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Surfactant Protein C Gene (*SFTPC*) Mutation–Associated Lung Disease: High-Resolution Computed Tomography (HRCT) Findings and its Relation to Histological Analysis

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Running title: HRCT and histological finding of *SFTPC* mutations

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Abstract:***Aim of the study***

Determine high-resolution tomography (HRCT) scan characteristics in children with *SFTPC* mutation and correlate them to histological findings.

Patients and Methods

This retrospective multicenter study included 15 children (7 females and 8 males) with *SFTPC* mutations. HRCT scans have been performed in all the children and lung biopsies in 8 children.

Results

From all signs assessed on initial HRCT scans, ground-glass opacities (n=14, 93 %) and lung cysts (n=6, 40 %) were predominant. Interlobular septal thickening (n=1, 7 %), air space consolidation (n=1, 7 %), paraseptal emphysema (n=2, 13 %), and pulmonary nodules (n=1, 7 %) were also found. Histological analysis revealed accumulation of macrophages in the alveolar lumen, type II pneumocyte hyperplasia, and alveolar septal thickening. Dilatation of the respiratory bronchiole and alveolar duct associated with muscular hyperplasia were also described. Interestingly, lung cysts on HRCT scans were associated with dilatation of terminal bronchioli and alveolar duct in lung biopsies.

Conclusion

In children with *SFTPC* mutations, HRCT scan finding were highly correlated to the histological findings and, as such, represent a useful tool to identify patients that may require *SFTPC* gene sequencing.

Introduction:

Idiopathic forms of interstitial lung disease (ILD) in children are associated with a wide spectrum of underlying pathologies. Until recently, a single classification system has been used, regardless of age, and children with ILD were forced into published adult disease classification systems¹. In 2007, a multi-disciplinary working group of pediatric pathologists, clinicians and radiologist (the Children's Interstitial Lung Disease (chILD) Research Cooperative) designed and conducted a retrospective clinical, radiographic and histopathologic review and proposed a new classification system for children with diffuse lung disease (chILD syndrome)². In this classification, surfactant genetic abnormalities account for more than 10 % of the patients reviewed. Pulmonary surfactant is a mixture of phospholipids and specific proteins secreted by type II epithelial cell to reduce the alveolar surface tension at the air-liquid interface and prevent end-expiratory atelectasis³. The hydrophobic surfactant protein SP-C, play an important role in the spreading and stabilization of phospholipids films in the alveolus³ highlighted by lung disease caused by inherited abnormalities in its genes^{4,5}. Since the first description by Nogee et al., more than 35 dominantly expressed mutations in the *SFTPC* gene have been identified in association with acute or chronic lung disease in patients ranging in age from newborn to adults⁶⁻¹⁰. These mutations, whether inherited or spontaneous are always found in an autosomal dominant form, the most frequently observed (nearly 50 %) being p.[Ile73Thr] or I73T). The phenotype associated with *SFTPC* mutations is eminently variable. Indeed, neonatal forms leading to death in the first years of life as well as childhood and adult forms with chronic respiratory disease have been observed. The effects of *SFTPC* mutations on surfactant homeostasis have not been clearly elucidated. In an *in vitro* study off cells with a *SFTPC* deletion, accumulation of misfolded proSP-C in the Endoplasmic reticulum (ER) causes disruption of the ubiquitin/proteasome system¹¹, activates apoptosis and interferes with non mutated protein

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3 synthesis. It results in the release of pro-inflammatory cytokines and enhances T-cell and
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5 fibroblast recruitment ¹².
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8 Although consensus exists on the need to consider early open lung in children with ILD,
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10 genetic testing for *SFTPC* mutation has become widespread and may be performed relatively
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12 quickly. In addition, recent advances in imaging techniques have contributed to more
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14 efficiently predict histological findings. Indeed, HRCT has been proposed for either diagnosis
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16 or following ILD precluding the need to obtain a surgical biopsy ^{13,14}. Thus, the aim of the
17
18 present study was to determine HRCT features of children with *SFTPC* mutation and to bring
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20 new insight in the correlation between HRCT and histological findings. To address these
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22 questions HRCT finding of a cohort of 15 children with interstitial chronic lung disease
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24 related to *SFTPC* gene mutations were retrospectively reviewed and subsequently correlated
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26 with histological analysis when lung biopsies were available.
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Patients and Methods

This study is a retrospective study performed in 2 university hospital-based pediatric pulmonology practice (Hôpital Armand Trousseau and Hôpital Necker enfants-malades, Paris, France) for a 15-year period (1993-2008). The protocol was accepted by the Committee for the protection of individuals in Biochemical research as required by French legislation and written informed consent was obtained from the patients or their next of kin included in this study.

Patients

The characteristics of patients are summarized in Table 1. Fifteen children, 7 females and 8 males with a definitive diagnosis of *SFTPC* gene mutation were included. All the patients were immunocompetent and screening for metabolic, infectious, and immunological etiologies was negative. Genetic and clinical characteristics of all these patients have been described in detail in previous reports^{8,15}. Three patients, members, or relatives of a same family, have also been reported previously⁶. Median age of onset was 2 (range 0-18) months. Three patients had neonatal respiratory distress, and symptom onset was associated with acute bronchiolitis in 7 (47 %) patients. Cough, tachypnea, and gastroesophageal reflux and failure to thrive were initially noticed in all the children. Physical examination at presentation revealed tachypnea and retraction in all patients. Low oxygen saturation (<95%) was observed initially in all patients. Treatment are summarized in table 1 and included methylprednisolone pulse (n=15), hydroxychloroquin (n=8) and azithromycin (n=5). Median follow-up was 3.1 (1-17.3) years. At the end of the study, all the children were alive but 11 patients (73 %) were still symptomatic with cough, dyspnea or exercise intolerance.

HRCT scan and image analysis

CT examinations were performed with two helical scanners and two multidetector CT scanners. In Armand Trousseau hospital, Elscint (CT Twin) with scan acquisition at 120 kV, 120 mAs and after 2003 a Philips (Mx 8000 IDT32) 16 slice multidetector CT was used with scan acquisition at the 90kV, 90mAs. In Necker Hospital, General Electric helical CT (Prospect) with scan acquisition at 140kV, 130mAs, and after 2005 a General Electric (LightSpeed) 64 slice multidetector CT was used with scan acquisition at the 100kV, 100mA. All the patients underwent non-contrast-enhanced incremental (at intervals of 3 to 10 mm) or helical HRCT scan with a collimation of 16 X 1mm. Scans were obtained from the level of apices to the diaphragm. Image reconstruction was performed with a high special frequency algorithm. All CT scans were performed before the diagnosis, and none of the patients were receiving treatment at the time of first HRCT. The HRCT scans (initial and follow-up) were reviewed and interpreted by 2 experienced pediatric radiologists who arrived at their interpretations by consensus without knowledge of histological parameters. HRCT scan analysis was performed with reference to a conventional model in line with Austin's definition

The scans were assessed for the presence, extent, and distribution of elementary lesions, such as areas of ground-glass opacity, lung cysts, lung consolidation, interlobular and intralobular septal thickening, thickening of bronchovascular bundles, and nodules (centrolobular or other). The anatomical distribution was classified as central (a predominance of abnormalities in the inner two-thirds of the lung), peripheral (a predominance of abnormalities in the outer one-third of the lung or along the interlobar fissures), or random (no central or peripheral predominance). The zonal predominance of abnormalities was assessed as being upper, intermediate lower, or random. Upper lung zone predominance was considered present when the extent of abnormalities was greatest above the level of the

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3 tracheal carina; intermediate was considered between the carina and inferior pulmonary veins,
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5 and lower zone predominance was considered present when disease extent was greatest below
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7 this level. Specific involvement or sparing of the subpleural region (<1cm from the pleura)
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9 was assessed. Ground-glass opacities describe lung that is higher in attenuation than a normal
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11 lung on inspiration but lower than the attenuation of bronchial walls and blood vessels so that
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13 these lung markings remain visible¹⁶. The term lung cyst is used to refer to a well-defined,
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15 rounded, and circumscribed lesion, with a wall that may be uniform or varied in thickness but
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17 which is usually thin (with thickness of less than 3 mm)¹⁷. Because this study was
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19 retrospective, the intervals of follow-up HRCT scanners were highly centre-dependent and
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21 based on the decision of the physician in charge of the child. Only HRCT scanners available
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23 were taking into consideration for analysis. Control HRCT scans were obtained from 6 age-
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25 matched patients with a definitive diagnosis of *ABCA3* (ATP-binding cassette subfamily A,
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27 member 3) mutations, a gene expressed in lamellar bodies of alveolar type II cells which has a
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29 critical role in surfactant storage and homeostasis¹⁸. Among them, 2 patients carried
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31 homozygous missense mutations, 3 patients carried compound heterozygous missense
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33 mutations and 1 child had compound heterozygous missense and nonsense mutations.
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35 Median age at HRCT scans was 2 months (range 1 month to 12 years).
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46 ***Lung biopsies and their analysis***

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48 Surgical lung biopsies ranging from 0.5 to 1cm were obtained from different lobes in 8
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50 children at the age of 3 to 7 months (mean age, 4 months) before treatment onset. Biopsy sites
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52 are summarized in Table 1. Specimens were further fixed in formalin and embedded in
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54 paraffin. A five-micrometer tissue section was stained with H and E stain (hematoxylin and
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56 eosin stain). Slides were examined by 2 pathologists. Radiology-pathology correlation was
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58 performed in 8 cases. All 8 children had only 1 open lung biopsy.
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Results:

Initial HRCT findings

The median age at initial HRCT scanner was 4 (range 1-40) months (one child had the scanner performed 40 months after the diagnosis for technical reasons). High-resolution CT findings are summarized in Table 2. The most frequent abnormalities identified on initial HRCT were ground-glass opacities (93 %) and lung cysts (40 %). Other abnormalities noted included interlobular septal thickening (7 %), air space consolidation (7 %), paraseptal emphysema (13 %), and pulmonary nodules (7 %). In comparison, ground-glass opacities were constantly observed in all of the 6 patients with ABCA3 mutations. However, opacities were more prominent with presence in some cases of alveolar condensations.

Bilateral ground-glass opacities were shown in 14 children (Table 3), either as an isolated finding (60 %) or as part of a mixed pattern with lung cysts (33 %), air space consolidation (7 %), paraseptal emphysema, and centrilobular nodules (7 %). They were homogeneously diffuse in 9 cases (60 %) (Figure 1A). When a patchy distribution was present, this was noted to have basal subpleural predominance in 1 case (7 %), predominance for the lower lobes in 2 cases (13 %) (Figure 1B), a central predominance in 1 case (7 %), and a predominance for the posterior portions of the lung in 1 case (7 %).

Thin-walled (<1mm) lung cysts of varying sizes were seen in 6 infants (40 %) (Table 4), either as an isolated finding (7 %) (Figure 2A), or as a mixed pattern with areas of ground-glass opacities (33 %), paraseptal emphysema (13 %) (Figure 2B), septal thickening (7 %), and non-septal thickening (7 %). A predominantly subpleural distribution was seen in 1 child (7 %); diffuse cysts with random distribution was seen in 4 cases (27 %) and upper lung zone predominance was seen in 1 case (7 %).

Follow-up HRCT scan

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3 Follow-up CT scans were performed on 11 of the 15 children. The median follow-up interval
4 was 8 (range 3-12) months. Eight patients had 1 follow-up HRCT scan, 2 patients had 2
5 follow-up HRCT scans (3 and 9 months and 4 and 9 months, respectively), and 1 patient had 3
6 follow-up HRCT scans (3, 6 and 12 months). Evaluation of the follow-up HRCT scans (Table
7 5) revealed the development of lung cysts in 5 patients (45 %) who had previously only had
8 ground-glass opacities (Figures 3A and 3B). These new cystic changes were associated with a
9 stable pattern of ground-glass opacities in 2 cases, decrease in the degree of ground-glass
10 opacities in 1 case, and an aggravation of this pattern in 2 cases. These cysts were diffuse in
11 all cases but showed a predilection for different regions of the lung: upper lobes (n=1), basal
12 subpleural (n=1), peripheral (n=1), and central (n=1). In 1 case, lung cysts were homogenous
13 and diffuse. Patients who had previously documented cysts on their initial HRCT scans also
14 demonstrated change when follow-up HRCT was performed. In 5 patients (45 %), we noted
15 an increase in the number or size of preexisting lung cysts (Figures 4A and 4B), which were
16 associated with stable ground-glass opacities (n=4), aggravated ground-glass opacities (n=1),
17 and progressive pulmonary fibrosis (n=1). The HRCT findings of pulmonary fibrosis
18 consisted of bilateral and diffuse honeycombing, septal and non-septal thickening, and
19 thickening of bronchovascular bundles and distortion of pulmonary fissures. In 1 patient, we
20 observed persistent isolated ground-glass opacities.

21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 ***Pathology findings***

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48 Eight of the 15 patients (53 %) underwent lung biopsy the median age at biopsy being 4
49 (range 3-7) months. Histological analysis revealed 2 main types of lesions. The first type
50 occurred in the alveolar lumen, where we observed the following changes: accumulation of
51 macrophages intermingled with few inflammatory cells and cholesterol cleft, type II
52 pneumocyte hyperplasia, and alveolar septal thickening by primitive mesenchymal cells and
53 lymphocytes either isolated or clustered (Figure 5A). These lesions were diffuse in 7 cases (90
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3 %) and patchy in 1 case (13 %). The second type of lesion corresponds to dilatation of the
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5 respiratory bronchiole and alveolar duct in 7 cases (88 %) associated with muscular
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7 hyperplasia in 2 patients (25%) (Figure 6A). There was no interstitial pulmonary fibrosis or
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9 alveolar proteinosis observed.
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12 13 14 15 ***Relation between HRCT and pathology findings***

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17 Radiological-pathological confrontation is summarized in Table 6. Ground-glass opacities on
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19 HRCT were observed in all the patients except 1 in which the CT scan was performed late (40
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21 months). All these children also have diffuse alveolar septal thickening by primitive
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23 mesenchymal cells and lymphocytes, intra-alveolar accumulation of macrophages, and type II
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25 pneumocyte hyperplasia. Small lung cysts were observed initially in half of the children but
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27 appeared during follow-up in all the cases. They were related with dilatation of the terminal
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29 bronchioles and alveolar duct which were noticed in all but one biopsy.
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36 **Discussion:**

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38 In the present study, we have shown for the first time in a large cohort of children with
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40 *SFTPC* mutation associated lung diseases that HRCT pattern were characterized with 2 main
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42 abnormalities: ground-glass opacities that were predominately diffuse but can be patchy in
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44 some cases and lung cysts with thin wall and different sizes.
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48 Ground-glass opacities have been commonly described in previous reports^{6,19,20}. They are
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50 predominately diffuse but can be patchy in some cases. In our series, they were present in all
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52 the patients except one in whom the HRCT scan was done late. We also note in our study the
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54 high frequency of cystic lesions on HRCT scan. They appeared in 45% of the children during
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56 follow-up and increased in number in 45%. Similar change during follow-up is typical of
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58 surfactant abnormality and was noted by Brody¹⁶. In our series, ground-glass attenuation
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3 decreased in intensity with age, whereas small lung cysts usually became more prominent
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5 over time.
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8 This HRCT presentation, namely the ground-glass opacities and cystic patterns is,
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10 nonetheless, not specific of *SFTPC* associated disorders. We have shown that this presentation
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12 may also be observed in children with *ABCA3* mutations as previously described ²¹. However,
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14 the high frequency of *SFTPC* associated disorders in children ILD ^{2,7}, the feasibility of *SFTPC*
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16 genetic analysis as well as the difficulty to perform lung biopsy in patients with fragile
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18 respiratory condition strongly encourage to perform *SFTPC* genetic screening before lung
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20 biopsy in children with ground-glass opacities associated with cystic lung changes on HRCT
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22 scanner.
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27 In the literature, there are histological studies of patients with *SFTPC* gene mutations showing
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29 4 histological patterns: desquamative interstitial pneumonitis (DIP), nonspecific interstitial
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31 pneumonitis(NSIP), (UIP) and pulmonary alveolar proteinosis ^{7,16,22} in children and usual
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33 interstitial pneumonitis (UIP) in adults. In children, DIP and NSIP have also been described in
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35 patients with mutations in the ATP-binding cassette transporter A3 (*ABCA3*) gene ²¹. Chibbar
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37 et al. showed that both children and adults with NSIP and UIP patterns, respectively, carried a
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39 common mutation within the *SFTPC* gene ²³. Thomas et al. described a mutation in *SFTPC*
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41 gene associated with large kindred with familial form of pulmonary fibrosis, including adults
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43 with the most common pathological subset of pulmonary fibrosis, UIP, and children with
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45 cellular NSIP ²⁴. Brasch reported histological patterns of NSIP and pulmonary alveolar
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47 proteinosis in a 13-month-old infant with a *de novo* heterozygous missense mutation of the
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49 *SFTPC* gene ²⁰. The histological findings were classified as DIP because of an increased
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51 number of macrophages in the alveolar lumen ²⁵ and as NSIP and UIP because of the
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53 numerous inflammatory cells in the alveolar septum ^{26,27}. In this study, we have related
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55 ground-glass opacities on HRCT to diffuse alveolar septal thickening by primitive
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3 mesenchymal cells and lymphocytes, intra-alveolar accumulation of macrophages, and type II
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5 pneumocytes hyperplasia. Small lung cysts were related to dilatation of the respiratory
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7 bronchioli and alveolar duct. On initial HRCT scan analysis, large cystic lung changes in the
8
9 periseptal and subpleural regions were that of subpleural emphysema. On lung biopsies,
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11 millimetric small cysts corresponding to a dilatation of respiratory bronchioli and alveolar
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13 ducts, sometimes in subpleural area and periseptal regions, were observed, whereas
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15 histological emphysema was not present. Large cyst (>1mm) probably corresponds to
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17 emphysema, whereas small cyst may be in part due to cystic dilatation of the proximal part of
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19 the acini.
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25 After natural exposure, CT scan is the greatest contributor to total radiation exposure and
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27 Brenner et al. notes that the evidence is "convincing" that the radiation dose from CT scans
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29 can lead to cancer induction in adults and "very convincing" in the case of children²⁸. Hence,
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31 it is of importance to adjust protocols in order to reduce unnecessary radiation exposure from
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33 CT. Exposure parameters have considerably decreased during the study, but these efforts must
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35 be sustained. In this context, we also recommend to use a sequential technique during follow-
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37 up, including for multi detector CT.

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39 With this report as our basis, the presence of bilateral ground-glass opacity or a combination
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41 of ground-glass opacities and cysts suggests the diagnosis of SFTPC mutation associated
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43 disorders and strongly encourage to perform genetic screening. We believe that this study is of
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45 value in highlighting this rare condition, and future prospective studies in children and adults
46
47 are needed to further characterize CT findings in surfactant dysfunction disorders.

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56 gov. number, NCT00493363.
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Figures:

Fig. 1: Four-year-old boy with *SFTPC* mutation. HRCT section through the carina shows diffuse and uniform ground-glass opacities (A). Two-year-old girl with *SFTPC* mutation. Initial HRCT (at 3 months) shows homogenous ground-glass opacity in the lower lobes (B). One month old girl with compound heterozygous missense *ABCA3* mutations. Initial HRCT shows ground-glass opacity in the lower lobes (C).

Fig. 2: Two-year-old girl with *SFTPC* mutation. Initial HRCT (at 16 months) shows paraseptal emphysema (curved arrow) associated with widespread cystic airspaces and diffuse ground-glass opacities (A). Seventeen-year-old boy with *SFTPC* mutation. The HRCT done at 14-year-old showed isolated lung cysts with thin wall and variable sizes (B). One month old girl with compound heterozygous missense *ABCA3* mutations. Initial HRCT shows association of ground-glass opacity, alveolar condensations and lung cysts (C).

Fig. 3: A one-year-old boy with *SFTPC* mutation. The initial computed tomography shows diffuse ground-glass opacities (A). The follow-up HRCT scan done 14 months later revealed the appearance of new lung cysts in lower lobes superimposed on diffuse ground-glass opacities (B).

Fig. 4: Two-year-old girl with *SFTPC* mutation. Initial HRCT scan diffuse shows lung cysts with subpleural predominance associated with diffuse ground-glass opacities (A). The follow-up HRCT scan done 6 months later, revealed an increase in the number and size of lung cysts associated with stable ground-glass opacities (B).

Fig. 5: A one-year old boy with *SFTPC* mutation who underwent lung biopsy in the middle lobe. High-power photomicrograph (original magnification $\times 200$, hematoxylin eosine [H- E] stain) (A) shows numerous macrophages in alveoli (M) and type II pneumocytes hyperplasia (straight arrows) with septal thickening. HRCT section through the middle lobe (B) shows diffuse ground glass opacity.

Fig. 6: A 3-year old girl with *SFTPC* mutation who underwent lung biopsy in the right upper lobe. High- power photomicrograph (original magnification $\times 200$, hematoxylin eosine [H- E] stain) (A) shows cystic distension of respiratory ducts (curved arrow), numerous macrophages in alveoli (M) and type II pneumocytes hyperplasia (straight arrows). HRCT section through the upper lobes (B) shows diffuse lung cysts associated with diffuse ground glass opacities.

Table 1: Characteristics of patients

Patient number	Sex	Biopsy (site)	Age at onset (months)	Mutation (protein level)	Familial (F)/de novo	Treatment	Oxygen status	Follow up (years)
1	F	Y(LIL)	2	p.[Ile73Thr]	F	MPP- A	SOO (47)	4
2	F	Y(LIL)	2	p.[Ile73Thr]	<i>de novo</i>	MPP- A	SOO (35)	3
3	F	Y(LIL)	4	p.[Ile73Thr]	<i>de novo</i>	MPP	OW (23)	5.3
4	M	N	Birth	p.[Cys189Tyr]	F	MPP	OW (9)	3
5	F	N	Birth	p.[Cys189Tyr]	F	MPP	OW (9)	3
6	M	Y(LSL)	4	p.[Ile73Thr]	F	MPP-H	OW (7)	3.6
7	F	N	5	p.[Ile73Thr]	F	MPP-H	OW (3)	2.6
8	M	N	2	p.[Ile73Thr]	<i>de novo</i>	MPP	OW (24)	5.5
9	M	Y(LSL)	2	p.[Leu188Pro]	<i>de novo</i>	MPP-H	OW (4)	17.3
10	M	N	15	p.[Ile73Thr]	F	MPP-H	OW (10)	3.3
11	F	N	2	p.[Ile73Thr]	F	MPP-H	OW (14)	2.9
12	F	Y(RIL)	4	p.[Val39Ala]	F	MPP-A	OW (14)	15.4
13	M	Y(RML)	2	p.[Leu194Pro]	F	MPP-A-H	OW (7)	1.8
14	M	Y(RSL)	2	p.[His142fs]	<i>de novo</i>	MPP-H	OW (7)	3.6
15	F	N	18	p.[Ile73Thr]	<i>de novo</i>	MPP-A-H	SOO (12)	1

Biopsy: LIL, left inferior lobe; LSL, left superior lobe; RIL, Right inferior lobe; RML, right medium lobe; RSL, right superior lobe. Treatment: MPP, methylprednisolone pulse; H, hydroxychloroquin; A, azithromycin. Oxygen status: OW, oxygen weaning; SOO, still on oxygen; duration of oxygen (months) in parentheses

Table 2: Initial HRCT signs of patients with *SFTPC* gene mutation

Initial HRCT findings	Patients (n=15)	Controls (<i>ABCA3</i> mutations) (n=6)
Ground-glass opacities	14 (93)	6 (100)
Lung cysts	6 (40)	3 (50)
Interlobular septal thickening	1 (7)	2 (30)
Consolidation	1 (7)	2 (30)
Paraseptal emphysema	2 (13)	0 (0)
Nodules	1 (7)	0 (0)
Lobe retractions	0 (0)	1 (17)

Percentage of total is indicated in parentheses.

Table 3: Distribution of ground-glass opacities on initial HRCT in patients with *SFTPC* gene mutation

	All patients (n=15)	Patients with lung biopsy (n=8)
Homogenous diffuse	9 (60)	5 (63)
Peripheral	1 (7)	1 (13)
Central	1 (7)	0 (0)
Posterior	1 (7)	0 (0)
Lower	2 (13)	1 (13)

Percentage of total is indicated in parentheses.

For Peer Review

Table 4: Distribution of lung cysts on initial HRCT in patients with *SFTPC* gene mutation

	<i>All patients (n=15)</i>	<i>Patients with lung biopsy (n=8)</i>
Diffuse	4 (27)	3 (38)
Upper lobe predominance	1 (7)	1 (13)
Subpleural predominance	1 (7)	0 (0)

Percentage of total is indicated in parentheses.

For Peer Review

Table 5: Findings on follow-up HRCT scans in patients with *SFTPC* gene mutation.

		All patients (n=11)
Ground-glass opacities n = 10	<i>Stable</i>	6 (55)
	<i>Increased</i>	3 (27)
	<i>Decreased</i>	1 (9)
Lung cyst n = 10	<i>Appeared</i>	5 (45)
	<i>Increased</i>	5 (45)
Pulmonary fibrosis		1 (9)

Percentage of total is indicated in parentheses.

Table 6: Radiological-pathological correlation.

Patient	Age at biopsy (months)	Age at initial HRCT (months)	Histology					HRCT findings			
			Cystic dilatation of the respiratory bronchioli	Cystic dilatation of the alveolar duct	Muscle hyperplasia of the respiratory bronchioli	Increased number of macrophages in the alveolar lumen	Alveolar septal thickening	Ground-glass opacities		Lung cysts	
								Initial	Follow-up	Initial	Follow-up
1	4	5	+	+	-	+	+	+	-	+	+
2	7	8	+	+	-	+	+	+	-	+	+
3	6	40	-	-	-	+	+	-	+	+	+
4	4	2	+	+	-	+	+	+	+	-	+
5	4	6	+	+	-	+	+	+	+	+	+
6	4	1	+	+	+	+	+	+	-	-	+
7	6	3	+	+	+	+	+	+	+	-	+
8	3	2	+	+	-	+	+	+	-	-	+

Figure 1
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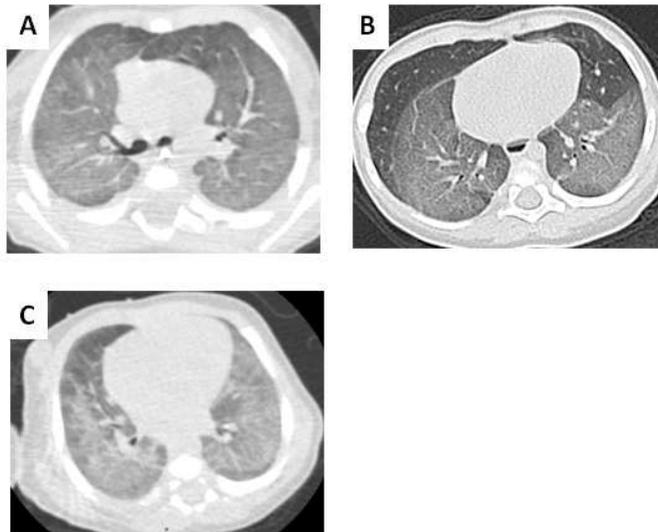


Fig. 1: Four-year-old boy with SFTPC mutation. HRCT section through the carina shows diffuse and uniform ground-glass opacities (A). Two-year-old girl with SFTPC mutation. Initial HRCT (at 3 months) shows homogenous ground-glass opacity in the lower lobes (B). One month old girl with compound heterozygous missense ABCA3 mutations. Initial HRCT shows ground-glass opacity in the lower lobes (C).
190x254mm (96 x 96 DPI)

Figure 2
Mechri et al

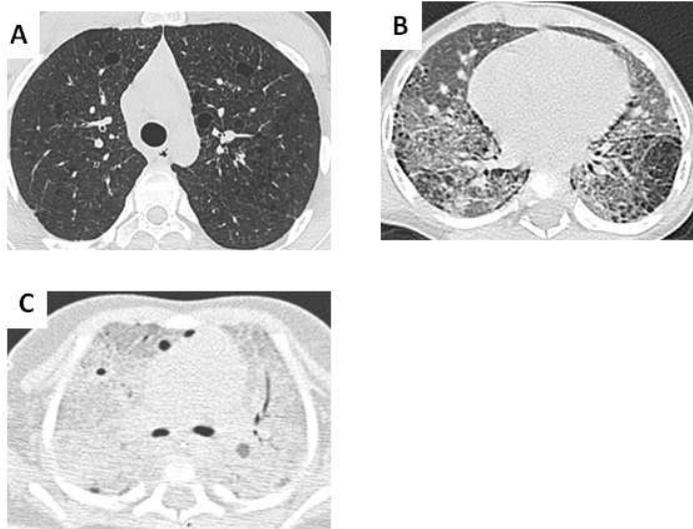
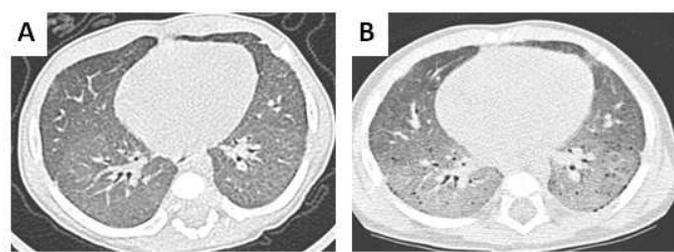


Fig. 2: Two-year-old girl with SFTPC mutation. Initial HRCT (at 16 months) shows paraseptal emphysema (curved arrow) associated with widespread cystic airspaces and diffuse ground-glass opacities (A). Seventeen-year-old boy with SFTPC mutation. The HRCT done at 14 -year -old showed isolated lung cysts with thin wall and variable sizes (B). One month old girl with compound heterozygous missense ABCA3 mutations. Initial HRCT shows association of ground-glass opacity, alveolar condensations and lung cysts (C).
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Figure 3
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A one-year-old boy with SFTPC gene mutation. The initial computed tomography shows diffuse ground-glass opacities (A). The follow-up HRCT scan done 14 months later revealed the appearance of new lung cysts in lower lobes surimposed on diffuse ground-glass opacities (B).
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Figure 4
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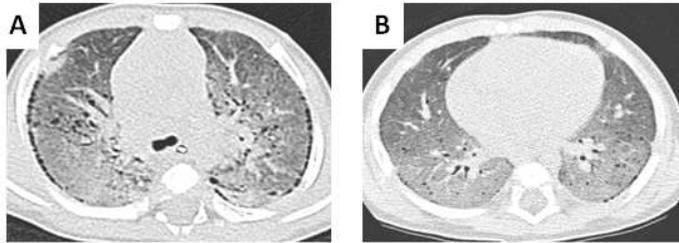
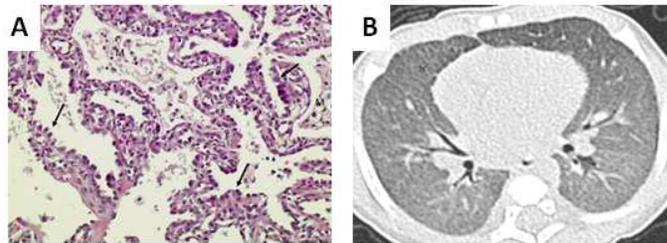


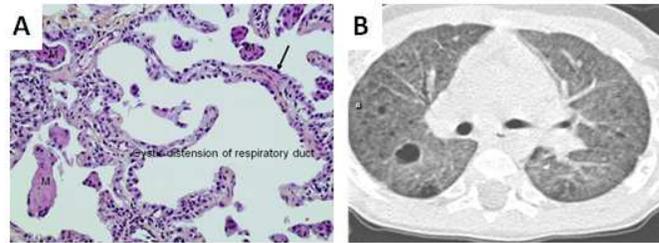
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190x254mm (96 x 96 DPI)

Figure 5
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A one-year old boy with SFTPC gene mutation who underwent lung biopsy in the middle lobe. High-power photomicrograph (original magnification $\times 200$, hematoxylin eosine [H- E] stain) (A) shows numerous macrophages in alveoli (M) and type II pneumocytes hyperplasia (straight arrows) with septal thickening. HRCT section through the middle lobe (B) shows diffuse ground glass opacity.
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Figure 6
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A 3-year old girl with SFTPC gene mutation who underwent lung biopsy in the right upper lobe. High- power photomicrograph (original magnification $\times 200$, hematoxylin eosine [H- E] stain) (A) shows cystic distension of respiratory ducts (curved arrow), numerous macrophages in alveoli (M) and type II pneumocytes hyperplasia (straight arrows). HRCT section through the upper lobes (B) shows diffuse lung cysts associated with diffuse ground glass opacities.
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Surfactant Protein C Gene (*SFTPC*) Mutation–Associated Lung Disease: High-Resolution Computed Tomography (HRCT) Findings and its Relation to Histological Analysis

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The authors have no conflicts of interest to disclose.

Running title: HRCT and histological finding of *SFTPC* mutations

Keywords: surfactant protein C, HRCT, Histological.

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Abstract:***Aim of the study***

Determine high-resolution tomography (HRCT) scan characteristics in children with *SFTPC* mutation and correlate them to histological findings.

Patients and Methods

This retrospective multicenter study included 15 children (7 females and 8 males) with *SFTPC* mutations. HRCT scans have been performed in all the children and lung biopsies in 8 children.

Results

From all signs assessed on initial HRCT scans, ground-glass opacities (n=14, 93 %) and lung cysts (n=6, 40 %) were predominant. Interlobular septal thickening (n=1, 7 %), air space consolidation (n=1, 7 %), paraseptal emphysema (n=2, 13 %), and pulmonary nodules (n=1, 7 %) were also found. Histological analysis revealed accumulation of macrophages in the alveolar lumen, type II pneumocyte hyperplasia, and alveolar septal thickening. Dilatation of the respiratory bronchiole and alveolar duct associated with muscular hyperplasia were also described. Interestingly, lung cysts on HRCT scans were associated with dilatation of terminal bronchioli and alveolar duct in lung biopsies.

Conclusion

In children with *SFTPC* mutations, HRCT scan finding were highly correlated to the histological findings and, as such, represent a useful tool to identify patients that may require *SFTPC* gene sequencing.

Introduction:

Idiopathic forms of interstitial lung disease (ILD) in children are associated with a wide spectrum of underlying pathologies. Until recently, a single classification system has been used, regardless of age, and children with ILD were forced into published adult disease classification systems¹. In 2007, a multi-disciplinary working group of pediatric pathologists, clinicians and radiologist (the Children's Interstitial Lung Disease (chILD) Research Cooperative) designed and conducted a retrospective clinical, radiographic and histopathologic review and proposed a new classification system for children with diffuse lung disease (chILD syndrome)². In this classification, surfactant genetic abnormalities account for more than 10 % of the patients reviewed. Pulmonary surfactant is a mixture of phospholipids and specific proteins secreted by type II epithelial cell to reduce the alveolar surface tension at the air-liquid interface and prevent end-expiratory atelectasis³. The hydrophobic surfactant protein SP-C, play an important role in the spreading and stabilization of phospholipids films in the alveolus³ highlighted by lung disease caused by inherited abnormalities in its genes^{4,5}. Since the first description by Nogee et al., more than 35 dominantly expressed mutations in the *SFTPC* gene have been identified in association with acute or chronic lung disease in patients ranging in age from newborn to adults⁶⁻¹⁰. These mutations, whether inherited or spontaneous are always found in an autosomal dominant form, the most frequently observed (nearly 50 %) being p.[Ile73Thr] or I73T). The phenotype associated with *SFTPC* mutations is eminently variable. Indeed, neonatal forms leading to death in the first years of life as well as childhood and adult forms with chronic respiratory disease have been observed. The effects of *SFTPC* mutations on surfactant homeostasis have not been clearly elucidated. In an *in vitro* study off cells with a *SFTPC* deletion, accumulation of misfolded proSP-C in the Endoplasmic reticulum (ER) causes disruption of the ubiquitin/proteasome system¹¹, activates apoptosis and interferes with non mutated protein

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3 synthesis. It results in the release of pro-inflammatory cytokines and enhances T-cell and
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5 fibroblast recruitment ¹².
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8 Although consensus exists on the need to consider early open lung in children with ILD,
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10 genetic testing for *SFTPC* mutation has become widespread and may be performed relatively
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12 quickly. In addition, recent advances in imaging techniques have contributed to more
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14 efficiently predict histological findings. Indeed, HRCT has been proposed for either diagnosis
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16 or following ILD precluding the need to obtain a surgical biopsy ^{13,14}. Thus, the aim of the
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18 present study was to determine HRCT features of children with *SFTPC* mutation and to bring
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20 new insight in the correlation between HRCT and histological findings. To address these
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22 questions HRCT finding of a cohort of 15 children with interstitial chronic lung disease
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24 related to *SFTPC* gene mutations were retrospectively reviewed and subsequently correlated
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26 with histological analysis when lung biopsies were available.
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Patients and Methods

This study is a retrospective study performed in 2 university hospital-based pediatric pulmonology practice (Hôpital Armand Trousseau and Hôpital Necker enfants-malades, Paris, France) for a 15-year period (1993-2008). The protocol was accepted by the Committee for the protection of individuals in Biochemical research as required by French legislation and written informed consent was obtained from the patients or their next of kin included in this study.

Patients

The characteristics of patients are summarized in Table 1. Fifteen children, 7 females and 8 males with a definitive diagnosis of *SFTPC* gene mutation were included. All the patients were immunocompetent and screening for metabolic, infectious, and immunological etiologies was negative. Genetic and clinical characteristics of all these patients have been described in detail in previous reports^{8,15}. Three patients, members, or relatives of a same family, have also been reported previously⁶. Median age of onset was 2 (range 0-18) months. Three patients had neonatal respiratory distress, and symptom onset was associated with acute bronchiolitis in 7 (47 %) patients. Cough, tachypnea, and gastroesophageal reflux and failure to thrive were initially noticed in all the children. Physical examination at presentation revealed tachypnea and retraction in all patients. Low oxygen saturation (<95%) was observed initially in all patients. Treatment are summarized in table 1 and included methylprednisolone pulse (n=15), hydroxychloroquin (n=8) and azithromycin (n=5). Median follow-up was 3.1 (1-17.3) years. At the end of the study, all the children were alive but 11 patients (73 %) were still symptomatic with cough, dyspnea or exercise intolerance.

HRCT scan and image analysis

CT examinations were performed with two helical scanners and two multidetector CT scanners. In Armand Trousseau hospital, Elscint (CT Twin) with scan acquisition at 120 kV, 120 mAs and after 2003 a Philips (Mx 8000 IDT32) 16 slice multidetector CT was used with scan acquisition at the 90kV, 90mAs. In Necker Hospital, General Electric helical CT (Prospect) with scan acquisition at 140kV, 130mAs, and after 2005 a General Electric (LightSpeed) 64 slice multidetector CT was used with scan acquisition at the 100kV, 100mA. All the patients underwent non-contrast-enhanced incremental (at intervals of 3 to 10 mm) or helical HRCT scan with a collimation of 16 X 1mm. Scans were obtained from the level of apices to the diaphragm. Image reconstruction was performed with a high special frequency algorithm. All CT scans were performed before the diagnosis, and none of the patients were receiving treatment at the time of first HRCT. The HRCT scans (initial and follow-up) were reviewed and interpreted by 2 experienced pediatric radiologists who arrived at their interpretations by consensus without knowledge of histological parameters. HRCT scan analysis was performed with reference to a conventional model in line with Austin's definition

The scans were assessed for the presence, extent, and distribution of elementary lesions, such as areas of ground-glass opacity, lung cysts, lung consolidation, interlobular and intralobular septal thickening, thickening of bronchovascular bundles, and nodules (centrolobular or other). The anatomical distribution was classified as central (a predominance of abnormalities in the inner two-thirds of the lung), peripheral (a predominance of abnormalities in the outer one-third of the lung or along the interlobar fissures), or random (no central or peripheral predominance). The zonal predominance of abnormalities was assessed as being upper, intermediate lower, or random. Upper lung zone predominance was considered present when the extent of abnormalities was greatest above the level of the

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3 tracheal carina; intermediate was considered between the carina and inferior pulmonary veins,
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5 and lower zone predominance was considered present when disease extent was greatest below
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7 this level. Specific involvement or sparing of the subpleural region (<1cm from the pleura)
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9 was assessed. Ground-glass opacities describe lung that is higher in attenuation than a normal
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11 lung on inspiration but lower than the attenuation of bronchial walls and blood vessels so that
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13 these lung markings remain visible¹⁶. The term lung cyst is used to refer to a well-defined,
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15 rounded, and circumscribed lesion, with a wall that may be uniform or varied in thickness but
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17 which is usually thin (with thickness of less than 3 mm)¹⁷. Because this study was
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19 retrospective, the intervals of follow-up HRCT scanners were highly centre-dependent and
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21 based on the decision of the physician in charge of the child. Only HRCT scanners available
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23 were taking into consideration for analysis. Control HRCT scans were obtained from 6 age-
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25 matched patients with a definitive diagnosis of *ABCA3* (ATP-binding cassette subfamily A,
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27 member 3) mutations, a gene expressed in lamellar bodies of alveolar type II cells which has a
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29 critical role in surfactant storage and homeostasis¹⁸. Among them, 2 patients carried
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31 homozygous missense mutations, 3 patients carried compound heterozygous missense
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33 mutations and 1 child had compound heterozygous missense and nonsense mutations.
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35 Median age at HRCT scans was 2 months (range 1 month to 12 years).
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46 *Lung biopsies and their analysis*

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48 Surgical lung biopsies ranging from 0.5 to 1cm were obtained from different lobes in 8
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50 children at the age of 3 to 7 months (mean age, 4 months) before treatment onset. Biopsy sites
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52 are summarized in Table 1. Specimens were further fixed in formalin and embedded in
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54 paraffin. A five-micrometer tissue section was stained with H and E stain (hematoxylin and
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56 eosin stain). Slides were examined by 2 pathologists. Radiology-pathology correlation was
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58 performed in 8 cases. All 8 children had only 1 open lung biopsy.
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Results:

Initial HRCT findings

The median age at initial HRCT scanner was 4 (range 1-40) months (one child had the scanner performed 40 months after the diagnosis for technical reasons). High-resolution CT findings are summarized in Table 2. The most frequent abnormalities identified on initial HRCT were ground-glass opacities (93 %) and lung cysts (40 %). Other abnormalities noted included interlobular septal thickening (7 %), air space consolidation (7 %), paraseptal emphysema (13 %), and pulmonary nodules (7 %). In comparison, ground-glass opacities were constantly observed in all of the 6 patients with ABCA3 mutations. However, opacities were more prominent with presence in some cases of alveolar condensations.

Bilateral ground-glass opacities were shown in 14 children (Table 3), either as an isolated finding (60 %) or as part of a mixed pattern with lung cysts (33 %), air space consolidation (7 %), paraseptal emphysema, and centrilobular nodules (7 %). They were homogeneously diffuse in 9 cases (60 %) (Figure 1A). When a patchy distribution was present, this was noted to have basal subpleural predominance in 1 case (7 %), predominance for the lower lobes in 2 cases (13 %) (Figure 1B), a central predominance in 1 case (7 %), and a predominance for the posterior portions of the lung in 1 case (7 %).

Thin-walled (<1mm) lung cysts of varying sizes were seen in 6 infants (40 %) (Table 4), either as an isolated finding (7 %) (Figure 2A), or as a mixed pattern with areas of ground-glass opacities (33 %), paraseptal emphysema (13 %) (Figure 2B), septal thickening (7 %), and non-septal thickening (7 %). A predominantly subpleural distribution was seen in 1 child (7 %); diffuse cysts with random distribution was seen in 4 cases (27 %) and upper lung zone predominance was seen in 1 case (7 %).

Follow-up HRCT scan

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3 Follow-up CT scans were performed on 11 of the 15 children. The median follow-up interval
4 was 8 (range 3-12) months. Eight patients had 1 follow-up HRCT scan, 2 patients had 2
5 follow-up HRCT scans (3 and 9 months and 4 and 9 months, respectively), and 1 patient had 3
6 follow-up HRCT scans (3, 6 and 12 months). Evaluation of the follow-up HRCT scans (Table
7 5) revealed the development of lung cysts in 5 patients (45 %) who had previously only had
8 ground-glass opacities (Figures 3A and 3B). These new cystic changes were associated with a
9 stable pattern of ground-glass opacities in 2 cases, decrease in the degree of ground-glass
10 opacities in 1 case, and an aggravation of this pattern in 2 cases. These cysts were diffuse in
11 all cases but showed a predilection for different regions of the lung: upper lobes (n=1), basal
12 subpleural (n=1), peripheral (n=1), and central (n=1). In 1 case, lung cysts were homogenous
13 and diffuse. Patients who had previously documented cysts on their initial HRCT scans also
14 demonstrated change when follow-up HRCT was performed. In 5 patients (45 %), we noted
15 an increase in the number or size of preexisting lung cysts (Figures 4A and 4B), which were
16 associated with stable ground-glass opacities (n=4), aggravated ground-glass opacities (n=1),
17 and progressive pulmonary fibrosis (n=1). The HRCT findings of pulmonary fibrosis
18 consisted of bilateral and diffuse honeycombing, septal and non-septal thickening, and
19 thickening of bronchovascular bundles and distortion of pulmonary fissures. In 1 patient, we
20 observed persistent isolated ground-glass opacities.

21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 ***Pathology findings***

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48 Eight of the 15 patients (53 %) underwent lung biopsy the median age at biopsy being 4
49 (range 3-7) months. Histological analysis revealed 2 main types of lesions. The first type
50 occurred in the alveolar lumen, where we observed the following changes: accumulation of
51 macrophages intermingled with few inflammatory cells and cholesterol cleft, type II
52 pneumocyte hyperplasia, and alveolar septal thickening by primitive mesenchymal cells and
53 lymphocytes either isolated or clustered (Figure 5A). These lesions were diffuse in 7 cases (90
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3 %) and patchy in 1 case (13 %). The second type of lesion corresponds to dilatation of the
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5 respiratory bronchiole and alveolar duct in 7 cases (88 %) associated with muscular
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7 hyperplasia in 2 patients (25%) (Figure 6A). There was no interstitial pulmonary fibrosis or
8
9 alveolar proteinosis observed.
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12 13 14 15 ***Relation between HRCT and pathology findings*** 16

17 Radiological-pathological confrontation is summarized in Table 6. Ground-glass opacities on
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19 HRCT were observed in all the patients except 1 in which the CT scan was performed late (40
20
21 months). All these children also have diffuse alveolar septal thickening by primitive
22
23 mesenchymal cells and lymphocytes, intra-alveolar accumulation of macrophages, and type II
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25 pneumocyte hyperplasia. Small lung cysts were observed initially in half of the children but
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27 appeared during follow-up in all the cases. They were related with dilatation of the terminal
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29 bronchioles and alveolar duct which were noticed in all but one biopsy.
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36 **Discussion:** 37

38 In the present study, we have shown for the first time in a large cohort of children with
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40 *SFTPC* mutation associated lung diseases that HRCT pattern were characterized with 2 main
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42 abnormalities: ground-glass opacities that were predominately diffuse but can be patchy in
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44 some cases and lung cysts with thin wall and different sizes.
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48 Ground-glass opacities have been commonly described in previous reports^{6,19,20}. They are
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50 predominately diffuse but can be patchy in some cases. In our series, they were present in all
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52 the patients except one in whom the HRCT scan was done late. We also note in our study the
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54 high frequency of cystic lesions on HRCT scan. They appeared in 45% of the children during
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56 follow-up and increased in number in 45%. Similar change during follow-up is typical of
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58 surfactant abnormality and was noted by Brody¹⁶. In our series, ground-glass attenuation
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3 decreased in intensity with age, whereas small lung cysts usually became more prominent
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5 over time.
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8 This HRCT presentation, namely the ground-glass opacities and cystic patterns is,
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10 nonetheless, not specific of *SFTPC* associated disorders. We have shown that this presentation
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12 may also be observed in children with *ABCA3* mutations as previously described ²¹. However,
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14 the high frequency of *SFTPC* associated disorders in children ILD ^{2,7}, the feasibility of *SFTPC*
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16 genetic analysis as well as the difficulty to perform lung biopsy in patients with fragile
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18 respiratory condition strongly encourage to perform *SFTPC* genetic screening before lung
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20 biopsy in children with ground-glass opacities associated with cystic lung changes on HRCT
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22 scanner.
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27 In the literature, there are histological studies of patients with *SFTPC* gene mutations showing
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29 4 histological patterns: desquamative interstitial pneumonitis (DIP), nonspecific interstitial
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31 pneumonitis(NSIP), (UIP) and pulmonary alveolar proteinosis ^{7,16,22} in children and usual
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33 interstitial pneumonitis (UIP) in adults. In children, DIP and NSIP have also been described in
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35 patients with mutations in the ATP-binding cassette transporter A3 (*ABCA3*) gene ²¹. Chibbar
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37 et al. showed that both children and adults with NSIP and UIP patterns, respectively, carried a
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39 common mutation within the *SFTPC* gene ²³. Thomas et al. described a mutation in *SFTPC*
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41 gene associated with large kindred with familial form of pulmonary fibrosis, including adults
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43 with the most common pathological subset of pulmonary fibrosis, UIP, and children with
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45 cellular NSIP ²⁴. Brasch reported histological patterns of NSIP and pulmonary alveolar
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47 proteinosis in a 13-month-old infant with a *de novo* heterozygous missense mutation of the
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49 *SFTPC* gene ²⁰. The histological findings were classified as DIP because of an increased
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51 number of macrophages in the alveolar lumen ²⁵ and as NSIP and UIP because of the
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53 numerous inflammatory cells in the alveolar septum ^{26,27}. In this study, we have related
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55 ground-glass opacities on HRCT to diffuse alveolar septal thickening by primitive
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3 mesenchymal cells and lymphocytes, intra-alveolar accumulation of macrophages, and type II
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5 pneumocytes hyperplasia. Small lung cysts were related to dilatation of the respiratory
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7 bronchioli and alveolar duct. On initial HRCT scan analysis, large cystic lung changes in the
8
9 periseptal and subpleural regions were that of subpleural emphysema. On lung biopsies,
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11 millimetric small cysts corresponding to a dilatation of respiratory bronchioli and alveolar
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13 ducts, sometimes in subpleural area and periseptal regions, were observed, whereas
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15 histological emphysema was not present. Large cyst (>1mm) probably corresponds to
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17 emphysema, whereas small cyst may be in part due to cystic dilatation of the proximal part of
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19 the acini.
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25 After natural exposure, CT scan is the greatest contributor to total radiation exposure and
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27 Brenner et al. notes that the evidence is "convincing" that the radiation dose from CT scans
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29 can lead to cancer induction in adults and "very convincing" in the case of children ²⁸. Hence,
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31 it is of importance to adjust protocols in order to reduce unnecessary radiation exposure from
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33 CT. Exposure parameters have considerably decreased during the study, but these efforts must
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35 be sustained. In this context, we also recommend to use a sequential technique during follow-
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37 up, including for multi detector CT.

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39 With this report as our basis, the presence of bilateral ground-glass opacity or a combination
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41 of ground-glass opacities and cysts suggests the diagnosis of SFTPC mutation associated
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43 disorders and strongly encourage to perform genetic screening. We believe that this study is of
44
45 value in highlighting this rare condition, and future prospective studies in children and adults
46
47 are needed to further characterize CT findings in surfactant dysfunction disorders.

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Figures:

Fig. 1: Four-year-old boy with *SFTPC* mutation. HRCT section through the carina shows diffuse and uniform ground-glass opacities (A). Two-year-old girl with *SFTPC* mutation. Initial HRCT (at 3 months) shows homogenous ground-glass opacity in the lower lobes (B). One month old girl with **compound heterozygous missense** *ABCA3* mutations. Initial HRCT shows ground-glass opacity in the lower lobes (C).

Fig. 2: Two-year-old girl with *SFTPC* mutation. Initial HRCT (at 16 months) shows paraseptal emphysema (curved arrow) associated with widespread cystic airspaces and diffuse ground-glass opacities (A). Seventeen-year-old boy with *SFTPC* mutation. The HRCT done at 14-year-old showed isolated lung cysts with thin wall and variable sizes (B). One month old girl with **compound heterozygous missense** *ABCA3* mutations. Initial HRCT shows association of ground-glass opacity, alveolar condensations and lung cysts (C).

Fig. 3: A one-year-old boy with *SFTPC* mutation. The initial computed tomography shows diffuse ground-glass opacities (A). The follow-up HRCT scan done 14 months later revealed the appearance of new lung cysts in lower lobes surimposed on diffuse ground-glass opacities (B).

Fig. 4: Two-year-old girl with *SFTPC* mutation. Initial HRCT scan diffuse shows lung cysts with subpleural predominance associated with diffuse ground-glass opacities (A). The follow-up HRCT scan done 6 months later, revealed an increase in the number and size of lung cysts associated with stable ground-glass opacities (B).

Fig. 5: A one-year old boy with *SFTPC* mutation who underwent lung biopsy in the middle lobe. High-power photomicrograph (original magnification $\times 200$, hematoxylin eosine [H- E] stain) (A) shows numerous macrophages in alveoli (M) and type II pneumocytes hyperplasia (straight arrows) with septal thickening. HRCT section through the middle lobe (B) shows diffuse ground glass opacity.

Fig. 6: A 3-year old girl with *SFTPC* mutation who underwent lung biopsy in the right upper lobe. High- power photomicrograph (original magnification $\times 200$, hematoxylin eosine [H- E] stain) (A) shows cystic distension of respiratory ducts (curved arrow), numerous macrophages in alveoli (M) and type II pneumocytes hyperplasia (straight arrows). HRCT section through the upper lobes (B) shows diffuse lung cysts associated with diffuse ground glass opacities.