic complications after lung transplantation in patients with telomerase complex mutations. *J Heart Lung Transplant*. 2015;34(4):538–46.
34. Newton CA, Kozlitina J, Lines JR, Kaza V, Torres F, Garcia CK. Telomere length in patients with pulmonary fibrosis associated with chronic lung allograft dysfunction and post-lung transplantation survival. *J Heart Lung Transplant*. 2017;36(8):845–53.
35. Phillips-Houlbracq M, Mal H, Cottin V, Gauvain C, Beier F, Sicre de Fontbrune F, et al. Determinants of survival after lung transplantation in telomerase-related gene mutation carriers: A retrospective cohort. *Am J Transplant*. 2022;22(4):1236–44.

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