ERJ Express. Published on February 5, 2015 as doi: 10.1183/09031936.00129414

ORIGINAL ARTICLE
IN PRESS | CORRECTED PROOF

Genotype alone does not predict the clinical course of *SFTPC* deficiency in paediatric patients

Carolin Kröner[1], Simone Reu[2], Veronika Teusch[3], Andrea Schams[1], Ann-Christin Grimmelt[1], Michael Barker[4], Joerg Brand[5], Monika Gappa[6], Richard Kitz[7], Boris W. Kramer[8], Lars Lange[9], Susanne Lau[10], Claus Pfannenstiel[11], Marijke Proesmans[12], Jürgen Seidenberg[13], Tugba Sismanlar[14], Ayse Tana Aslan[14], Claudius Werner[15], Stefan Zielen[5], Ralf Zarbock[1], Frank Brasch[16], Peter Lohse[17] and Matthias Griese[1]

Affiliations: [1]Department of Pediatric Pneumology, Hauner Children's Hospital, Ludwig-Maximilians University, Comprehensive Pneumology Center Munich (CPC-M), German Center for Lung Research (DZL), Munich, Germany. [2]Department of Pathology, Ludwig-Maximilians University, Munich, Germany. [3]Department of Pediatric Radiology, Hauner Children's Hospital, Ludwig-Maximilians University, Munich, Germany. [4]Department of Pediatrics, HELIOS Klinikum Emil von Behring, Berlin, Germany. [5]Department of Pediatrics, Johann Wolfgang Goethe University, Frankfurt am Main, Germany. [6]Department of Pediatric Pneumology, Marien-Hospital GmbH, Wesel, Germany. [7]Department of Pediatric Pneumology, Clementinen Children's Hospital, Frankfurt am Main, Germany. [8]Department of Pediatrics, Maastricht University Medical Center, Maastricht, Netherlands. [9]Department of Pediatrics, St. Marien Hospital, Bonn, Germany. [10]Department of Pediatric Pneumology and Immunology, Charity University, Berlin, Germany. [11]Pediatric Consultation Center Laurensberg, Aachen, Germany. [12]Department of Pediatric Pneumology, University Hospital Leuven, Leuven, Belgium. [13]Department of Pediatric Pneumology, Klinikum Oldenburg gGmbH, Medizinischer Campus Universität Oldenburg, Oldenburg, Germany. [14]Department of Pediatric Pneumology, Gazi University Hospital, TR-Ankara, Turkey. [15]Department of Pediatric Pneumology, University Hospital Münster, Münster, Germany. [16]Department of Pathology, Academic Teaching Hospital Bielefeld, Bielefeld, Germany. [17]Institute of Laboratory Medicine and Human Genetics, Singen, Germany.

Correspondence: M. Griese, Hauner Children's Hospital, Ludwig-Maximilians-University Munich Lindwurmstraße 4, D-80337 Munich, Germany. E-mail: matthias.griese@med.uni-muenchen.de

ABSTRACT Patients with interstitial lung disease due to surfactant protein C (*SFTPC*) mutations are rare and not well characterised.

We report on all subjects collected over a 15-year period in the kids-lung register with interstitial lung disease and a proven *SFTPC* mutation. We analysed clinical courses, interventions and outcomes, as well as histopathological and radiological interrelations.

17 patients (seven male) were followed over a median of 3 years (range 0.3–19). All patients were heterozygous carriers of autosomal dominant *SFTPC* mutations. Three mutations (p.L101P, p.E191 K and p.E191*) have not been described before in the context of surfactant protein C deficiency. Patients with alterations in the BRICHOS domain of the protein (amino acids 94–197) presented earlier. At follow-up, one patient was healthy (2 years), six patients were sick-better (2.8 years, range 0.8–19), seven patients were sick-same (6.5 years, 1.3–15.8) and three patients were sick-worse (0.3 years, 0.3–16.9). Radiological findings changed from ground-glass to increasing signs of fibrosis and cyst formation with increasing age. Empiric treatments had variable effects, also in patients with the same genotype.

Prospective studies with randomised interventions are urgently needed and can best be performed in the framework of international registers.

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Genotype alone does not predict the clinical course of surfactant protein C deficiency in children and young adults http://ow.ly/GRhCc

This article has supplementary material available from erj.ersjournals.com

Received: July 16 2014 | Accepted after revision: Dec 05 2014

Conflict of interest: None declared.

Support statement: Funding information for this article has been deposited with FundRef

Introduction

Interstitial lung diseases (ILD) in children represent a broad array of clinical entities. Only for some distinct groups is the cause known [1]. The surfactant dysfunction disorders are the group best defined molecularly, since the discovery of mutations in genes involving surfactant proteins with important functions in the lungs. Among these proteins is surfactant protein (SP) C, a hydrophobic protein critical for surfactant homeostasis, encoded by the surfactant protein C gene (*SFTPC*) on chromosome 8p21.3 and expressed as a pro-protein in type II pneumocytes. After extensive post-translational processing, mature SP-C is secreted into the alveolar space [2]. Since the first description of ILD being associated with an alteration of the *SFTPC* gene in 2001 [3], about 50 different mutations have been reported [4], mainly as case reports and small series.

The most common mutation is p.I73T (c.218T>C), which hampers the secretion of SP-C through aberrant protein folding and intracellular processing [2]. Mutations are also located in the so called BRICHOS domain, which is a highly conserved region at the C-terminal end (p.F94–p.I197) of the SP-C pro-protein [4]. This domain normally prevents the protein from forming amyloid-like fibrils [5], which deposit in patients with SP-C-related ILDs as insoluble, fibrillar β-sheet polymers [6]. Lung disease caused by different *SFTPC* mutations covers a broad range of phenotypes [7–10]. Whether mutation-specific pathophysiological mechanisms are associated with distinct clinical phenotypes remains elusive. The total number of reported cases per mutation is still very low, and the phenotypic description of these cases is frequently not complete. We intended to widen the spectrum of genetically defined patients and to provide details on the individual disease courses as well as the histopathological and radiological findings in order to allow more precise estimates of outcomes and effects of therapeutic interventions.

Methods

Patients

All children identified in the programme for rare lung diseases of the Kids-Lung Register (Hauner Children's Hospital, Munich, Germany) between 1998 and 2013 with a heterozygous dominant mutation in the *SFTPC* gene were included in this study. 17 patients were identified, including three monozygous triplets. Three patients (5, 12 and 15) have been reported previously [11–13], but were included into this cohort because of additional information. Retrospective and prospective clinical data were collected since the start of the study. In addition, bronchoalveolar lavage (BAL) levels of SP-C and SP-B were determined, and lung biopsy specimens as well as radiological studies were evaluated. The study was approved by the Ethics committee of the University of Munich (Munich, Germany) (Protocol 257-10). All parents or the guardians of the children gave their informed consent, and an older youth assented.

Clinical follow-up

The following definitions were used to group patients into follow-up groups. The status was compared with the beginning of the individual observation periods: "sick-worse" was used for patients with a worse clinical status (more severe symptoms), "sick-same" for patients with the same clinical status (same degree of severity of symptoms), "sick-better" was defined as an improvement of clinical status (less therapy, less symptoms) and "healthy" was defined as having had no clinical symptoms and no therapy for at least 12 months.

Genetic analysis of the SFTPC *gene*

The five protein-coding exons of the *SFTPC* gene were amplified *via* PCR and sequenced by standard methods.

Structure-function analysis of novel SFTPC *mutations*

In silico prediction of the effect of *SFTPC* mutations on protein function was performed with the computer programmes PROVEAN [14], SIFT [15] and PolyPhen2 [16]. Conservation of amino acids across species was determined by comparison of the SP-C sequences of *Homo sapiens, Mus musculus, Bos taurus, Rattus norvegicus, Sus scrofa, Pan troglodytes, Felis catus, Columba livia and Xenopus tropicalis*.

Analysis of total protein and of SP-B and SP-C concentration in BAL

Total protein was measured using the Bradford method as described previously [16]. SP-B and SP-C were determined as described [17, 18]: proteins were separated on NuPage 10%; Bis-Tris gels (Novex X-cell Ii Mini Cell System; Novex, San Diego, CA, USA), transferred onto nitrocellulose membranes by Western blot in Nupage Blot modules (Novex) and incubated with antibodies as described [17]. For quantification, two standards were run on each gel; SP-B at 10 and 20 ng and SP-C at 10 and 25 ng. The assay for SP-B was linear between 1 and 40 ng, the one for SP-C between 3 and 80 ng. The inter-assay coefficient of variation was 10% for SP-B and 19% for SP- C. The membranes were activated with an enhanced

DOI: 10.1183/09031936.00129414

chemiluminescence assay and exposed to X-ray films (Hyperfilm ECL; Amersham Biosciences, Amersham, UK). After development of the films, bands were quantified using the Diana III chemiluminescence detection system and Advanced Image Data Analyzer software, Version 4.04.032 (Raytest, Straubenhardt, Germany).

Lung biopsy and histopathology

All available lung biopsies were collected as paraffin blocks or as histopathological slides. The slides were examined by a pathologist (F. Brasch) experienced in the field of paediatric ILD. They were then independently scored by a second pathologist (S. Reu) specialising in pulmonary diseases.

Radiological studies

All available radiological studies were reviewed and scored by a paediatric radiologist according to the criteria of the Fleischner Society [19]. Computed tomography (CT) scans were rated in six areas. Each lung was divided into three sections: apex to carina; carina to lower pulmonary vein; and lower pulmonary vein to diaphragm. The incidence of hilar lymph nodes, ground glass opacities, cysts, peribronchial thickening, bronchiectasis, honeycombing, interlobular septal thickening, intralobular septal thickening, crazy paving, paraseptal emphysema/hyperinflation, nodular opacities, lobe retraction and consolidations was evaluated for each lung section. The findings were graded into four levels according to the severity (none, mild, moderate and extensive).

Statistical analysis

Fisher's exact test and the Mann–Whitney test (GraphPad Prism, version 4.00; GraphPad Software, San Diego, CA, USA) were used to compare frequencies between patient groups. Fisher's exact test was used to analyse contingency tables. The unpaired t-test (GraphPad Prism) was used to compare the means of CT scan rating between different groups. A p-value ≤ 0.05 was considered significant.

Results

Clinical presentation

About half of the 17 patients presented with neonatal respiratory symptoms. The others had respiratory symptoms during infancy or later (table 1, and table s1 and fig. s1 in the online supplementary material). Failure to thrive was the most important extra-pulmonary manifestation (15 out of 17 patients) and treated in subjects by percutaneous endoscopic gastrostomy tube in 10 subjects.

Genetic results

All 17 patients were of Caucasian descent; eight patients carried the hot spot mutation p.I73T (table 2, and table s1 and fig. s2 in the online supplementary material). Eight patients had sequence alterations, likely to be damaging enough to explain the phenotype, except in patient 17 (table 2, and table s2 in the online supplementary material). In this patient (compound heterozygous p.L101P; p.E191K), genetic analysis of relatives revealed that the grandfather carried p.L101P and suffered from usual interstitial pneumonia (UIP), while the patient's mother (also carrying p.L101P) was reported to be healthy. The patient's father, in contrast, was heterozygous for p.E191K and healthy. This amino acid is conserved among mammals and its exchange is predicted to be possibly damaging. In total, 10 patients had *de novo* and three familial mutations, while four parents refrained from genetic testing (table 2).

Follow-up

Median (range) follow-up was 3 years (0.3–19) (table 3). Empiric therapy included neonatal surfactant, corticosteroids (continuous and pulse therapy), colchicine, hydroxychloroquine, antibiotic therapy including azithromycin and whole lung lavage for initial alveolar proteinosis presentation. One patient received a left lung volume reduction due to the development of bullous emphysema. The effect of therapy was judged retrospectively based on patient reports. Therapy with hydroxychloroquine moderately improved the clinical course in six patients and had a good effect in another six individuals, shortly after initiation of therapy (table 4, and table s3 in the online supplementary material). Only two patients developed significant side effects: one patient (12) developed an erythema exsudativum multiforme after the start of hydroxychloroquine and one patient (17) developed Cushing's syndrome under steroid therapy.

Overall, the patients' outcomes were classified as healthy in one (patient 4, p.I73T, after 2 years), sick-same in seven patients (median 6.5 years, range 1.3–15.8), sick-better in six patients (median 2.8 years, range 0.8–19) and sick-worse in three patients (at 0.3, 0.8 and 16.9 years) (table 3, and table s3 in the online supplementary material). All patients survived. The localisation of the mutations inside or outside the BRICHOS domain did not affect the outcome.

TABLE 1 Clinical presentation of 17 patients with *SFTPC* mutations

n	Female/ male n	Gestational age	Age at onset	Neonatal symptoms n	Post neonatal symptoms n	Mechanical ventilation n (age)	Failure to thrive n	Other diagnosis n
All patients								
17	10/7	39 (36–43) weeks	2 (0–132) months	9 yes, of whom: 3 RDS; 4 tachy/ dyspnoea; 1 cyanosis; 1 staphylodermia	17 yes, of whom: 17 tachypnoea, oxygen demand; 3 spontaneous pneumothorax; 2 persistent pulmonary hypertension; 2 cyanosis; 5 abnormal skeletal thorax; 17 recurrent infections of which: 5 RSV pneumonia; 2 *Chlamydophilia pneumoniae* pneumonia; 3 *Pseudomonas aeruginosa* pneumonia; 1 influenza pneumonia; 1 chronic disseminated cytomegalovirus infection; 1 methicillin-resistant *Staphylococcus aureus* pneumonia	11 (1 (0–24) month)	15 yes, of whom: 9 percutaneous endoscopic gastrostomy; 4 gastro-oesophageal reflux disease; 1 cow milk allergy	10 yes, of whom: 5 cardiac anomalies; 2 mental retardation; 8 other diagnosis[#]
Non-BRICHOS domain mutations								
10	6/4	39 (36–40) weeks	0.3 (0–11) years	3 yes, 1 unknown	10 yes	7 yes (4.8 (0–24) months)	9 yes	4 yes, 1 unknown
BRICHOS domain mutations								
7	4/3	39 (36–43) weeks	0 (0–3) months	6 yes	7 yes	4 yes (0 (0–19) months)	5 yes	5 yes
p-value[¶]				0.06	NS	NS	NS	NS

Data are presented as median (range), unless otherwise stated. RDS: respiratory distress syndrome; RSV: respiratory syncytial virus; PEG: percutaneous endoscopic gastrostomy; NS: not significant. [#]: see table s1 in the online supplementary material [¶]: Fisher's exact test comparing frequencies between BRICHOS/non-BRICHOS mutations.

DOI: 10.1183/09031936.00129414

TABLE 2 Genetic information and family history of 17 patients with *SFTPC* mutations

Patient	Mutation	De novo or inherited mutation	Consanguinity	Family history
1[#]	p.I73T	*De novo*	No	Father and grandfather: Barrett's oesophagus
2[#]	p.I73T	*De novo*	No	Father and grandfather: Barrett's oesophagus
3[#]	p.I73T	*De novo*	No	Father and grandfather: Barrett's oesophagus
4	p.I73T	*De novo*	No	No
5	p.I73T	*De novo*	No	No
6	p.I73T	Unknown	No	2 abortions, 1 postnatal death
7	p.I73T	*De novo*	Yes	No
8	p.I73T	Unknown	Unknown	Mother: thalassaemia minor, G5P2 (five pregnancies, two live births), hyperprolactinaemia, intrauterine fetal death; father: haemoglobin E haemoglobinopathy; parents: cow milk's protein intolerance
9	p.H59R	Father H59R, mother negative, brother negative	Yes	Father healthy, brother: spontaneous pneumothorax at age 19 years, bullous paraseptal emphysema
10	p.E191*[¶]	*De novo*	No	No
11	p.G74V	*De novo*	Yes	No
12	p.C121F	*De novo*	No	No
13	p.A112T	Unknown	Unknown	No
14	p.C121F	Unknown	No	No
15	p.C121G	*De novo*	No	No
16	p.A53T and p.L181V	Father p.A53T and p.L181 V, sister unknown	No	Mother: asthma, atopic dermatitis, neurinoma, gestational diabetes, one abortion; father: atopic dermatitis, recurrent otitis, sister: recurrent otitis
17	p.L101P[¶] and p.E191K[¶]	Mother, grandfather p.L101P, father p.E191 K	No	Grandfather: usual interstitial pneumonia

[#]: monozygous triplets; [¶]: novel mutations.

Surfactant proteins in BAL fluid

BAL data were available for eight patients. Mature SP-C (4 kDa) was lacking in all six patients with BRICHOS domain mutations and available BAL. In patients with p.I73T, SP-C was present at young age, in particular when alveolar proteinosis was demonstrated histologically, but not later. SP-B was present in all patients as a physiological dimer (16 kDa). Its concentration was relatively low in three patients and elevated in two (table 5, and table s4 in the online supplementary material).

TABLE 3 Follow-up of 17 patients with *SFTPC* mutations

Age at last observation	Symptoms at last observation (age)
3 (0.3–19) years	2 global respiratory insufficiency, mechanical ventilation (0.3 and 0.8 years) 1 fibrosis, listed for lung transplantation (16.9 years) 1 home ventilation, mainly at night (1.3 years) 4 hypoxia (6.5 (6.5–13) years) 3 mild tachypnoea (0.8, 1.6 and 2.6 years) 1 hypoxaemia with exercise (15.8 years) 1 severe pneumonia in past years (19 years) 2 mild upper airway infections (3 and 6.7 years) 1 oxygen demand during infection (2.5 years) 1 healthy (2 years)

Data are presented as median (range).

TABLE 4 Therapy and response of 17 patients with *SFTPC* mutations

Treatment	Patients n	BRICHOS/non-BRICHOS mutation n
Surfactant treatment		
Not done	10	4/6
No improvement	5	4/1
Moderate improvement	1	0/1
Good response	1	0/1
Systemic steroids		
Not done	2	2/0
No improvement	1	1/0
Moderate improvement	13	5/8
Good response	1	0/1
Colchicin		
Not done	14	11/3
No improvement	3	0/3
Moderate improvement	0	0
Good response	0	0
Hydroxychloroquine		
Not done	3	1/2
No improvement	2	1/1
Moderate improvement	6	2/4
Good response	6	4/2
Other treatments		
Antibiotic therapy	17	7/10
Azithromycin	8	2/6
Whole lung lavage	5	1/4
Left lung volume reduction	1	0/1
General response to all treatments at end of observation		
No long-term treatment	3	1/2
Moderate improvement	7	2/5
Good improvement	7	5/2

Radiology

Six early CT scans were available, six at early follow-up and four at late follow-up (table 6). During the first 9 months ground glass pattern was present in all investigated patients in addition to intralobular septal thickening and a mild degree of cysts and bronchiectasis. At follow up, ground glass pattern was less

TABLE 5 Analysis of surfactant proteins B (SP-B) and C (SP-C) from bronchoalveolar lavage fluid

Patient	Age at BAL years	SP-C ng mL^{-1}		SP-B ng mL^{-1}		
		4 kDa	8 kDa	8 kDa	16 kDa	24 kDa
1	1	16	16	400	533	yes
5	1.9	58	223		2316	421
5	4.1	0	0		723	275
8	0.5	120	56	0	1697	494
10	0.1	0	0		370	673
10	0.4	0	0	536	506	413
11	19.2	14	0		229	216
12	1.3	0	0		1941	Yes
14	0.5	0	0		26	Yes
17	0.1	0	0	0	1326	1350
Mean±SE	2.9±1.8	20.8±12.4	29.5±22.2	234±138	967±251	549±145
Reference values in healthy children mean±SE; n		116±14; 61	140±81; 26	68±22; 13	580±53; 67	288±89; 18

BAL: Bronchoalveolar lavage

DOI: 10.1183/09031936.00129414

TABLE 6 Development of chest computed tomography scans[#] at diagnosis, early follow-up and late follow-up

	At diagnosis	Early follow up	Late follow up	p-value[¶]
Available studies	6	6	4	
Mean (range) age at investigation years	0.4 (0.1–0.7)	1.4 (0.9–2.0)	14.2 (8.3–19.2)	
Hilar lymphnodes (present/all invest)	1/6	2/6	2/4	NS
Ground glass opacities[#]	1.9	1.8	0.2	0.011
Cysts[#]	0.2	0.5	2.6	<0.001
Peribronchial thickening	0.1	0.5	0.2	0.006
Bronchiectasis	0.8	1	0.4	NS
Honeycombing	0	0.3	1.5	NS
Interlobular septal thickening	0	0.1	0	NS
Intralobular septal thickening	1.5	1.2	1.5	NS
Crazy paving	0.7	0.3	0	NS
Paraseptal emphysema/hyperinflation	0	0	0.8	NS
Nodular opacities	0.1	0	0	NS
Lobe retraction	0	0	0	NS
Attenuations	0	0.1	0.1	NS

[#]: Scans were rated in six areas (apex to carina, carina to lower pulmonary vein, below pulmonary vein on left and right side) for the indicated findings as none=0, mild=1, moderate=2 and severe=3; the results were averaged per CT scan in an individual subject and the mean values were calculated for the number of subjects indicated. [¶]: Explorative comparison between "at diagnosis" and "late follow up" by unpaired t-test. NS: not significant (p>0.05).

prominent, giving way to increased signs of fibrosis, including bronchial wall thickening and more severe development of cysts (table 6 and fig. 1, and fig. s3 in the online supplementary material). Differentiation between BRICHOS and non-BRICHOS domain mutations was not possible due to the limited number of available studies. Two patients (patients 9 and 13) showed the typical features of a combined pulmonary fibrosis and emphysema syndrome (CPFE), an entity which has recently been recognised [20]. Both patients had pronounced upper lobe emphysema at ages 13.6 and 19.2 years and signs of fibrosis, including intralobular septal thickening, diffuse honeycombing, and cyst formation.

Histopathology

Lung biopsies were done in 10 subjects (table 7, and table s5 in the online supplementary material), available for review in six patients; in four patients only written results of the lung biopsies were available. Most patients had a combined picture of interstitial fibrosis and interstitial chronic inflammation, type II pneumocyte hyperplasia, alveolar septal thickening, inflammation, enlargement as well as alveolar macrophage and material accumulation, and the accumulation of mesenchymal cells in the alveolar

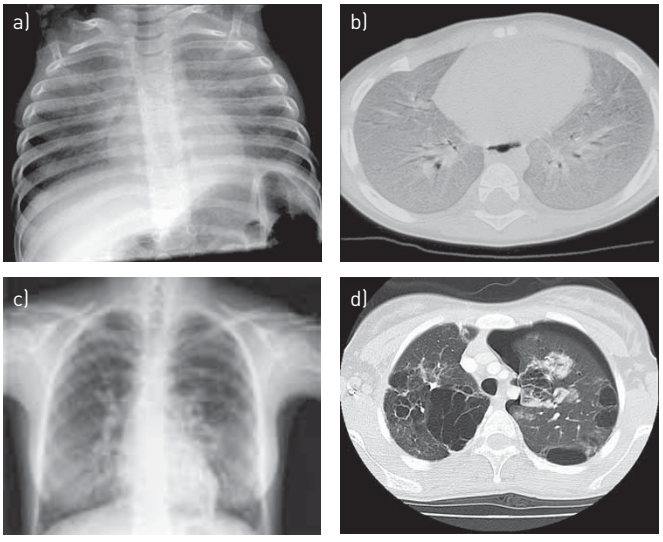

FIGURE 1 Exemplary radioimaging of two patients with surfactant protein C mutations. a, b) chest radiograph and computed tomography scan of patient 1 at 18 months showing diffuse ground glass pattern and interstitial thickening. c, d) chest radiograph and computed tomography scan of patient 9 at 13.6 years showing emphysema, cysts, bullae and left-sided pneumothorax.

TABLE 7 Histological classification, mutation and age at biopsy of patients with *SFTPC* mutations and lung biopsy

Histological picture	Patient	Mutation	Age at biopsy years
PAP	5	p.I73T[#]	1.2
	11[¶]	p.G74V[#]	0.3
	13[¶]	p.A112T	0.3
NSIP	2	p.I73T[#]	0.5
	7[¶]	p.I73T[#]	2.2
	9	p.H59R[#]	13.7
	14[¶]	p.C121F	0.3
	17	p.L101P[+] and p.E191K[+]	0.1
Combined NSIP, PAP, DIP	8[§]	p.I73T[#]	0.5
	10	p.E191*[+]	0.8

PAP: pulmonary alveolar proteinosis; NSIP: non-specific interstitial pneumonia; DIP: desquamative interstitial pneumonia. [#]: BRICHOS mutations; [¶]: only report available;[+]: novel mutations; [§]: reviewer scored NSIP only.

interstitium. Histological pattern identified were pulmonary alveolar proteinosis (PAP) (three patients) and non-specific interstitial pneumonia (NSIP) (five patients) and combined NSIP, PAP and desquamative interstitial pneumonia (DIP) (two patients). In four patients (2, 5, 8 and 17) both, report and biopsy material were available. Histological pattern recognized by two pathologists were identical in patients 2, 5 and 17, for patient 8 one pathologist classified as NSIP, one pathologist as a combined picture of NSIP, DIP and PAP (table 7).

Discussion

The goal of this study was to increase the number of genetically defined patients with SP-C deficiency, to provide detailed patient information, and to identify recognisable patterns which may be helpful for the physician to identify such rare individuals in clinical practice. The diagnosis of ILD as a consequence of *SFTPC* mutations is not easy due to the variable clinical presentation; however, indispensable knowledge of the phenotypes and a sufficient index of suspicion can serve to initiate specific genetic testing in order to rule out or confirm the suspected diagnosis. From our cohort, together with the experiences reported by others, we can differentiate three periods with different but typical characteristics depending on the age of the subject.

The first type of presentation is neonatal respiratory distress syndrome, mostly in the mature neonate. In our series patients with a mutation in the BRICHOS domain present more frequently in the first week of life, confirming the results of one other study [8]. These patients also require early mechanical ventilation. Family history may reveal lung disease, previous abortions or postnatal death in about one-third of cases.

A second type of presentation is the infant with tachydyspnoea (seven out of 17 of our patients) and failure to thrive (eight out of 17). The start of symptoms was often insidious (seven out of 17 of our patients) and possibly triggered by a viral infection (six out of 17). On chest CT imaging, ground-glass pattern is the predominant finding in patients during the first 6–9 months of age. If histology was done, a pattern of PAP, NSIP and DIP was observed, alone and in combination. This is consistent with other studies [8].

The third, late-onset type of presentation from later childhood to adolescence or even adulthood is characterised by tachydyspnoea, hypoxia and possibly repeated infections. This agrees with case reports describing manifestation of *SFTPC* mutations in adults with interstitial lung disease [10, 21, 22]. However, it may well be that several of these patients are not diagnosed earlier due to reduced perception of signs or are lost during follow-up.

Clinical outcome at follow-up varied from healthy (age 2 years) to persistent severe respiratory insufficiency (age 0.3 and 16.9 years) and was not associated with the type of mutation, the location in the BRICHOS domain, or the age at presentation. *SFTPC* mutations have been described before with severity varying from neonatal respiratory insufficiency [9] to children and adults with mild-to-severe ILD [3, 23–25]. In an elegant study, follow-up of five patients with *SFTPC* mutations was reported after three decades, as previously published cases can be genetically analysed later [10]. Three subjects (p.I38T, p.I73T and p.V39L) had no pulmonary symptoms at age 29, 32, and 37 years, respectively, and two (p.I73T) suffered from stable interstitial lung disease at age 28 and 32 years.

DOI: 10.1183/09031936.00129414

It is obvious that the phenotype of SP-C deficiency is not predicted by the *SFTPC* mutation alone. It is likely that other genes and also environmental factors, including viral infections, may strongly influence the disease course. Therefore, it is difficult in single cases to judge on the significance of some sequence variations. In the present study, we had two complex genotypes: in patient 17, the two p.L101P and p.E191K mutations were located in trans, as demonstrated by investigations of the parents and were predicted to be at least possibly damaging. In patient 16, carrying p.A53T and p.L181V was likely disease-causing, as the leucine is highly conserved across species and the variation is predicted to be protein damaging or probably damaging by SIFT and PolyPhen-2. Additionally, p.L181V has been reported in one adult with idiopathic pulmonary fibrosis (IPF) [26] and in one child with ILD [27]. In an epidemiological study, p.A53T in contrast was associated with a two-fold increased risk of asthma [28], but not ILD or chronic obstructive pulmonary disease.

Imaging over time by CT scan revealed significant changes from initial homogeneous ground glass attenuation to increasing signs of fibrosis with honeycombing, peribronchial, interlobular, and intralobular septal thickening as well as cyst formation. Qualitatively similar observations have been made in other cohorts [7, 8]. Interestingly, patients 9 and 13 exhibited typical features of a CPFE, an entity which was only recently recognised [20]. CPFE is reported to be associated especially with smoking, male sex, IPF, hypersensitivity pneumonitis, asbestosis and connective tissue disorders [20]. One recent case report described CPFE in a 32-year-old woman with a familial I73T mutation [22]. Here, we add two more patients with *SFTPC* mutations (p.H59R and p.A112T) and typical radiological features of combined upper lobe emphysema and fibrosis. Patient 9 required a left lung volume reduction due to the emphysema.

Biochemical analysis of BALF for SP-C yielded low or absent levels and as such directs the suspicion towards surfactant dysfunction disorders. However, although indicative of a *SFTPC* mutation, this finding is not specific, as reduced values have also been described in SP-B [29] and ABCA3 (ATP-binding cassette, sub-family A (ABC1), member 3) deficiency [30].

Empirical treatment with various drugs is frequently done and the results reported here may suggest some efficacy for hydroxychloroquine. Thus, in a first step, a randomised controlled trial is needed to investigate an overall effect, followed by a mutation and a stage-specific evaluation. Such studies are currently in preparation (www.childeu.net).

Acknowledgements

We thank Traudl Wesselak (Department of Pediatric Pneumology, Hauner Children's Hospital, Ludwig-Maximilians University, Munich, Germany) who helped in assembling the patient data. The work of Matthias Griese was supported by DFG-970/8-1, BMBF-Goldnet, chILD-EU (FP7, No. 305653) and the "Else Kröner-Fresenius-Stiftung".

C. Kröner, A-C. Grimmelt, R. Zarbock and M. Griese conceived and designed the study and collected and analysed the data. S. Reu and F. Brasch diagnosed, analysed and scored the histological material. V. Teusch diagnosed, analysed and scored the radiological imaging. A. Schams, M. Griese and R. Zarbock performed the biochemical analysis. P. Lohse performed the sequencing of the SFTPC gene. C. Kröner, A-C. Grimmelt, M. Griese, M. Barker, J. Brand, M. Gappa, R. Kitz, BW. Kramer, L. Lange, S. Lau, C. Pfannenstiel, M. Proesmans, J. Seidenberg, T. Sismanlar, C. Werner and S. Zielen provided patient information and material. C. Kröner and M. Griese drafted the manuscript and all authors critically revised, read and approved the final manuscript. M. Griese is responsible for the study concept and validity of the data.

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DOI: 10.1183/09031936.00129414