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# Similarities and differences of interstitial lung disease associated with pathogenic variants in SFTPC and ABCA3 in adults

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# Abstract

## Background and objective:

Variants in surfactant genes SFTPC or ABCA3 are responsible for interstitial lung disease (ILD) in children and adults, with few studies in adults.

## Methods:

We conducted a multicentre retrospective study of all consecutive adult patients diagnosed with ILD associated with variants in SFTPC or ABCA3 in the French rare pulmonary diseases network, OrphaLung. Variants and chest computed tomography (CT) features were centrally reviewed.

## Results:

We included 36 patients (median age: 34 years, 20 males), 22 in the SFTPC group and 14 in the ABCA3 group. Clinical characteristics were similar between groups. Baseline median FVC was 59% ([52-72]) and DL<sub>CO</sub> was 44% ([35-50]). An unclassifiable pattern of fibrosing ILD was the most frequent on chest CT, found in 85% of patients, however with a distinct phenotype with ground-glass opacities and/or cysts. Nonspecific interstitial pneumonia and usual interstitial pneumonia were the most common histological patterns in the ABCA3 group and in the SFTPC group, respectively. Annually, FVC and DL<sub>CO</sub> declined by 1.87% and 2.43% in the SFTPC group, respectively, and by 0.72% and 0.95% in the ABCA3 group, respectively (FVC,  $p = 0.014$  and DL<sub>CO</sub>,  $p = 0.004$  for comparison between groups). Median time to death or lung transplantation was 10 years in the SFTPC group and was not reached at the end of follow-up in the ABCA3 group.

## Conclusion:

SFTPC and ABCA3-associated ILD present with a distinct phenotype and prognosis. A radiologic pattern of fibrosing ILD with ground-glass opacities and/or cysts is frequently found in these rare conditions.

# INTRODUCTION

Variants in surfactant-related genes have been linked to interstitial lung disease (ILD) in children<sup>1</sup> and adults.<sup>2,3</sup> ILD caused by variants in SFTPC, encoding the surfactant protein C, are rare and may be underestimated in adults due to the lack of specific presentation and/or absence of family history.<sup>4–12</sup> The inheritance pattern is autosomal dominant with incomplete penetrance. Variants in ABCA3, encoding the lipid transporter ATP-binding cassette subfamily A member 3, can cause neonatal fatal respiratory distress syndrome<sup>13</sup> and ILD in children and adults.<sup>14</sup> Inheritance pattern is autosomal recessive and heterozygosity for ABCA3 variants may play a disease modifier role in other surfactant-related ILDs.<sup>7,10</sup> Despite belonging to surfactant-related genes associated with onset of ILD in children, ABCA3 and SFTPC differ regarding the supposed pathophysiology of ILD. ABCA3 pathogenic variants are loss of function variants,<sup>15</sup> whereas p.(Ile73Thr), the most frequent SFTPC variant, is responsible for protein dysfunction and cell toxicity.<sup>16</sup> We hypothesized that SFTPC- and ABCA3-associated ILD in adults may share an identifiable phenotype but with distinct characteristics. Here, we report the phenotypical description of ILD in a cohort of adult patients carrying pathogenic variants in either SFTPC or ABCA3.

## METHODS

### Subjects

A multicentre retrospective study was conducted within the French rare pulmonary diseases network OrphaLung. Data from participating centres were matched with those from the molecular reference genetic centre for surfactant-related diseases (Armand Trousseau Hospital, Paris, France) to include all consecutive patients diagnosed with ILD and SFTPC or ABCA3 variants from 1 January 2002 until 1 June 2022. The inclusion criteria were patients >18 years of age, the presence of ILD on chest computed tomography (CT) and pathogenic/likely pathogenic SFTPC or ABCA3 variant(s). Exclusion criteria were missing radiologic or pulmonary function data, absence of ILD on CT and the presence of only monoallelic ABCA3 variant.

### Data collection

Data were collected using a standardized and anonymous form. For patients diagnosed before the age of 18-years, paediatric descriptive data (functional, radiological, histopathological and treatment-related) were censored, and the radiological analysis was carried out from the first chest CT available after the age of 18-years. The retrospective analysis of histopathology was based on the biopsy reports and the multidisciplinary discussion reports provided by the reporting centre for each patient. Lung biopsies had been reviewed by expert pathologists in all cases. Pulmonary hypertension at right heart catheterization (RHC) was defined as per guidelines.<sup>17</sup>

### Genetic analysis

Genetic data were reviewed retrospectively by molecular geneticists (ML, SA). Variants were classified as class 4 (likely pathogenic) or class 5 (pathogenic) variants according to the international classification.<sup>18</sup>

## **Radiological analysis**

Chest CT scans were reviewed by two independent thoracic radiologists (SSM, PYB) blinded to the clinical and genetic data, using a pre-determined list of features. Whenever possible, patterns were categorized according to the 2018 international guidelines on idiopathic pulmonary fibrosis (IPF),<sup>19</sup> the 2013 classification of idiopathic interstitial pneumonias<sup>20,21</sup> or other patterns such as pleuroparenchymal fibroelastosis (PPFE).<sup>22</sup> Radiologic patterns of fibrosing ILD that did not fit into existing categories were termed ‘fibrotic ILD, unclassifiable pattern’<sup>23,24</sup> and classified between three subgroups based on the presence of (1) predominant ground glass opacities (GGO) (unclassifiable pattern—GGO), (2) prominent cystic changes (unclassifiable pattern—cysts) and (3) combination of GGO and cystic changes (unclassifiable pattern—mixed). The extent of ILD was determined by visual assessment between four categories (0%–25%, 26%–50%, 51%–75% and 75%–100% of the lung parenchyma). Discrepancies between the independent radiological reviews were resolved by consensus.

## **Statistical analysis**

Comparisons between groups were made with Wilcoxon test for quantitative variables and Fisher’s exact test for qualitative variables. A random effect linear model was used to evaluate forced vital capacity (FVC) and diffusing capacity for carbon monoxide (DLCO) changes over time. Survival was analysed with the Kaplan–Meier method, using the date of ILD diagnosis as baseline (including for cases diagnosed in childhood). All statistical analyses were performed using the RStudio software (RStudio, version 2022.02.0 Build 443).

# **RESULTS**

## **Study population and characteristics**

Fifty-four patients were initially reported by clinicians (Figure S1 in the Supporting Information). Eighteen patients were excluded: 11 with a variant in SFTPC and 7 with variants in ABCA3. Reasons for exclusion in the SFTPC group were death before the age of 18 years (n=1), lack of data available (n=3), lack of data available prior to lung transplantation (n=2), SFTPC variant of uncertain significance (class 3, n=3) and absence of ILD in asymptomatic carriers (n=2). Reasons for exclusion in the ABCA3 group were the presence of only monoallelic variant of ABCA3 (n=6) and ABCA3 variants of uncertain significance (class 3, n=1). Thirty-six patients from 29 families were included (22 in the SFTPC group and 14 in the ABCA3 group), 12 of whom belonging to 5 kindreds (all in the SFTPC group). Eight patients diagnosed before the age of 18-years had reached the adult age and were included (five in the SFTPC group and three in the ABCA3 group). Only one patient (in the SFTPC group) presented with an asymptomatic ILD, discovered during familial screening. Six patients 25–30 were previously reported.

## **Genetic findings**

Variants in SFTPC are reported in Table S1 in the Supporting Information. The most frequent mutation was the p.(Ile73Thr) (12 patients in 10 families, 54.5%). Variants in ABCA3 are reported in Table S2 in the Supporting Information. Three patients (21%) had homozygous pathogenic variants: p.[(Gly974Asp;Ala1027Pro)], p.(Thr1173Arg) and p.(Asp253His),

respectively. The other patients were compound heterozygous. The most common variant was p.(Glu292Val), found in five patients (35.7%).

## Clinical findings

The main clinical characteristics are reported in Table 1. Median age of ILD diagnosis was 38.9 years (IQR 23.2–47.6 years) in the SFTPC group and 31.5 years (IQR 19.1–40.6 years) in the ABCA3 group ( $p=0.24$ ). Both groups were similar except for sex (77% of males in the SFTPC group vs. 21.4% in the ABCA3 group,  $p=0.002$ ) and the familial history of ILD (81.8% in the SFTPC groups vs. 28.6% in the ABCA3 group,  $p=0.004$ ), compatible with the autosomal dominant pattern of inheritance of SFTPC variants. Dyspnoea at presentation and inspiratory crackles were common on examination, found each in 86.1% of the patients. Cough and clubbing were present in more than half of the cases. Seven patients (19.4%) had pectus excavatum. Overall, 27.8% of patients were smokers or former smokers.

**TABLE 1** Demographics and clinical findings.

	Overall population (n = 36)	SFTPC (n = 22)	ABCA3 (n = 14)	p-value
<b>Demographics</b>				
Age at diagnosis of ILD (years), median (IQR)	34 (19.3–44.2)	38.9 (23.2–47.6)	31.5 (19.1–40.6)	0.24
Male sex (%)	20 (55.6)	17 (77)	3 (21.4)	0.002
Premature birth (%)	1 (2.8)	0 (0)	1 (7.1)	0.39
Respiratory symptoms during childhood (%)	11 (30.6)	6 (27.3)	5 (35.7)	0.72
History of neonatal respiratory distress (%)	5 (22.7)	2 (9.1)	3 (21.4)	0.36
Familial history of ILD (%)	22 (61.1)	18 (81.8)	4 (28.6)	0.004
Ever smoker (%)	10 (27.8)	7 (31.8)	3 (21.4)	0.71
Pack-year, mean (SD)	3 (7.5)	3 (6.1)	3 (9.8)	0.52
<b>Clinical findings</b>				
Dyspnoea at presentation (%)	31 (86.1)	18 (81.8)	13 (92.9)	0.63
Cough (%)	19 (52.8)	10 (45.5)	9 (64.3)	0.32
Crackles (%)	31 (86.1)	19 (86.4)	12 (85.7)	1.0
Clubbing at any time (%)	20 (55.6)	11 (50)	9 (64.3)	0.5
Pectus excavatum (%)	7 (19.4)	2 (9.1)	5 (35.7)	0.08

Abbreviation: IQR, interquartile range.

## Pulmonary function tests

Baseline PFTs are reported in Table 2. Out of the 35 patients with available FVC at baseline, 29 (82.9%) had a FVC <80% predicted (15 out of 21 in the SFTPC group and all 14 in the ABCA3 group). Two patients presented an obstructive pattern. Twenty-eight patients had available baseline DLco, which was <80% predicted for 27 (96.4%) of them. Forced expiratory volume in 1 second (FEV1, % predicted) and DLco were lower in the ABCA3 group as compared to SFTPC (Table 2).

## Radiological findings

The chest CT characteristics of 34 patients are provided in Table 3, and representative examples are showed in Figures 1 and 2.

In 85.3% of patients, the ILD pattern was categorized as unclassifiable, including 44.1% percent of patients with a non-classifiable pattern with predominance of GGO, 8.8% with an unclassifiable pattern with predominance of cysts, and 32.4% with an unclassifiable pattern with both GGO and cysts. Other patterns were found in five patients (14.7%): two patients



with a pattern suggesting PPFE, one with a radiological pattern of fibrosing nonspecific interstitial pneumonia (NSIP) (Figure 2E), one with cystic changes with minimal fibrosis in the SFTPC group and one with a pattern of both PPFE and GGO in the ABCA3 group. There was no significant difference of predominant patterns between groups. Pulmonary cysts, present in all CT scans except two, were oval or irregularly shaped (Figure 1). In half of the patients, cysts showed an upper lobe predominance, with no difference between groups. GGO were present in 31 patients (91.2%), were patchy in 21 patients (67.7%) and colocalized with the cysts in most of the cases (Figure 1), with no difference between groups. Thirteen patients (38.2%) had emphysematous lesions on CT scan with random distribution. The presence of emphysema was not associated with tobacco use ( $p=0.17$ ) or age at diagnosis of ILD ( $p=0.85$ ). Features of lung fibrosis, including bronchial distortion and reticulations (Figure 1), were present in 91.2% of patients. Honey combing was absent from all CT scans. Fibrotic lesions were reported as the main feature on CT scan in only five patients (14.7%), all in the SFTPC group. Upper lobe sub-pleural consolidations evoking PPFE lesions were seen in 30 patients (88.2%; Figure 2C, D, F).

**TABLE 2** Baseline pulmonary function tests.

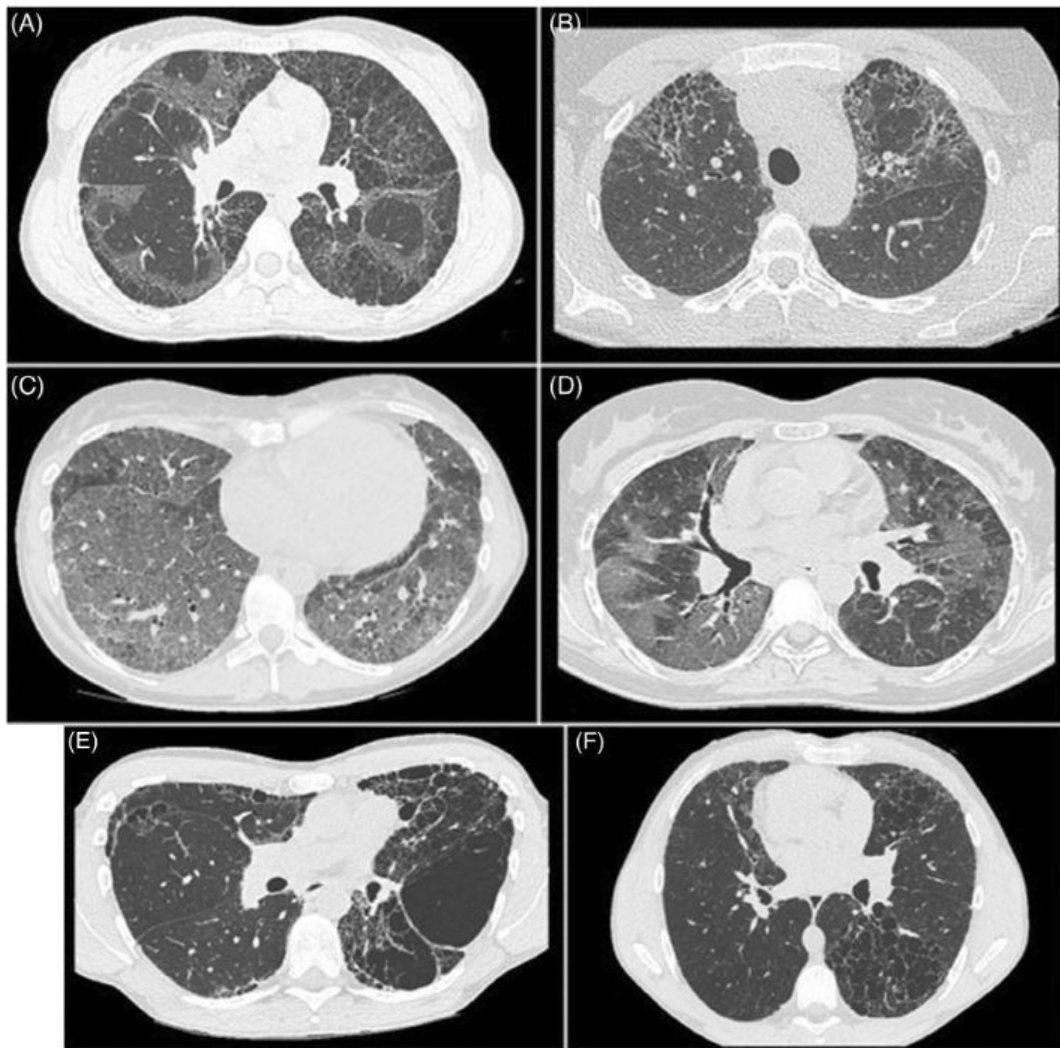
Pulmonary function tests, median (IQR)				
	Overall population ( <i>n</i> = 36)	SFTPC ( <i>n</i> = 22)	ABCA3 ( <i>n</i> = 14)	<i>p</i> -value
Time from diagnosis of ILD to baseline PFT (years)	0.8 (0.2–8.7)	0.8 (0.3–7.9)	0.8 (0.2–7.6)	0.84
FVC (L) ( <i>n</i> = 32)	2.47 (1.89–3.0)	2.86 (2.21–3.22)	2.09 (1.63–2.54)	-
FVC %predicted ( <i>n</i> = 35)	59 (52–72)	66 (45–80)	55.5 (52–62.5)	0.17
FEV <sub>1</sub> (L) ( <i>n</i> = 30)	2.02 (1.55–2.51)	2.48 (1.89–3.13)	1.79 (1.24–2.05)	-
FEV <sub>1</sub> %predicted ( <i>n</i> = 30)	63.2 (48.3–73.3)	71 (54.8–86.8)	55 (43.3–60.3)	0.01
FEV <sub>1</sub> /FVC ( <i>n</i> = 30)	0.9 (0.78–0.92)	0.9 (0.81–0.92)	0.8 (0.74–0.88)	0.093
TLC (L) ( <i>n</i> = 26)	4.14 (3.15–4.59)	4.25 (3.99–5.17)	3.44 (2.81–4.29)	-
TLC %predicted ( <i>n</i> = 29)	65 (59–76)	65 (55.3–78.3)	64 (60.5–73.5)	0.89
DL <sub>CO</sub> %predicted ( <i>n</i> = 28)	44 (35.4–50)	46 (39.5–53.5)	36.1 (26–38)	0.01
KCO %predicted ( <i>n</i> = 23)	67.8 (52.4–82.5)	76 (63.3–91.5)	52.8 (36–68)	0.11

Abbreviations: DL<sub>CO</sub>, diffusing capacity for carbon monoxide; FEV<sub>1</sub>, forced expiratory volume in 1 second; FVC, forced vital capacity; IQR, interquartile range; KCO, carbon monoxide transfer coefficient; TLC, total lung capacity.

**TABLE 3** Radiological features.

Feature		Overall population ( <i>n</i> = 34)	SFTPC ( <i>n</i> = 20)	ABCA3 ( <i>n</i> = 14)	<i>p</i> -value
Predominant pattern	Unclassifiable pattern—GGO, <i>n</i> (%)	15 (44.1)	6 (30)	9 (64.3)	0.24
	Unclassifiable pattern—cysts, <i>n</i> (%)	3 (8.8)	2 (10)	1 (7.1)	
	Unclassifiable pattern—mixed, <i>n</i> (%)	11 (32.4)	8 (40)	3 (21.4)	
	Others [see text], <i>n</i> (%)	5 (14.7)	4 (20)	1 (7.1)	
Pulmonary cysts, <i>n</i> (%)		32 (94.1)	19 (95)	13 (92.9)	1.0
Emphysema, <i>n</i> (%)		13 (38.2)	8 (40)	6 (42.9)	1.0
GGO, <i>n</i> (%)		31 (91.2)	17 (85)	14 (100)	0.25
Enlarged mediastinal lymph nodes, <i>n</i> (%)		23 (67.6)	11 (55)	12 (85.7)	0.08
Fibrosis	Bronchial distortion, <i>n</i> (%)	23 (67.6)	15 (75)	8 (57.1)	0.46
	Reticulations, <i>n</i> (%)	28 (82.4)	16 (80)	12 (85.7)	1
	Fibro-elastosis features, <i>n</i> (%)	30 (88.2)	18 (90)	12 (85.7)	1
Extent of ILD	0%–25%, <i>n</i> (%)	12 (35.3)	6 (30)	6 (42.9)	0.32
	26%–50%, <i>n</i> (%)	9 (26.5)	7 (35)	2 (14.3)	
	51%–75%, <i>n</i> (%)	7 (20.6)	5 (25)	2 (14.3)	
	76%–100%, <i>n</i> (%)	6 (17.6)	2 (10)	4 (28.6)	

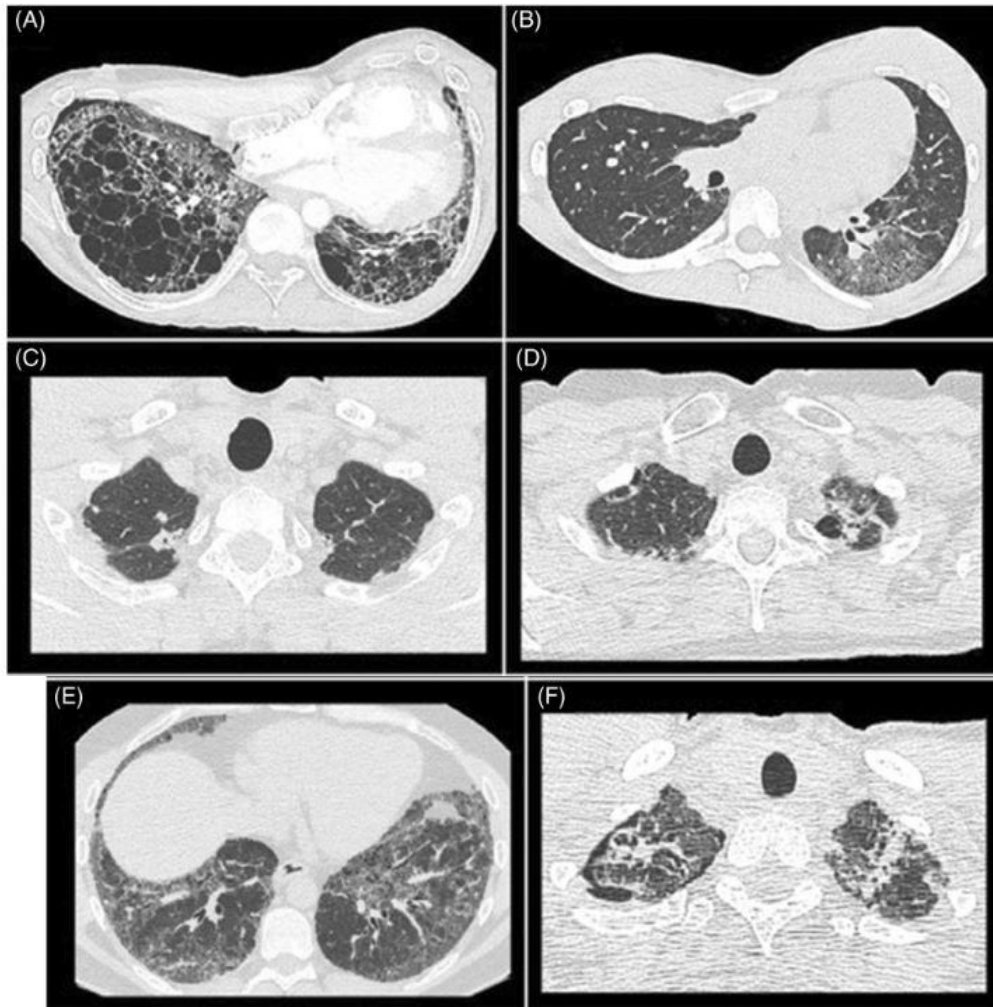
Abbreviations: GGO, ground-glass opacities; ILD, interstitial lung disease.



**FIGURE 1** Chest computed tomography patterns of interstitial lung disease (ILD) in patients with variant(s) in *SFTPC* or *ABCA3*. (A,B): pattern of 'unclassifiable fibrosing ILD with mixed GGO and cysts' (uILD-mixed) in the *ABCA3* group (A) or *SFTPC* group (B); (C,D): pattern of 'unclassifiable fibrosing ILD with predominant GGO' (uILD-GGO), both in the *ABCA3* group; (E,F): pattern of 'unclassifiable fibrosing ILD with predominant cysts' (uILD-cysts), in the *ABCA3* group (E) or *SFTPC* group (F). Emphysematous lesions and pectus excavatum can be seen in E. GGO, ground-glass opacities.

## Histopathological findings

Seventeen patients (47.2%) underwent video-assisted thoracoscopic surgical lung biopsy, nine in the *SFTPC* group and eight in the *ABCA3* group. Data from an additional patient in the *SFTPC* group were available on the explanted lung after transplantation. Fifteen histopathological results (9 in the *SFTPC* group and 7 in the *ABCA3* group) were included in the analysis because one biopsy in each group were performed in patients <18-year-old at the time and therefore censored. The most frequent histopathological pattern observed was NSIP (one patient in the *SFTPC* group and five in the *ABCA3* group). Usual interstitial pneumonia (UIP) was reported in three patients, and end-stage fibrotic lung disease in two patients (all in the *SFTPC* group). Additional reported findings included inflammatory fibrosis, emphysema, cysts and fibroelastosis foci (Tables S1, S2 and S3 in the Supporting Information). Cysts were described as subpleural with either thin walls or walls thickened by fibrosis. Two patients had accessory histological features of alveolar proteinosis. No patient had available data regarding bronchoalveolar lavage fluid staining with periodic acid-Schiff.



**FIGURE 2** Chest computed tomography ancillary finding in patients with interstitial lung disease associated with variant(s) in *SFTPC* or *ABCA3*. (A,B): pectus excavatum (both in the *SFTPC* group); (C,D): apical caps evoking pleuroparenchymal fibroelastosis lesions in the *SFTPC* group (C) and the *ABCA3* group (D); (E): nonspecific interstitial pneumonia pattern in the *SFTPC* group; (F): pleuroparenchymal fibroelastosis lesions and right pneumothorax in the *ABCA3* group.

## Outcomes

The median follow-up duration was 9 years (IQR 3–14.5 years) in the overall population, 7.7 years (IQR 2.4–11.3 years) in the *SFTPC* group and 10.6 years (IQR 4.6–15.9 years) in the *ABCA3* group. Drugs prescribed for ILD are reported in Table S4 in the Supporting Information. Nine patients (25% underwent lung transplantation (eight in the *SFTPC* group and one in the *ABCA3* group). Median time from diagnosis to transplantation was 3.2 years (IQR 2.4–9 years). Five patients (four in the *SFTPC* group and one in the *ABCA3* group) died without undergoing lung transplantation. Death was attributed to acute exacerbation of ILD (n=3), acute respiratory insufficiency due to bilateral pneumothorax (n=1) and evolution of the ILD (n=1). Median time from diagnosis to death was 5.7 years (IQR 2.1–9.5 years) in the overall population. Figure 3 shows the survival estimates of the two groups from diagnosis to a composite event of death or transplantation. Median time to the composite event was 10 years in the *SFTPC* group and was not reached at the end of follow-up in the *ABCA3* group. Survival to death or lung transplantation tended to be worse in the *SFTPC* group but the difference did not reach statistical significance (p=0.07)

Follow-up data on FVC were available for 21 patients in the *SFTPC* group and 14 patients in the *ABCA3* group while follow-up data on DLCO were available for 20 patients in the *SFTPC* group



and 13 patients in the ABCA3 group. Figure S4 in the Supporting Information shows the individual decline of lung function in both groups. Using random effect linear model, FVC declined annually by 1.87% of predicted (CI95% [2.58; 1.16],  $p < 0.001$ ) in the SFTPC group and by 0.72% of predicted (CI95% [1.43; 0.004],  $p = 0.048$ ) in the ABCA3 group (absolute change,  $p = 0.014$  for comparison between groups). DLCO declined annually by 2.43% of predicted (CI95% [3.24; 1.62],  $p < 0.001$ ) in the SFTPC group and by 0.95% of predicted (CI95% [1.83; 0.07],  $p = 0.03$ ) in the ABCA3 group (absolute change,  $p = 0.004$  for comparison between groups).

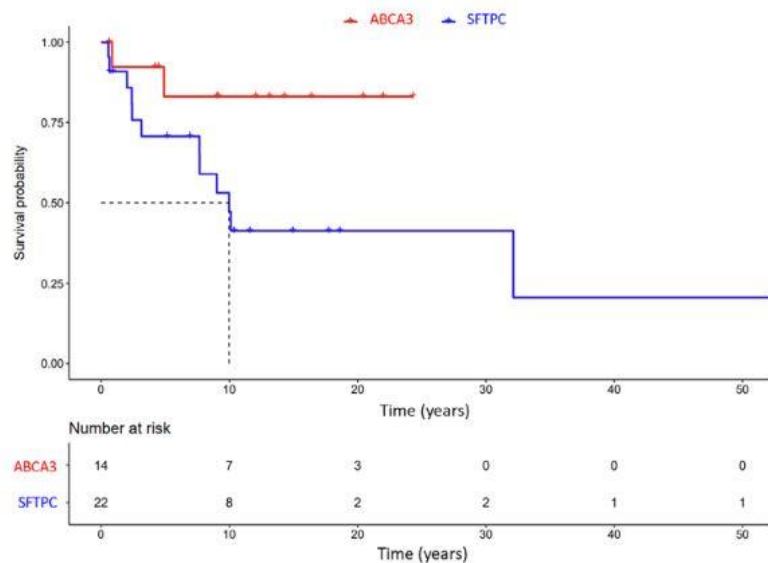
Fifteen patients (41.6%) presented at least one exacerbation of ILD (45.5% in the SFTPC group vs. 35.7% in the ABCA3 group,  $p = 0.73$ ). In eight patients, pulmonary hypertension was diagnosed by RHC (seven in the SFTPC group, 31.8%; one in the ABCA3 group, 7.1%,  $p = 0.12$ ).

## DISCUSSION

The present study describes the characteristics of adult patients with ILD associated with pathogenic variants in SFTPC or ABCA3. The main findings of the study were that SFTPC- and ABCA3-associated ILD shares unusual CT features distinct from UIP, with three predominant radiological patterns combining fibrotic features with GGO and cysts in a young adult, yet with differences between SFTPC and ABCA3.

Despite good descriptions in paediatric series, few studies to date have reported ILD associated with SFTPC or ABCA3 pathogenic variants in adults. Therefore, one purpose of our study was to provide information to pulmonologists to better recognize the possibility of ILD caused by such variants.

**FIGURE 3** Survival analysis. Kaplan–Meier survival analysis showing time to death or lung transplantation of the 36 patients with ILD associated with SFTPC ( $n = 22$ ) or ABCA3 ( $n = 14$ ) variant(s). Age at diagnosis was used as a starting point.  $p = 0.07$  between the two curves (log-rank test).



SP-C is a small, extremely hydrophobic, valine-rich protein of 35 amino-acid residues.<sup>31, 32</sup> Its hydrophobic properties help to stabilize surfactant at low lung volumes, thus preventing alveolar end-expiratory collapse. The SFTPC gene, located on chromosome 8, encodes a 197-amino-acid precursor protein selectively expressed in the alveolar type II epithelial cells of the lung alveoli.<sup>33</sup> The COOH flanking domain of the precursor protein can be separated into a 35 amino acids linker domain and a 104 amino acids BRICHOS domain. Mutations in the BRICHOS domain of other proteins have been linked to various diseases including dementia, chondrosarcoma and stomach cancer.<sup>34</sup> The ABCA3 gene encodes a 1704-amino acids protein consisting of two homologous sequences comprising 12 transmembrane helices, the signature ATP-binding cassette motif, two nucleotide binding domains, two regulatory domains and two extracellular domains.<sup>35, 36</sup> ABCA3 is involved in the formation of

lamellar bodies and is located at their outer membrane where it drives the transportation of phospholipids. 37

Variants in SFTPC induce different cellular effects depending on the localization of the mutation. Proposed mechanisms of cellular injury include blockade of macroautophagy, 38 impairment of endosomal machinery<sup>39</sup> and induction of endoplasmic reticulum stress. Mutations in the BRICHOS domain alter the correct SP-C folding and result in aggregation in endosomal compartments, also inducing endoplasmic reticulum stress.<sup>40</sup> In line with a dominant dysfunction resulting in cell toxicity, all the pathogenic variants identified in SFTPC in our study were missense ones, except one three amino-acid deletion and one late frameshift mutation (p.(Ser 153 Leu fs\*27)) that may escape nonsense-mediated mRNA decay and result in the production of a moderately truncated protein. Variants in ABCA3 can affect the cellular localization, the function of the protein or both.<sup>41</sup> Some variants lead to retention of the mutant protein in the endoplasmic reticulum, inducing endoplasmic reticulum stress and cellular toxicity, <sup>41, 42</sup> whereas others impair lipid transport<sup>43</sup> or ATPase activity.<sup>44</sup>

In the study by van Moersel et al. the authors identified SFTPC variants in 5 adults in a cohort of 20 patients with familial pulmonary fibrosis, including the p.(Ile73Thr) variant in three patients.<sup>11</sup> The chest CT scans of all variant carriers showed unclassifiable patterns of ILD, reported as cystic ILD with diffuse GGO and nodular septal thickening. The histological analysis available in four patients showed a UIP pattern in all.<sup>11</sup>

To our knowledge, only 12 adult patients with ILD associated with bi-allelic variants in ABCA3 were previously reported.<sup>4, 14, 25, 26, 29, 45, 46</sup> Consistent with our findings, chest CT scan often showed diffuse GGO with or without super-imposed reticulonodular opacities. Wambach et al. suggested that the clinical phenotype of lung disease associated with ABCA3 mutations relates to the type of mutation. Null/null ABCA3 genotypes seem incompatible with life, while null/other or other/other genotypes display phenotypic heterogeneity.<sup>47</sup> In line with this assessment, no patient in our study had a null/null genotype. Of note, 5 out of 14 patients carried the pathogenic hypomorphic p.(Glu292-Val) mutation in the compound heterozygous state. This is in keeping with the relatively high frequency of this variant in the general population (e.g., about 0.86% of controls from European descent are heterozygous for this variant in the gnomAD database).

Although the imaging features did not belong to an existing category, we were able to subcategorize radiologic patterns of fibrosing ILD according to the predominance of GGO, cysts or both. Almost all patients presented GGO and pulmonary cysts. It has been previously hypothesized that ground-glass opacities on chest CT scan in children with SFTPC variants are the radiological expression of alveolar septal thickening, alveolar accumulation of macrophages and type II pneumocytes hyperplasia, whereas cystic lesions may be related to dilatation of the bronchioles and alveolar ducts.<sup>48</sup> Moreover, sustained chronic lung inflammation and activation of the NFκB pathway could explain the fibrotic phenotype observed in most patients.<sup>16, 49</sup> Emphysema was a common feature and may lead to combined pulmonary fibrosis with emphysema syndrome, as previously described in adults with SFTPC or ABCA3 variants.<sup>27, 29, 50</sup> Emphysema was not associated with tobacco use or age at diagnosis, suggesting different pathogenic effects in patients sharing the same variant. Additionally, alveolar injury could also contribute to emphysema.<sup>51</sup>

Of note, two seemingly different subsets of patients can be distinguished based on imaging: some patients display retraction of the lung, with reduced lung volumes and chest wall deformity, and some patients display lung distension from cysts and emphysema (Figure S2 in

the Supporting Information). No patient presented a UIP pattern on chest CT. Although few histological results were available, only a small number of patients had histologic UIP. Therefore, ILD associated with mutations in SFTPC and ABCA3 can generally be distinguished from IPF. Interestingly, there seems to be a continuum between the centrilobular and perilobular in filtration (i.e., GGO and reticular pattern). We hypothesize that imaging abnormalities may follow the subsequent progression: GGO, then cystic changes often intertwined with reticulations, possibly leading to emphysema and/or established fibrotic changes (Figure S3 in the Supporting Information).

Based on our results, we propose that a diagnosis of ILD associated with variants in SFTPC or ABCA3 should be suspected in a patient presenting with:

- A relatively young age at diagnosis (before the seventh decade),
- A CT pattern of fibrosing ILD with pulmonary cysts and GGO,
- A familial history of ILD (for SFTPC variants, or for ABCA3 variants in inbred individuals).

In our series, ILD was severe with a significant number of patients experiencing disease progression to death or lung transplantation, and nearly half of them had an acute exacerbation of ILD. However, the median survival was 10 years after diagnosis in patients with SFTPC variant, while it is less than 5 years in IPF.<sup>52</sup> Patients with variants in SFTPC more often presented with a fibrosing phenotype than those with ABCA3 variants, and more frequently received antifibrotic medications (Table S4 in the Supporting Information).<sup>6, 9, 11, 12</sup>

Patients with ABCA3 variants tended to be younger at ILD onset and more severe but with better prognosis. These results are consistent with the recent reported Dutch cohort of 12 SFTPC and 3 ABCA3 adult variant carriers with ILD.<sup>53</sup> Moreover, they indicate that further research is warranted to develop specific targeted therapies based on both the genotype and the pulmonary phenotype.

Although this study is the first to characterize a sizable number of adult patients with ILD associated with variants in both these genes, it is however limited by its retrospective design and heterogeneity. Whilst chest imaging was centrally reviewed, lung biopsies were not. Functional assessment of the variants was not performed. Respiratory functional decline analysis must be interpreted with caution, owing to the lack of data for a proportion of patients and the small observed difference between groups, which can be skewed by intra-individual measurement variability. The changes of clinical practices over the large period of data collection, the presence of patients diagnosed during childhood or adulthood and the heterogeneity of the treatment strategies used in this study population hinder its ability to describe the effect of treatments. Prospective studies are needed to define the best possible course of action in managing these patients.

In conclusion, variants in the surfactant-related genes SFTPC and ABCA3 are associated with a non-UIP phenotype of fibrotic ILD in adult patients, with severe prognosis. A radiologic pattern of fibrosing ILD with ground-glass opacities and/or cysts is frequently found in these rare conditions.

## AUTHOR CONTRIBUTIONS

**Rémi Diesler:** Conceptualization (lead); data curation(lead); formal analysis (lead); investigation (lead); methodology (lead); project administration (lead); supervision (lead);validation (lead); visualization (lead); writing–original draft(lead); writing–review and editing (lead).

**Marie Legendre:** Conceptualization (supporting); data curation (equal);investigation (supporting); writing–review and editing(supporting).

**Salim Si-Mohamed:** Investigation (equal); methodology (supporting); writing–review and editing(supporting).

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Rémi Diesler (0000-0003-0746-357X) reported support for attending meetings and/or travel by Asdia.

Pierre-Yves Brillet reported payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events, support for attending meetings and/or travel by Boehringer Ingelheim and Sanofi, and participation on a Data Safety Monitoring Board or Advisory Board for Boehringer Ingelheim.

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## **DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## **HUMAN ETHICS APPROVAL DECLARATION**

The study was registered in the Commission Nationale Informatique et Liberté of the Hospices Civils de Lyon and approved by the Institutional Review Board (number21\_636). All patients signed an informed consent for genetic analysis.

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