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Case report

Heterozygous *TERT* gene mutation associated with familial idiopathic pulmonary fibrosis

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ABSTRACT

Idiopathic pulmonary fibrosis (IPF) is a chronic interstitial lung disease of unknown cause that occurs sporadically, but it can also occur in families and so named as Familial Pulmonary Fibrosis (FPF). Some forms of FPF overlaps IPF features, namely the radiological and histological pattern of usual interstitial pneumonia (UIP). Genetic and environmental factors commonly play an important role in the pathogenesis of FPF and the most commonly identified mutations involve the telomerase complex. Here, we report a rare case of FPF in a male at the age of 44, in whom genetic testing showed heterozygous variants for the telomerase reverse transcriptase gene (*TERT*). Our report highlights the importance of compiling a thorough family history in younger patients identified with UIP serving as a resource for identifying the current and future genetic links to disease. Families with UIP hold a great promise in defining UIP pathogenesis, potentially suggesting targets for the development of future therapies.

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a specific form of progressive fibrotic pulmonary disease of unknown origin that occurs mainly in middle-aged or elderly adults and it is characterized by an underlying radiological and histopathological pattern of usual interstitial pneumonia (UIP). The etiology of the disease is still under investigation, but a combination of genetics and environmental factors seem to play an important role [1]. When IPF is identified in two or more direct or first-degree members of a family, Familial Pulmonary Fibrosis (FPF) is defined [1,2]. In the present study, we report a rare case of a young man with an early onset of familial IPF at the age of 44.

2. Case report

A 44-year-old male, a metallurgical painter, was referred to our department with a complaint of dry irritating cough and progressively worsening dyspnea for the past year. He was a smoker of three packs years and he had a family history of pulmonary fibrosis. We knew that pulmonary fibrosis was the responsible for his mother and three brothers death at age of 62, 52, 47 and 42, respectively. The patient's family tree is shown in Fig. 1.

On physical examination, he was tachypneic (22 cpm) and digital clubbing and hair with early depigmentation (since 22) were observed. During auscultation, bilateral basal inspiratory fine crackles were noticed. His pulmonary function tests revealed a restrictive pattern of disease with a markedly decreased diffusion capacity: Forced Vital Capacity (FVC) 54.6%, Forced Expiratory Volume in 1 second [FEV1] 61.8%, Diffusion Capacity of the Lung to transfer Carbon monoxide (DLCO) 39.3% of predicted. The distance at a six-minute walk test (6MWT) was 281m with 76% as lower oxygen saturation. Air blood gas analysis in room air showed decreased oxygen pressure with normocapnia (pH 7.44, pO2 72.4 mmHg, pCO2 38.4 mmHg). White and red blood cell counts were within normal range, but platelet counts revealed thrombocytopenia (90 × 109/sL). Blood chemistry tests disclosed altered liver function: aspartate aminotransferase 50 U/L, alanine aminotransferase 55 U/L, gamma-glutamyl transferase 280 U/L. Erythrocyte sedimentation was 36 mm per hour. An autoimmune serology panel was negative.

His chest-X-ray displayed reduced lung volume and a diffuse interstitial pattern. High resolution computed tomography (HRCT) of the lungs demonstrated honeycombing and reticulation with subpleural

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Abbreviations

6MWT	six-minute walk test
BALF	bronchoalaveolar lavage fluid
DKC1	Dyskerin Pseudouridine Synthase 1
DLCO	Diffusion Capacity of the Lung to transfer Carbon monoxide
FEV1	Forced Expiratory Volume in 1 second
FPF	Familial Pulmonary Fibrosis
FVC	Forced Vital Capacity
HRCT	High resolution computed tomography
IPF	Idiopathic pulmonary fibrosis
PARN	Poly(A)-specific ribonuclease
RTEL1	Regulator Of Telomere Elongation Helicase 1
TERT	telomerase reverse transcriptase
UIP	Usual interstitial pneumonia

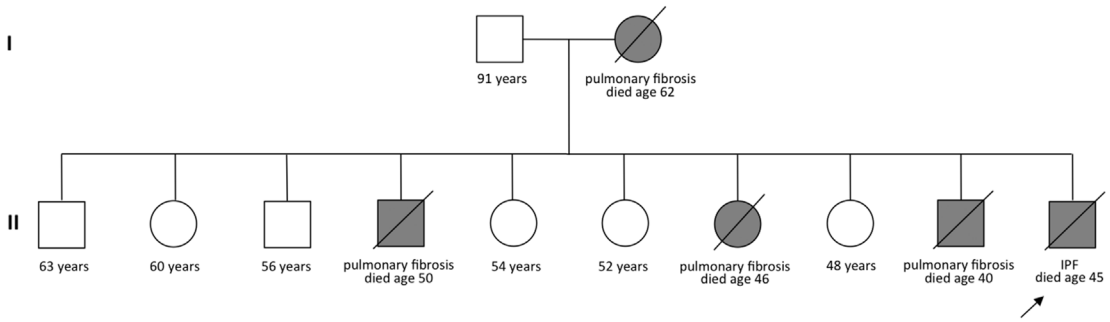

Fig. 1. Family tree. This study represents a 2-generation pedigree in which individuals from 2 generations had pulmonary fibrosis. Grey and unfiled shapes represent affected and unaffected individuals, respectively. Squares represent males and circles represent females. Strikethrough symbol (/) denotes deceased individuals. The patient is indicated by an arrow.

predominance along with traction bronchiectasis affecting all lobes of the lungs. These radiological features were consistent with usual interstitial pneumonia (UIP) (Fig. 2). Liver morphological changes suggestive of cirrhosis were also observed. The patient underwent ultrasound-mediated transient elastometry (FibroScan) to measure liver fibrosis and the results showed liver fibrosis F0 (no evidence of hepatic cirrhosis). The patient was kept under surveillance in gastroenterology and started medication with ursodeoxycholic acid.

He was admitted to our hospital for further evaluation. The bronchoalaveolar lavage fluid (BALF) obtained from middle lobe showed elevated total cell counts (2.4×10^5/mL) with a normal cytological differential count. Microbiological examination of BALF revealed no evidence of infection. Transbronchial lung cryobiopsies obtained from the right lower lobe demonstrated scattered fibroblast foci

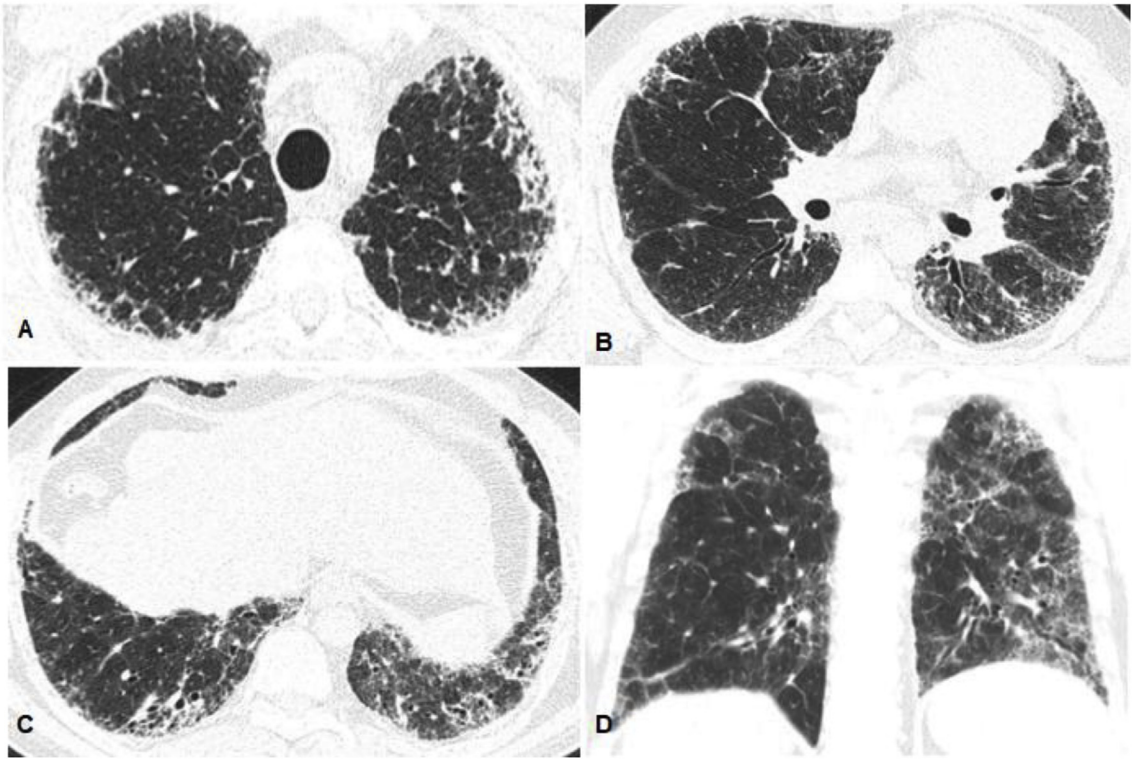

Fig. 2. High resolution computed tomography (HRCT) scans at a presentation. **(A–C)** Transverse CT section and **(D)** coronal reconstruction showing honeycombing and reticulation along with traction bronchiectasis involving mainly the subpleural segments of middle and upper lobe but also the basal segments of the lower lobes. The overall radiologic pattern was typical for radiologic usual interstitial pneumonia (UIP).

in a background of chronic interstitial fibrosis, consistent with histologic UIP pattern, although no honeycomb change was seen (Fig. 3).

We diagnosed IPF based on his clinical presentation, laboratory investigations, pulmonary function, radiographic abnormalities and histological examination of lung biopsy. A younger age and a prominent family history of pulmonary fibrosis coupled with his clinical course suggested he most likely had FPF. Due to the similarities with IPF, antifibrotic therapy was allowed to prescribe and nintedanib plus a pulmonary rehabilitation program was then initiated. He was also forward to evaluation for lung transplantation. Nintedanib was well tolerated with no adverse events or increased of liver enzymes levels. His clinical presentation, early pulmonary fibrosis, early hair depigmentation, sustained thrombocytopenia and altered liver function led us to hypothesize telomeropathy. Therefore, a genetic evaluation was carried out. A heterozygous mutation (c.2701C > T) located in exon 11 of the *TERT* gene that replaces arginine by tryptophan (Arg901Trp) was identified (Fig. 4). Genomic DNA was extracted from blood samples to evaluate the telomerase length using a multiplex quantitative *polymerase chain reaction* (qPCR) as described previously [3]. The length of telomeres was expressed by the ratio of the telomere repeat copy number (T) and single copy gene copy number (S). A reference DNA sample was used so that relative quantities of T and S could be determined from standard curves (T/S ratios). Short telomeres were defined as a telomere length less than or equal to the first percentile of the normal telomere length distribution.

After six months of treatment with nintedanib, he exhibited rapid clinical and radiological deterioration. He complained of worsening dyspnea with limitation of daily life activities. His pulmonary function was slowly declining: FVC 41%, FEV1 49.2% and DLCO could not be measured because he had difficulty taking a deep breath. At 6MWT he walked 175 m with significant desaturation from 89% to 76%. Air blood gas analysis in room air showed decreased oxygen pressure (pH 7.44, paO_2 60 mmHg, $paCO_2$ 38 mmHg). The new HRCT showed progressive honeycomb pattern (Fig. 5). He started long-term oxygen therapy and, subsequently, listed for lung transplantation. Nine months after the diagnosis of FPF, a deceased donor was identified and he underwent a right single lung transplantation. However, on the 37th postoperative day, he developed a septic shock and bacteraemia by multi-drug resistant *Klebsiella pneumoniae* and died.

3. Discussion

IPF is a devastating interstitial lung disorder of unknown etiology that typically leads to respiratory failure and death within 3–5 years after diagnosis [1,2,4]. It is usually a sporadic condition and rarely it is identified in families, those cases identified as Familial Pulmonary Fibrosis (FPF) [1]. FPF is defined as an idiopathic diffuse parenchymal lung disease affecting two or more members of the same primary biological family [5]. The exact prevalence of FPF is unknown, however, it is thought to represent about 5% of IPF cases [6]. Vertical transmission in families, suggests that FPF is inherited in an autosomal dominant fashion, although with incomplete penetrance (since not all of them develop disease in the presence of mutated genes). The age of onset is often younger than in sporadic IPF, approximately 55 years [2,4–6]. Several studies have compared the clinical, radiological, histological features and outcome of sporadic and familial IPF and they concluded that both diseases shared characteristics [7–9]. As FPF occurs in families and is virtually indistinguishable from sporadic IPF, this familial condition provides an opportunity to better investigate IPF disease. Unfortunately, there are still few FPF case reports in literature.

The most frequent mutations in FPF involve genes of the telomerase complex such as *TERT*, *TERC*, *RTEL1*, *PARN* or *DKC1* [1]. Heterozygous mutations of protein component of telomerase (TERT) are the most frequently evidenced mutations observed in about 15% of affected families, while heterozygous mutations of RNA component of the enzyme (*TERC*) are more rare (a few per cent) [1,6,10,11]. Recently, two other genes, *RTEL1* and *PARN*, have been associated with shortened telomere lengths and FPF [12]. These genes are related to premature shortening of telomeres in the peripheral blood and lungs, assessed as short telomere syndrome. It is believed that the loss of function of the telomerase complex may influence the turnover and the healing of alveolar epithelial cells after a damaging stimulus, thus triggering IPF. Moreover, mutations in *TERT/TERC* are also associated with extra-pulmonary abnormalities, including premature hair greying, bone marrow failure and liver cirrhosis. The phenotype may be heterogeneous, even in patients with the same mutation [2,10,13–15]. In our case, the result of genetic test showed a mutation in the *TERT* gene (c.2701C > T) that leads to a short telomeric score (1st percentile of the normal telomere length distribution).

The therapeutic recommendation for IPF includes two antifibrotic drugs, pirfenidone and nintedanib, which demonstrated efficacy in slowing functional decline and disease progression [16–18]. However, there are no specific therapeutic recommendations for patients who carry a *TERT* or *TERC* mutations. In a multicentre retrospective study, a beneficial effect of pirfenidone on the decline of lung function could not be demonstrated in patients with a *TERT/TERC* mutation [19]. Therefore, the role for antifibrotic therapy in these patients is still unclear and it should be managed with caution once there is a risk of liver injury associated [18]. Recently, danazol, a synthetic androgen, showed to increase telomere length and to stabilize FVC and DLCO during the treatment [20]. Gene therapy has been suggested, however no strategy reached the stage of clinical trials for gene correction in telomerase complex mutation carriers [21].

The final outcome of our case of patient death after 37 days of lung transplantation reinforced previous findings that associated *TERT* mutations with reduced transplantation survival. This failure may be the result of the increased rates of bone marrow failure, infection and renal and allograft dysfunction after lung transplant that IPF patients with

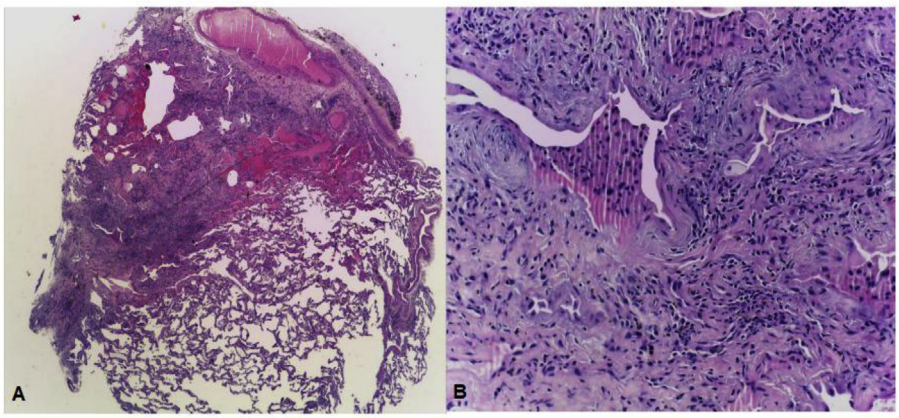

Fig. 3. Transbronchial lung cryobiopsy showing aspects suggestive of a pattern of usual interstitial pneumonia (UIP). **(A)** Low magnification showing pulmonary parenchyma with architecture distortion; heterogeneous appearance with areas of fibrosis and parenchymal areas more preserved (H & E, 25x). **(B)** Higher magnification reveals typical fibroblast foci with myofibroblast accumulation adjacent to areas of fibrosis (H & E, 200x).

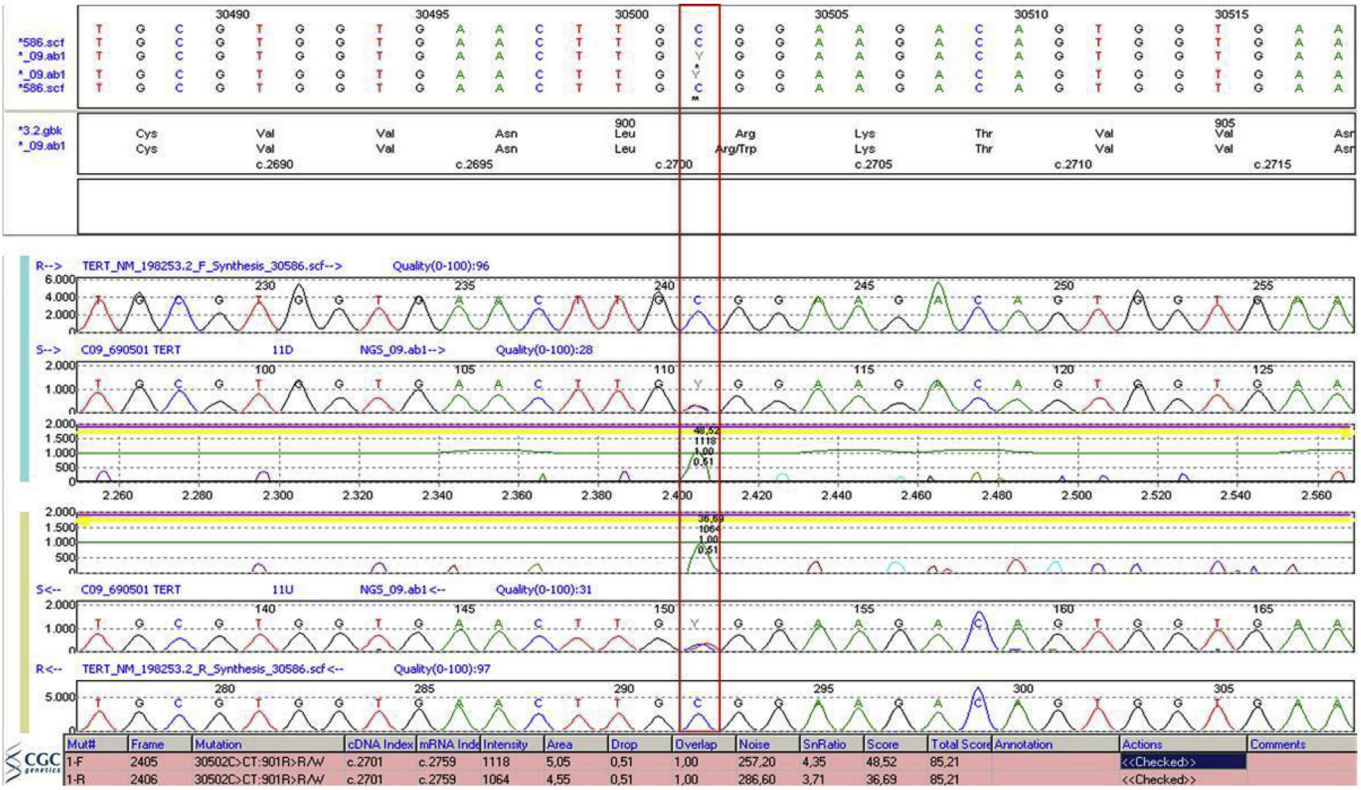

Fig. 4. Sequence of the *TERT* gene in wild type (above) and patient DNA (bellow). A heterozygous missence mutation (c.2701C > T), located in exon 11 in *TERT* gene was identified in the patient (red square indicates the position of the mutation).

TERT mutations are predisposed [22,23].

Overall, our case highlights how challenging the diagnosis of very rare diseases such as FPF is. Physicians must consider FPF in younger patients with a family history of fibrotic lung disease, and, more importantly, all clinical findings should be appreciated and considered, including extra-pulmonary signs suggestive of telomere syndrome in order to ensure an early diagnosis. Genetic investigations, in particular the detection of telomerases mutations should be performed and reported when FIP is diagnosed because these data will allow to understand the underlying pathogenesis. Only with better recognition of the pathophysiology of different forms of fibrotic pneumonias, new therapeutic strategies may arise and facilitate disease management.

Conflicts of interest

Authors have no conflicts of interest to disclose.

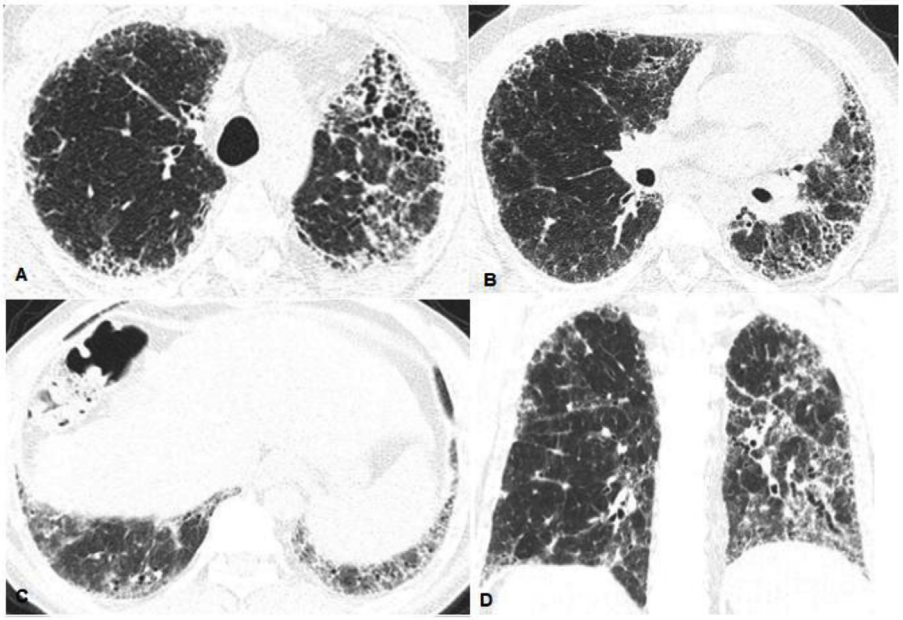

Fig. 5. High resolution computed tomography (HRCT) scans six months after treatment with nintedanib. **(A–C)** Transverse CT section and **(D)** coronal reconstruction showing progression of diffuse honeycombing involving mainly left lung.

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