NKX2.1 (TTF1) germline mutation associated with pulmonary fibrosis and lung cancer

To the Editor:

The high prevalence of lung cancer in patients with idiopathic pulmonary fibrosis (3–30%) has been confirmed by several studies, pointing to specific diagnostic and therapeutic issues. The co-occurrence is associated with worse survival than with each disease alone [1]. Because cigarette smoking is a risk factor for both diseases, smoking is an ideal culprit for their co-occurrence, despite several common pathogenic mechanisms such as common genetic risk factors.

Several germline mutations have been associated with pulmonary fibrosis [2]. Mutation of telomere-related genes is the most frequent (25–30% of familial cases) [2], with mutation of surfactant-associated genes (including *SFTPC* (location: 8p21.3), *SFTPB* (2p11.2), *SFTPA1* (10q22.3), *SFTPA2* (10q22.3), *ABCA3* (16p13.3) and *NKX2.1* (14q13.3)) second in frequency (1–5% of familial cases) [2]. *SFTPA1* and *SFTPA2* germline mutations are unique in that they are associated with a high prevalence of lung cancer, although the mechanisms are poorly understood [3].

The *NKX2.1* gene codes for thyroid transcription factor 1 (TTF1), which is implicated in lung development and the expression of surfactant proteins [4]. *NKX2.1* heterozygous mutations have been associated with "brain–lung–thyroid syndrome", characterised by central nervous system abnormalities, hypothyroidism and interstitial lung disease (ILD), with an inconstant triad [5]. Severe ILD can be the only manifestation in up to 25% of *NKX2.1* mutation carriers [5]. Only three patients with lung cancer associated with *NKX2.1* mutation have been reported, with few data (figure 1a) [6]. Here, we describe a woman with lung fibrosis and chorea associated with *NKX2.1* mutation, complicated by lung cancer.

A 42-year-old woman, without previous respiratory symptoms, was referred to our department in 2014 for a diagnosis of ILD. She had a history of chorea and subclinical hypothyroidism. The chorea led to the identification, at age 32 years, of a *de novo* c.267dupG *NKX2.1* mutation [9]. Neither of her parents had ILD, chorea or hypothyroidism, and neither carried the mutation. The patient received prenatal screening during pregnancy and none of her asymptomatic children carried the mutation. She had a 10-pack-year smoking history and no other toxic lung exposures. She did not present any clinical or biological signs of an autoimmune disease. Clinical evaluation showed lung crackles and a few abnormal involuntary movements. Laboratory test results were within the normal range. High-resolution chest computed tomography (CT) revealed a pattern indeterminate for usual interstitial pneumonia, with honeycombing, ground-glass opacities, reticulations and traction bronchiectasis with ventral and basal predominance (figure 1b–e). Because the distribution of lung fibrosis did not suggest any specific aetiology, the chest CT was considered truly indeterminate and not suggestive of an alternative diagnosis. Bronchoalveolar lavage analysis revealed 335 000 cells per mL (68% macrophages, 10% lymphocytes, 15% neutrophils and 7% eosinophils) and Golde score 46 (which evaluates haemosiderin-laden macrophages, a score >100 suggesting diffuse alveolar haemorrhage). Bronchial biopsy showed mild, nonspecific bronchial inflammation.

Surgical lung biopsy was declined considering the severity of the disease at diagnosis: forced vital capacity 1.36 L (46% predicted) and diffusing capacity of the lung for carbon monoxide 26% predicted. On right heart catheterisation, mean pulmonary artery pressure was 40 mmHg, pulmonary capillary wedge pressure

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Germline surfactant-associated genes mutations are associated with ILD and increased risk of lung cancer https://bit.ly/3CkkXgD

a)

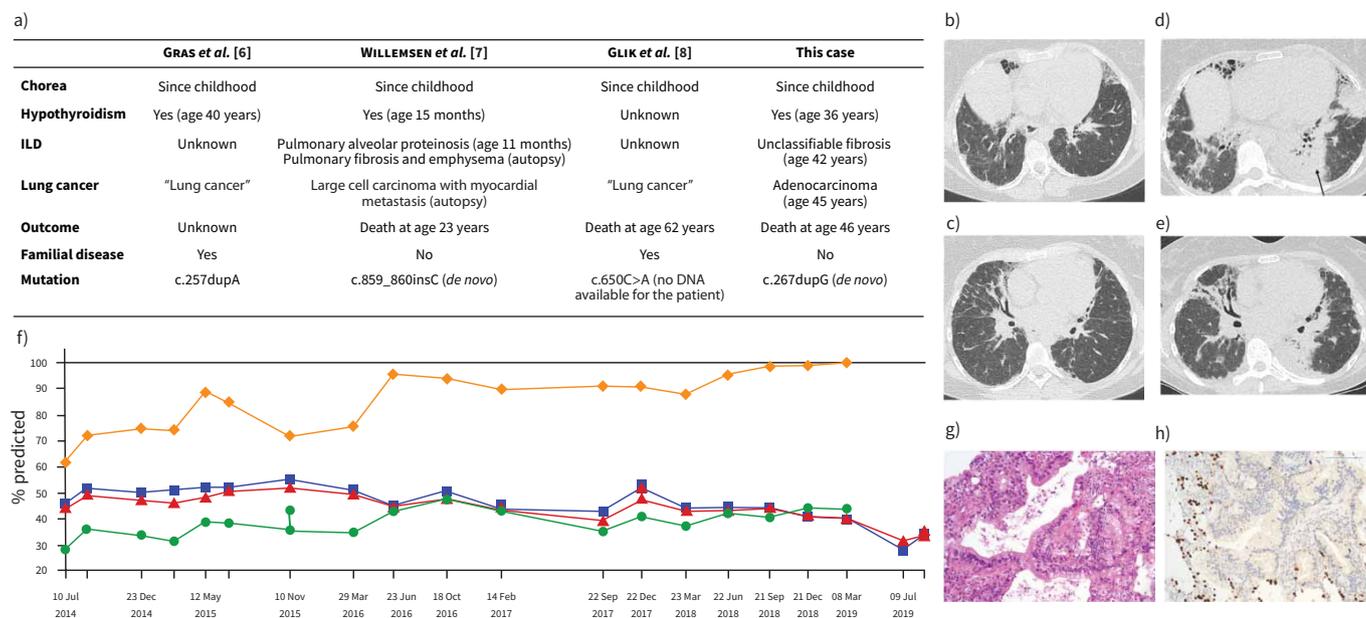|	GRAS *et al.* [6]	WILLEMSEN *et al.* [7]	GLIK *et al.* [8]	This case
Chorea	Since childhood	Since childhood	Since childhood	Since childhood
Hypothyroidism	Yes (age 40 years)	Yes (age 15 months)	Unknown	Yes (age 36 years)
ILD	Unknown	Pulmonary alveolar proteinosis (age 11 months) Pulmonary fibrosis and emphysema (autopsy)	Unknown	Unclassifiable fibrosis (age 42 years)
Lung cancer	"Lung cancer"	Large cell carcinoma with myocardial metastasis (autopsy)	"Lung cancer"	Adenocarcinoma (age 45 years)
Outcome	Unknown	Death at age 23 years	Death at age 62 years	Death at age 46 years
Familial disease	Yes	No	Yes	No
Mutation	c.257dupA	c.859_860insC (*de novo*)	c.650C>A (no DNA available for the patient)	c.267dupG (*de novo*)

FIGURE 1 a) Reported cases of lung cancer associated with germline *NKX2.1* mutation. b and c) Chest computed tomography (CT) image of a 42-year-old woman at interstitial lung disease (ILD) diagnosis, showing honeycombing associated with ground-glass opacities, reticulations and traction bronchiectasis with ventral and basal predominance. d and e) Chest CT image at lung cancer diagnosis: emergence of several bilateral subpleural condensations. Arrow: transthoracic core biopsy. f) Evolution of pulmonary function test results after ILD diagnosis. Blue: forced vital capacity; red: forced expiratory volume in 1 s; green: diffusing capacity of the lung for carbon monoxide; orange: carbon monoxide transfer coefficient. g) Transparietal biopsy: invasive mucinous adenocarcinoma (haematoxylin and eosin stain, original magnification 20×). h) Immunohistochemistry for thyroid transcription factor 1: positive for pneumocytes and negative for tumour cells (original magnification 20×). Scale bar=100 µm.

20 mmHg, cardiac output 8.5 $L·min^{-1}·m^{-2}$ and pulmonary vascular resistance 4.7 Wood units. After multidisciplinary discussion, the diagnosis was unclassifiable pulmonary fibrosis [10].

The patient received azithromycin (250 mg three times a week) from July 2014 to December 2014 and prednisone 40 $mg·day^{-1}$ progressively tapered from December 2014 to June 2016. She did not show significant functional or radiological improvement. She also received first pirfenidone and then nintedanib in 2016 for a few weeks but experienced nausea and abdominal pain, without abnormal laboratory findings, and she decided to stop any antifibrotic therapy.

The disease was slowly progressive up to July 2019, when a chest CT scan showed several bilateral subpleural consolidations, with several nodules in the left lower lobe (figure 1b–e). ^{18}F-fluorodeoxyglucose positron emission tomography–CT revealed five hypermetabolic nodules (maximal standardised uptake value 5.8) without any extrapulmonary involvement or mediastinal or hilar hypermetabolic lymphadenopathy. Cerebral magnetic resonance imaging findings were normal. Transthoracic core biopsy (arrow, figure 1d) showed invasive mucinous adenocarcinoma TTF1/ALK/ROS1/PD-L1 0% (figure 1h). Next-generation sequencing did not reveal any driver somatic mutation/translocation associated with lung cancer (*ALK*, *BRAF*, *EGFR*, *KRAS*, c-*MET* exon 14, *NRG1*, *NTRK1*, *NTRK3*, *RET* and *ROS1*).

The patient underwent chemotherapy with carboplatin (area under the curve 5 $mg·mL^{-1}·min^{-1}$) and pemetrexed, followed by four cycles of maintenance pemetrexed. Lung cancer progressed locally with maintenance chemotherapy. In June 2020, she experienced an acute exacerbation, perhaps related to the chemotherapy, and received parenteral antibiotics and high-dose corticosteroids, but eventually died.

We describe a rare case of lung cancer and ILD associated with a germline *NKX2.1* mutation. This case suggests a specific risk of lung cancer in adults with surfactant-associated gene mutations.

Lung cancer may develop in up to one third of patients with *SFTPA1* and *SFTPA2* germline mutations, which suggests a specific risk of lung cancer with these mutations [3, 11]. ILD and lung cancer have been observed in the same family with *SFTPA1* or *SFTPA2* mutations but not necessarily in the same patient, so

lung cancer development may be independent of the ILD [3, 11]. Ageing might be an important factor in the development of lung cancer in patients with surfactant-associated gene mutations. Indeed, *SFTPA1* and *SFTPA2* germline mutations are usually detected in adults (mean age 43 years), whereas many *SFTPB*, *SFTPC*, *ABCA3* and *NKX2.1* mutations have been mostly reported in children, and may not allow the necessary development time for cancer [12]. For instance, homozygous *SFPTB* and *ABCA3* null mutations are associated with neonatal distress leading to death or lung transplantation before age 1 year [12]. Inhaled toxins, such as tobacco smoke, may be cofactors for carcinogenesis, as evidenced in our patient with a 10-year exposure to tobacco smoke.

Almost 150 patients with *NKX2.1* mutation have been reported, 60% with ILD [9]. The pathophysiology of ILD in patients with an *NKX2.1* mutation is unknown but the main hypothesis relates to endoplasmic reticulum stress and caspase pathway activation in type II cells [13]. Corticosteroids, azithromycin and/or hydroxychloroquine might ameliorate the ILD related to *SFTPC* or *ABCA3* mutation, but evidence in adults is lacking [14]. Because *NKX2.1* interferes with *SFTPC* promoters, the same treatment may be effective [4]. Our patient received azithromycin and prednisone without objective improvement, as well as pirfenidone and nintedanib as fibrosis progressed but did not tolerate them. Prospective data and clinical trials are urgently needed to define better the optimal treatment for these patients. The patient had only a limited germline genetic analysis in 2014, which did not include *RTEL1* and other telomere-related gene sequencing since associated with familial pulmonary fibrosis. Whether we should offer or repeat next-generation sequencing or whole-exome sequencing to families with suspected genetic cause of pulmonary fibrosis is an important question and is the subject of an ongoing dedicated European Respiratory Society task force.

No known addictive somatic mutation was evidenced in the cancer, which is another argument for an original carcinogenesis. Most frequent germline mutations associated with increased risk of lung cancer involve *EGFR* and p53 in Li-Fraumeni familial syndrome. In addition to *SFTPA* genes, *NKX2.1* may be another germline risk factor for lung cancer, as supported by its role in the differentiation of the terminal respiratory unit cells and peripheral lung development, as a lineage-survival oncogene in lung adenocarcinoma and its recently evidenced crosstalk with EGFR/ERBB3 [15]. Indeed, lung cancer was reported in three other patients with *NKX2.1* mutation, with almost no data about the lung cancer history in two cases (figure 1a) [6]. The third case was a 23-year-old man confirmed to carry an *NKX2.1* mutation, with localised lung cancer associated with ILD, although the three diagnoses were established *post mortem* [7].

From a therapeutic point of view, the association of cancer and ILD is a major concern. Pre-existing ILD limits the possibility of surgery or radiotherapy [1]. Moreover, this unique pathophysiology does not suggest an effective targeted therapy or immunotherapy, as evidenced by negative next-generation sequencing findings and lack of PD-L1 expression.

Although we cannot rule out a coincidence, *NKX2.1* mutation, as well as mutations in other surfactant-associated genes, is associated with ILD and possibly increased risk of lung cancer. A lung cancer screening strategy should be evaluated for these patients, with the balance of the risk of radiation exposure with repeated CT scanning and the difficulty of therapeutics in patients with ILD. Furthermore, owing to the rarity of lung transplantation for cancer, the conclusions that can be drawn about lung transplantation for this indication are limited. In addition, the ethical balance of how to allocate a scarce resource, such as a donor lung, remains an unresolved dilemma given the uncertainties regarding long-term survival [16].

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