Genetic disorders of the surfactant system: focus on adult disease

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Genes involved in monogenic surfactant-related parenchymal lung disease associate with age, ethnicity and pulmonary phenotype but not with sex. High disease penetrance and emerging genotype–phenotype correlations advocate genetic analysis in the clinic. https://bit.ly/2PrjAp6

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ABSTRACT Genes involved in the production of pulmonary surfactant are crucial for the development and maintenance of healthy lungs. Germline mutations in surfactant-related genes cause a spectrum of severe monogenic pulmonary diseases in patients of all ages. The majority of affected patients present at a very young age, however, a considerable portion of patients have adult-onset disease. Mutations in surfactant-related genes are present in up to 8% of adult patients with familial interstitial lung disease (ILD) and associate with the development of pulmonary fibrosis and lung cancer.

High disease penetrance and variable expressivity underscore the potential value of genetic analysis for diagnostic purposes. However, scarce genotype–phenotype correlations and insufficient knowledge of mutation-specific pathogenic processes hamper the development of mutation-specific treatment options.

This article describes the genetic origin of surfactant-related lung disease and presents spectra for gene, age, sex and pulmonary phenotype of adult carriers of germline mutations in surfactant-related genes.

Surfactant-related genes

Mutations in surfactant-related genes have been recognised as an important cause of severe pulmonary disease. This review aims to assemble and discuss what is known on the mutated genes and the phenotype of the affected patients. As several excellent reviews for surfactant-related paediatric disease exist [1–3], we have focused on aggregating data for adult cases.

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Pulmonary surfactant consists of a mixture of lipids and proteins that are essential for lowering alveolar surface tension and preventing alveolar collapse at the end of expiration. Furthermore, surfactant proteins play an important role in modulation of the immune response and of inflammatory processes [4–11].

The genes *SFTPA1*, *SFTPA2*, *SFTPB*, *SFTPC* and *SFTPD* encode the apolipoproteins that form approximately 8% of the surfactant fluid (consisting of surfactant proteins A (SP-A; 5.5%, comprising of SP-A1 and SP-A2), B (SP-B; 1%), C (SP-C; 1%) and D (SP-D; 0.5%)) [10]. These proteins can be divided into two groups which differ highly in biological processing and function: the hydrophilic proteins (SP-A and SP-D) and the hydrophobic proteins (SP-B and SP-C). The latter require so-called "lamellar bodies" for biosynthetic processing, transport and secretion into the alveolar lumen. A critical component of these lamellar bodies is encoded by the gene ATP binding cassette subfamily A member 3 (*ABCA3)* [1].

TABLE 1 Characteristics of genes involved in monogenic parenchymal lung disease

Characteristic	*SFTPA1*	*SFTPA2*	*SFTPB*	*SFTPC*	*ABCA3*	*NKX2-1*
Genomic						
Genomic location	10q22.3	10q22.3	2p11.2	8p21.3	16p13.3	14q13.3
Size kb	4.5	4.5	9.5	9.8	65	3.8
Number of exons (coding)	6 (4)	6 (4)	11 (10)	6 (5)	33 (30)	3 (3)
Exons encoding mature protein	3–6	3–6	6–7	2	4–33	1–3
Number of amino acids in mature protein (numbering)	227 (21–248)	227 (21–248)	79 (201–279)	35 (24–58)	1704 (1–1704)	401 (1–401)
Molecular weight of mature protein kDa	35	35	8	4	191	39
Disease						
Inheritance	Autosomal dominant	Autosomal dominant	Autosomal recessive	Autosomal dominant	Autosomal recessive	Autosomal dominant
Children at diagnosis	Rare (one infant)	Not reported	Neonatal RDS Rarely ILD in childhood	ILD in childhood Some neonatal RDS	Neonatal RDS ILD in childhood	Neonatal RDS ILD in childhood
Adults at diagnosis	Interstitial pneumonitis/ pulmonary fibrosis Lung cancer	Interstitial pneumonitis/ pulmonary fibrosis Lung cancer	Not reported	Interstitial pneumonitis/ pulmonary fibrosis	Rarely Interstitial pneumonitis/ pulmonary fibrosis	Rarely Interstitial pneumonitis/ pulmonary fibrosis
Mutation sites	CRD	CRD	Coding sequence	Mature peptide C-terminal linker BRICHOS domain	Coding sequence	Gene deletion Exon 2–3
Most frequent mutation						
Known as	NA	NA	121ins2	I73T	E292V	NA
DNA notation			c.361delCinsGAA[#]	c.218T>C	c.875A>T	
Protein			p.(Pro121GlufsTer95)[#]	p.(Ile73Thr)	p.(Glu292Val)	
Transcript			NM_000542.5	NM_003018.3	NM_001089.3	
RS number			rs35328240	rs121917834	rs149989682	
Classification			Pathogenic	Pathogenic	Pathogenic	
Mutation frequency			>60% of mutated alleles	>25% of mutated alleles	<10% of mutated alleles	
Origin			Haplotype of NW European descent	Mutation hotspot	Unique haplotype associated with European descent	
GnomAD allele frequency[¶]			0.0004[+]	0.000	0.004	

RDS: respiratory distress syndrome; ILD: interstitial lung disease; CRD: carbohydrate recognition domain; NA: not applicable; NW: North-Western. [#]: depending on the transcript referred to as c.397delCinsGAA and p.Pro133GlnfsTer95; [¶]: GnomAD frequency for European non-Finnish; [+]: frequency of rs779795233, the read data shows this concerns rs35328240.

Transcription of the surfactant genes is regulated by the transcription factor *NKX2-1*. Mutations in the surfactant-related genes *SFTPA1*, *SFTPA2*, *SFTPB*, *SFTPC*, *ABCA3* and *NKX2-1*, but not *SFTPD*, have been established as an important cause of monogenic parenchymal lung disease. Table 1 provides details of the disease-associated genes and their most frequent mutations [2, 6, 12–19].

Surfactant proteins B and C

The hydrophobic proteins SP-B and SP-C increase the rate of surfactant spreading over the alveolar surface. Furthermore, they enhance surface activity, contribute to innate immune defence of the lung and may be involved in surfactant catabolism through enhancement of the uptake of surfactant phospholipids [5, 20–23].

Synthesis occurs in alveolar Type II (AT2) epithelial cells and involves production of large precursor proteins, known as pro-SP-B and pro-SP-C, followed by intracellular proteolytical processing into the final mature forms. Mature SP-B and SP-C are secreted in the alveolar space *via* fusion of the lamellar body membrane with the alveolar cell membrane. Presence of SP-B in lamellar body membranes has been shown to be essential for correct processing of SP-C, whereas SP-C is not essential for processing of SP-B [12, 20, 24–29].

SFTPB gene mutations

The first mutation in *SFTPB* was identified in a full-term neonate with SP-B deficiency and alveolar proteinosis, who was homozygous for the so-called "121ins2" mutation [12] (table 1). The 121ins2 mutation in exon 4, officially referred to as c.361delCinsGAA, causes a frame-shift and a premature stop codon in exon 6, resulting in an unstable transcript as well as an absence of mRNA and SP-B protein [12]. Inheritance of disease due to mutations in *SFTPB* is autosomal recessive, with patients carrying bi-allelic homozygous or compound heterozygous mutations [2, 30, 31]. Approximately 50 different mutations have now been reported throughout the gene, of which the 121ins2 mutation is the most common and may account for over half of the *SFTPB* cases in white cohorts with respiratory distress syndrome (RDS) [2, 32–37]. Whereas homozygosity is deleterious, parents carrying the mutation in a heterozygous state are not affected, suggesting that a 50% decrease in SP-B has no major health effects.

Disease due to *SFTPB* mutation typically presents in neonates as RDS with a fatal outcome within the first months after birth. However, some patients are reported to have longer survival times and later onset of disease. These patients carry mutations that allow partial production of functional SP-B protein [2, 34–36, 38]. Although numbers are low, it is therefore suggested that a genotype–phenotype correlation may exist, with mutations causing complete deficiency in SP-B associating with fatal neonate RDS and mutations causing partial deficiency in SP-B associating with delayed onset and prolonged survival. However, survival is rare and, although some children age, no adults with pathogenic bi-allelic *SFTPB* mutations have been reported thus far.

SFTPC gene mutations

The first family with a pathogenic mutation in *SFTPC* included not only a 6-week old, full-term infant with tachypnoea and cyanosis, but also her mother [39]. Candidate gene sequencing of *SFTPC* yielded a heterozygous mutation (c.460+1 G→A) that causes a deletion of exon 4 [39]. The mother, who died after delivery from respiratory insufficiency, had been diagnosed with desquamative interstitial pneumonitis (DIP) at the age of 1 year and was treated with corticosteroids until the age of 15.

Thereafter, several families with pulmonary fibrosis were discovered and not all included children [40–42]. In contrast with *SFTPB*, inheritance of *SFTPC*-mediated disease is dominant and affects subjects of all ages ranging from newborns to the elderly [40, 43, 44]. As an exception, several bi-allelic mutation carriers were reported with early-disease onset [15, 45].

The pathogenic effect of an *SFTPC* mutation is primarily the result of aberrantly processed mutant protein, which has toxic consequences for the AT2 epithelial cell. These intracellular effects are associated with the position of the mutation in the gene and the type of mutation [8, 42, 46]. The most common *SFTPC* mutation in both children and adults is the so-called "I73T" linker domain mutation. *SFTPC* I73T, officially named c.218T>C, alters trafficking of the pro-peptide to early endosomes [47] and causes dysregulated proteostasis in AT2 epithelial cells [48]. Furthermore, alteration of surfactant lipid composition and activation of immune cells are reported for this mutation [49]. Other mutations, situated in the C-terminal BRICHOS domain, cause an increase in endoplasmic reticulum (ER) stress and activation of the unfolded protein response (UPR) [50–53] in AT2 epithelial cell experiments, leading to a toxic intracellular accumulation of the pro-protein and apoptosis [8, 54, 55].

SFTPC mutations have been frequently reported in neonates and juveniles suffering from RDS or chronic interstitial lung disease (ILD) and many were *de novo* findings [15, 39–41, 43, 44, 56–66]. Mortality is high, with disease worsening in approximately 50% of paediatric cases [61]; however, children have also been reported to experience disease stability or recovery with (or even without) long-term immunomodulation therapy [39, 61, 62, 67]. It does appear, however, that after years of relative stability symptoms often resurface or aggravate unexpectedly and disease progresses towards treatment refractory pulmonary fibrosis. Studies using knock-in *Sftpc* I73T mice show that induced expression in heterozygous mice results in early inflammation, which does not proceed to fibrosis, whereas, in homozygous mice, progression towards fibrosis does occur [68]. This shows that fibrogenesis is preceded by an early inflammatory phase and that dosage of the mutant allele influences disease evolution. Adults with *SFTPC* mutations present at all ages with a form of idiopathic interstitial pneumonitis (IIP) characterised by progressive pulmonary fibrosis, while no pulmonary disease is usually detected in childhood [39–41, 43, 44, 56, 57, 63–66]. Disease penetrance is extremely high and only a few asymptomatic family members carrying a pathogenic *SFTPC* mutation have been reported. Pulmonary screening of these relatives often unveils subclinical disease with below normal lung function or the presence of ILD changes on high-resolution computed tomography (HRCT) [15, 69].

Lamellar body protein gene *ABCA3*

The lamellar body ATP-binding cassette 3 protein encoded by the *ABCA3* gene is essential for intracellular processing and transport of surfactant proteins B and C [20]. Given the important role of lamellar bodies in surfactant metabolism, the gene was investigated in 21 ethnically diverse infants with severe neonatal surfactant deficiency of unknown aetiology. Fourteen infants had bi-allelic, mostly homozygous *ABCA3* mutations and aberrantly formed small dense bodies in their AT2 epithelial cells [70].

Mutations in *ABCA3* are now known as a common cause of both fatal RDS in the neonatal period and chronic ILD in older infants and children [31, 70, 71]. Inheritance of disease due to mutations in *ABCA3* is autosomal recessive, with patients carrying homozygous or compound heterozygous mutations. The most frequent mutation is c.875A>T, known as "E292V", which was first discovered in paediatric patients with ILD [72]. Studies have shown a significant reduction in lamellar body size and secreted phospholipids in homozygous E292V knock-in mice, which developed alveolitis and age-dependent lung remodelling [73]. Homozygosity for E292V is rare, with only one infant with fatal neonatal respiratory distress and one adult with idiopathic pulmonary fibrosis (IPF) reported [64, 74]. The E292V mutation is most commonly found in compound heterozygous patients.

Five-year survival in subjects with two disease-causing *ABCA3* mutations is <20% [61]. To date, over 200 different mutations have been discovered and genotype–phenotype correlations are beginning to emerge, although the role of many mutations is still unknown and difficult to predict. Most frequent are null mutations (frame-shift and nonsense mutations) predicting complete absence of functional ABCA3. A study of 185 children with bi-allelic *ABCA3* mutations has shown that patients who are homozygous for null mutations have respiratory failure at birth, resulting in either a fatal outcome or lung transplantation, whereas absence of null mutations on one or both alleles frequently results in later onset and better survival [74]. Similar results have been retrieved in a study of 40 European patients [75].

The non-null mutations result in partial functional impairment or induce cellular toxicity [1] and correlate with better survival. Although extremely rare, a recent review and case series [76] describes seven adult patients with interstitial pneumonitis who carry bi-allelic mutations in *ABCA3*, of which only one patient carried a null mutation [71, 75–78].

Carrying a single *ABCA3* missense mutation may also increase risk for disease and has been found to be associated with increased risk of neonatal RDS in late preterm infants [79, 80]. Moreover, it has been suggested that heterozygous carriers of a single *ABCA3* mutation might influence disease development of carriers with an SFTPC mutation [69, 81]. Very few such cases are documented however and, due to the variable disease course in *SFTPC* mutation carriers, the link is difficult to study [40, 69, 81].

Surfactant proteins A and D

The hydrophilic surfactant proteins SP-A and SP-D are structurally related and play an important role in adaptive and innate immunity [6, 7]. The proteins consist of four domains: a short N-terminal involved in oligomerisation, a collagen-like domain, a coiled neck region important for oligomerisation and spacing the lectin domain and the C-terminal carbohydrate recognition domain (CRD). The CRD induces opsonisation by binding carbohydrates at the surface of pathogens [6, 82].

Expression of SP-A and SP-D is not limited to the lung, and both RNA and immunohistochemical expression have been reported in the epithelia of multiple organs in congruence with a role in host defence

[6, 82–84]. In the lung, SP-A and SP-D expression appears highest in AT2 epithelial cells, but is also observed in sub-mucosal and club cells [85, 86]. In contrast with SP-B and SP-C, secretion of SP-A and SP-D in AT2 epithelial cells bypasses the lamellar bodies [87]. Within AT2 epithelial cells, SP-A localises mainly in the small vesicles and multivesicular bodies [88], whereas SP-D is highly localised in the ER, as well as being present in the Golgi complex and in multivesicular bodies [86]. Furthermore, SP-A is enriched in the outer membranes of unwinding lamellar bodies in the alveoli [88] and plays a role in tubular myelin formation. In mice lacking SP-A, tubular myelin was missing and increased susceptibility to pulmonary infections was observed [89].

Two slightly different forms of SP-A (SP-A1 and SP-A2) exist and are encoded by *SFTPA1 and SFTPA2*. These genes are highly similar, sharing 94% sequence homology [6], with the main difference consisting of four amino acids in the collagen-like domain [82, 90]. However, both quantitative and qualitative differences exist: SP-A2 is more efficiently translated, is more abundant and more effectively enhances bacterial phagocytosis than SP-A1 [91, 92].

To date, only mutations in *SFTPA1* and *SFTPA2* have been associated with monogenic lung disease. Common variants in *SFTPD* are associated with numerous respiratory diseases, as recently reviewed by Sorensen *et al.* [82], but mutations that specifically cause monogenic parenchymal lung disease are not known. The absence of *SFTPD* mutations suggests that either such mutations are deleterious *in utero*, do not cause a pulmonary phenotype, or may be resolved without pathogenic consequences. For instance, the low production of SP-D in AT2 epithelial cells may preclude the deleterious consequences of mutant alleles.

SFTPA2 gene mutations

Involvement of the surfactant biolectin genes in monogenic lung disease was discovered through genome linkage analysis in a large family with adult-onset pulmonary fibrosis. The analysis pointed towards a large region at the long arm of chromosome 10 which contained about 120 genes including *SFTPA1*, *SFTPA2* and *SFTPD* [93]. Subsequent sequencing revealed a heterozygous mutation (GGG/GTG) in codon 231 of *SFTPA2* (c.692G>T; p.(G231V)) which segregated with disease in the family. Additional sequencing in 58 unrelated probands with familial pulmonary fibrosis revealed a second heterozygous mutation in *SFTPA2*. Both mutations involve amino acid substitutions in exon 6 (which encodes the CRD) and were predicted to destabilise the protein [93]. In transfected A549 cells, ER retention of the mutant protein and ER-stress were observed; furthermore, the mutated protein was not present in the patient's lavage fluid [94]. Later studies not only showed that harmful mutations are limited to exon 6, but also that, next to pulmonary fibrosis, lung cancer was always present in mutation carrying families [64, 95]. Six mutations are now known and inheritance of disease due to mutations in *SFTPA2* is autosomal dominant. Disease has only been reported in adults [64, 93, 95], although the age range is wide. The youngest patient presented at the age of 20 years with a significantly reduced forced vital capacity (FVC) of 57% predicted and diffusing capacity of the lung for carbon monoxide (D_{LCO}) of 49% predicted [95] (suggestive of preclinical onset of disease pathogenesis at an even younger age). It is our experience that survival is limited, mimicking the outcome in IPF and that lung transplantation is a successful therapy in adults.

SFTPA1 gene mutations

The involvement of mutations in the *SFTPA2* gene in families with both pulmonary fibrosis and lung cancer, led to the compilation of a small cohort characterised by the co-existence of both diseases. Candidate sequencing of *SFTPA2* in the 12 probands yielded no results; however in *SFTPA1*, a disease segregating heterozygous mutation (c.631T>C) causing an amino acid substitution at position p.(W211R) was detected. Similar to previous *SFTPA2* findings, the mutation was located in exon 6 and inheritance was autosomal dominant [96]. Experiments with transfected HEK293T cells showed an absence of mutated protein in the cell medium. Although the study involved families with adult-onset pulmonary fibrosis, a baby of 9 months old who died from severe respiratory disease was a member of the mutation carrying family. The child's biopsy demonstrated excessive intra-cytoplasmic SP-A staining in hyperplastic AT2 epithelial cells, highly suggestive of a deleterious effect due to mutated protein in the postnatal period [96].

Thereafter three other families were described, each with their own unique exon 6 *SFTPA1* mutation [97–99], including the homozygous adult sons of consanguineous heterozygous Japanese parents [99]. The parents were asymptomatic but, upon examination, subclinical disease with a D_{LCO} <63% predicted was detected [100].

Multisystem diseases: NKX2-1

There are several other genes directly or indirectly related to surfactant homeostasis, in which mutations may cause pulmonary fibrosis. For these genes, pulmonary disease is often only reported in paediatric

patients or in the context of a multi-system disease in children and adults [101, 102]. One of these genes is the transcription factor *NKX2-1*, which directly regulates expression of all the above surfactant proteins and ABCA3, as well as its own expression *via* a positive feedback loop [103]. Heterozygous mutations in *NKX2-1* cause a spectrum of pulmonary phenotypes, presenting as RDS in neonates or ILD in older children or adults affected with pulmonary fibrosis or recurrent pulmonary infections. *NKX2-1* associated disease is expressed as brain–lung–thyroid syndrome. While neurological features are most common, the respiratory problems may appear isolated in up to 25% of patients and can improve or become life threatening [104, 105].

Inheritance of disease is autosomal dominant and caused by haploinsufficiency, mostly conferred by nonsense and frame-shift mutations or by complete gene deletions [106]. Both penetrance and expressivity are extremely variable, corresponding with an overall lack of genotype–phenotype correlations [104–107]. Many patients survive into adulthood; however, diagnosis at adulthood without prior severe respiratory problems is only described in four subjects. These subjects had an age at diagnosis of between 25 and 40 years. Two subjects had pulmonary fibrosis, one had ILD and one was asymptomatic with early signs of pulmonary fibrosis [104, 105].

Among the many syndromes associated with pulmonary fibrosis and surfactant homeostasis, Hermansky–Pudlak syndrome (HPS) is worth mentioning. HPS is an autosomal recessive, multisystem disease characterised by oculocutaneous albinism. The disease is caused by dysfunctional vesicles in multiple organs, such as the skin and the eyes, as well as the blood and AT2 epithelial cells. Mutations in the *HPS1*, *AP3B1* (HPS2) and *HPS4* genes, encoding major components of lamellar bodies, are involved in the development of chronic progressive pulmonary fibrosis [108]. Onset of pulmonary fibrosis usually occurs at the age of 30–40 years and medium survival is approximately 10 years [109]. HPS has one of the best organised patient societies, facilitating not only patient empowerment but also scientific research and conductance of clinical trials (www.hpsnetwork.org).

Pathogenesis

Two different kinds of deleterious surfactant-related mutation exist. The first, loss-of-function mutation, results in an absence or a decrease in functional protein. This type of mutation is generally found in recessive disease and indeed is an important mode of action of mutations in *ABCA3* and *SFTPB*; however, loss-of-function is also the mechanism behind the dominant *NKX2-1* disease. Loss-of-function mutations cause reduced protein content resulting in dysfunctional lamellar bodies or disrupted surfactant homeostasis. The second mechanism, gain-of-function mutation, involves an increase in the amount of a protein or alters its functionality. This type is most common in dominant disease and is the main mode of action of mutations in *SFTPC*, *SFTPA1* and *SFTPA2*. Several studies have shown that mutations in these genes result in increased protein misfolding, aberrant protein trafficking, intracellular retention or accumulation, increased ER stress, activation of the UPR, increased pro-apoptotic signalling and apoptosis [47, 55, 94]. The commonality involved is not the mutation specific aberrant process but the involvement of the AT2 epithelial cell, the alveolar progenitor cell that is key to alveolar maintenance and repair [110]. Several studies have provided evidence that injurious processes in AT2 epithelial cells, which may be mediated by apoptosis, are sufficient for the development of pulmonary disease [111]. However, not all studies could detect involvement of apoptosis, even when increased ER-stress could be detected [94]. Recently, a different form of regulated cell death, necroptosis (also known as programmed necrosis) has been detected in surfactant-related disease. Knock-in mice carrying the *Sftpa1* c.622T>C mutation spontaneously developed pulmonary fibrosis at 20 weeks of age, with further deterioration on aging or on infection with influenza virus. Most importantly, enhanced necroptosis rather than apoptosis of AT2 epithelial cells was detected in the knock-in mice and in the affected patient biopsies [99].

Overall, it can be concluded that the pathogenesis of surfactant-related mutations is only partly overlapping, which complicates the development of targeted therapeutic interventions.

Mutation spectrum

Mutations in surfactant-related genes are an accepted cause of severe pulmonary disease, but the overall relative and gene-specific contribution to disease is not well known. Figure 1a provides the mutation spectrum in 221 adult cases (>18 years old) of probands with familial pulmonary fibrosis in the national Dutch ILD biobank at St Antonius Hospital, using whole exome sequencing (WES) and mutation detection in all genes associated with familial pulmonary fibrosis. Mutations in surfactant-related genes are present in nearly 8% of probands, whereas nearly 36% had a telomere-related gene mutation and in 56% no causal mutation was found. These data are highly congruent with the previously published mutation spectrum for French patients suspected of monogenic pulmonary fibrosis [112].

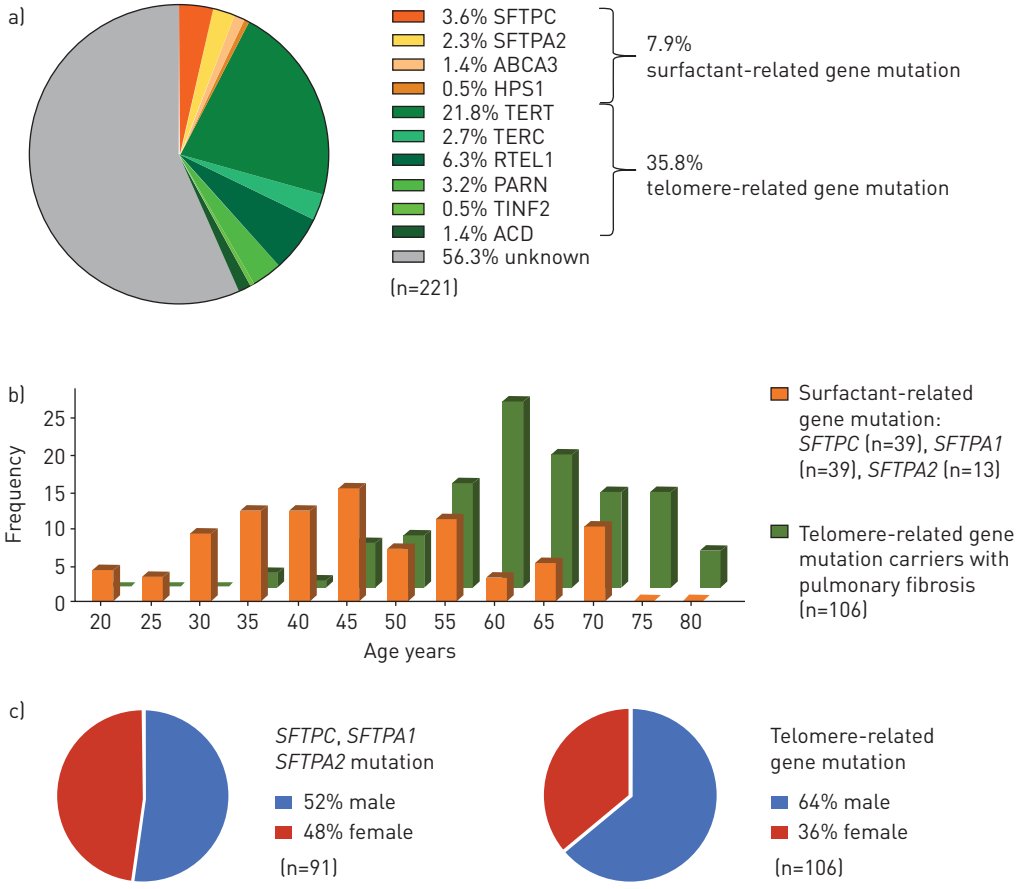

FIGURE 1 Mutation spectrum, age and sex of patients with surfactant-related mutations in comparison with telomere-related mutations. a) Mutation spectrum of adult Dutch probands with familial pulmonary fibrosis (mutation spectrum of the St. Antonius cohort, consisting of the probands (>18 years) of families with pulmonary fibrosis screened with whole exome sequencing. b) Age distribution of patients with a surfactant- or telomere-related gene mutation (age distribution of probands and family members with pulmonary fibrosis). Telomere-related mutation data is from the St. Antonius cohort. Surfactant mutation data is from the St. Antonius cohort and all adult cases reported in the literature. Age is the patient's age at disease diagnosis or the age reported in the literature. c) Male-to-female ratio of adult patients with monogenic pulmonary fibrosis (cohort composition is similar to that of b).

The mutation frequency varies widely in paediatric cohorts of RDS and ILD, but experts suggest that 10–20% of cases have monogenic surfactant-related disease [2, 3, 113]; however, the specific genes involved are dependent on age, ethnicity and disease phenotype.

Comparing the results of multiple studies shows that involvement of the recessive genes *SFTPB* and *ABCA3* differs between populations [16, 35–37, 114–119]. Furthermore, next to clinical differences between the studied cohorts, the background frequency of deleterious recessive alleles also differs between populations. Haplotype and population analysis have shown that in some healthy white populations the most frequent *SFTPB* 121ins2 and *ABCA3* E292V mutations have allele frequencies of 0.03–0.1% and 0.3–0.4%, respectively, but are absent or extremely rare in healthy African and Asian cohorts [16, 17, 33]. Furthermore, the origin of the parents has been shown to influence the mutation spectrum (*e.g.* one study has found that in patients of Middle-Eastern descent, not E292V but Y1515X is the most frequent mutation [74]). Homozygosity for deleterious alleles is frequent, however, this is not due to high background frequencies, but is instead caused by consanguinity [74, 75].

For lethal dominant gene mutations there is no population background frequency. The most frequent *SFTPC* mutation (I73T) is absent in all healthy cohorts but present in disease worldwide [15, 17, 40, 58, 64, 65, 120]. Haplotype analysis in three Dutch families with pulmonary fibrosis and the I73T mutation has shown that the mutations are of independent origin [40]. The *SFTPC* I73T mutation clearly represents a mutational hotspot, although extremely high disease penetrance and lethal expressivity prevent it from spreading in the general population. When comparing the frequency of *SFTPC* I73T and *ABCA3* E292V between our 120 probands with familial pulmonary fibrosis and the GnomAD database (table 1), we

found that the three *SFTPC* I73T mutations were statistically over-represented in familial pulmonary fibrosis, whereas the three *ABCA3* E292V mutations in familial pulmonary fibrosis may be expected on the basis of an overall low background frequency in white subjects.

Age

The involvement of surfactant-related genes in disease is highly associated with age. Mutations in the recessive *SFTPB* and *ABCA3* genes predominantly involve neonates and infants, while in children with ILD mutations are most commonly present in the *ABCA3* and *SFTPC* genes [121]. The age spectrum of adult surfactant cases was not studied previously. As such, we compiled an age spectrum based on all reported adult cases (n=84) together with unpublished cases from our own cohort (n=7) [39–41, 43, 44, 56, 57, 63–66, 71, 93, 95–100]. Comparison between the age spectrum of this aggregated surfactant cohort and our Dutch pulmonary fibrosis cohort with telomere-related gene mutations shows that patients with surfactant mutations present with early-onset disease significantly more often (figure 1b). Median age in adult patients with surfactant-related mutations is 45 years *versus* 62 years in patients with telomere-related mutations ($p<0.0001$ by Mann–Whitney U-test). To further analyse differences between surfactant genes, we subdivided the aggregated cohort. The median age in the *SFTPC* group is 37 years, which is significantly lower than the median age of 48 years in the *SFTPA1*/*SFTPA2* group ($p=0.0036$ by Mann–Whitney U-test). The age difference is in congruence with the rarity of *SFTPA* mutations in childhood (only one documented case *versus* 51 adult cases) and suggests that *SFTPA* may be considered as a disease for adults, while *SFTPC*-related disease may present at all ages.

While genotype–phenotype correlations arise for the recessive *SFTPB* and *ABCA3* genes, with complete protein deficiency causing fatal neonatal disease and partial functionality enabling prolonged survival, no such age-associated correlations exist for the dominant genes. Evaluation of paediatric *SFTPC* cases suggests that patients with mutations in the BRICHOS domain present at an earlier age than non-BRICHOS cases [61, 62]. However, there is no significant difference ($p=0.1$) between the age of adult patients with BRICHOS mutations (n=21; mean age 38 years) *versus* non-BRICHOS mutations (n=17; mean age 45 years) in this review.

The gene-specific age spectrum, for which only data from patients diagnosed at age 18 years or older is used, is shown in figure 2a. Even so, it remains possible that the patients included have had either earlier preclinical onset of disease or an episode of clinical disease caused by the as yet unrecognised surfactant disorder during childhood. In figure 2b we present a model for the relative contribution of each gene to

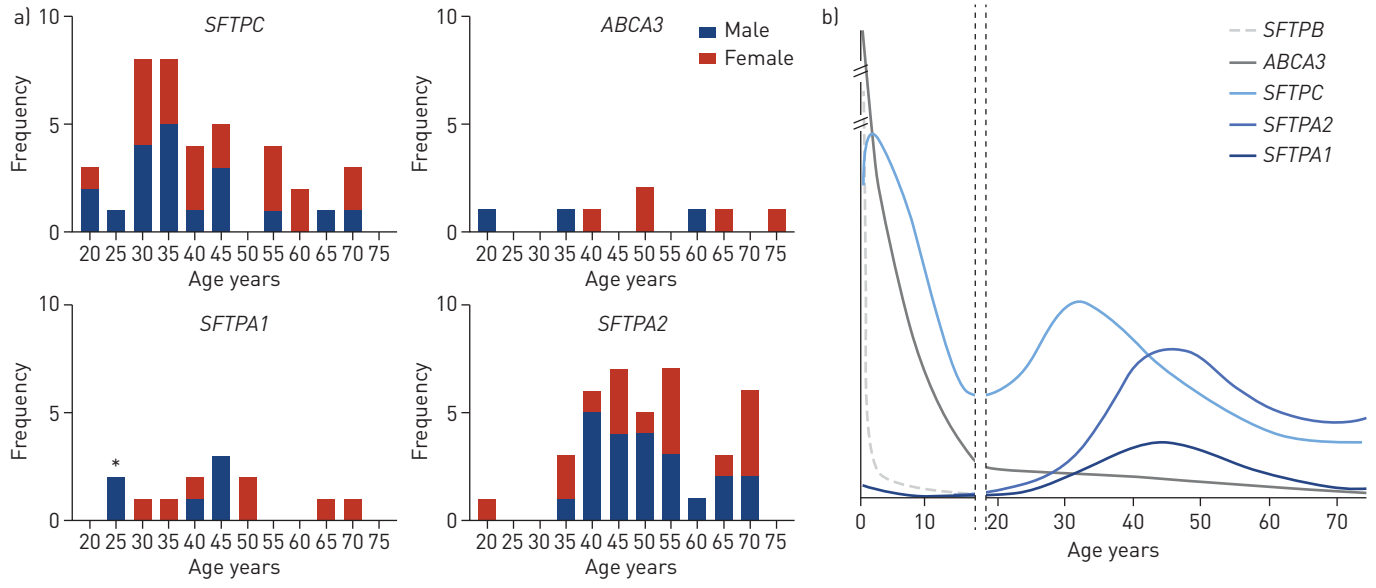

FIGURE 2 Age at disease onset spectrum for genes associated with monogenic surfactant-related parenchymal lung disease. a) Distribution of age and sex in patients with adult-onset disease, including all cases reported in the literature and from the St. Antonius cohort (columns represent age categories for subjects with an age ±2.5 years around the column label). Age is the patient's age at disease diagnosis or the age in the original report. b) Model for monogenic surfactant-related disease, showing the relative contribution of each gene to monogenic parenchymal lung disease (the break at age 18 years represents the lack of knowledge of how paediatric data relates to adult data, while the dashed line for *SFTPB* is representative for US patients of North-Western origin, the frequency being much lower in other cohorts). *SFTPC*, *SFTPA1* and *SFTPA2* are heterozygous cases except for two brothers included in *SFTPA1* (*).

disease. The break at age 18 years represents the lack of knowledge on how frequencies below and above 18 years relate to each other. For *SFTPC*, it is not yet known whether the risk of developing disease is greater in childhood or adulthood (many studies report paediatric *SFTPC* cases, but reports have also been made on many families dominated by adult cases).

Sex in adult-onset disease

Differences in lung growth and production of surfactant are known to exist between males and females. Premature male infants are more susceptible to RDS and adult males are more susceptible to pulmonary fibrosis [122]. Knock-in mice with induced *Sftpc* I73T expression show significantly worse survival in male mice *versus* female mice [68]; furthermore, males predominate in telomere-related disease [123, 124]. That males (52%) and females (48%) are equally affected by surfactant mutations is shown in figure 1c. In addition, both sexes appear equally distributed across all ages (figure 2a). This suggests that in monogenic surfactant-related disease sex, as well as environmental factors related to sex, do not have a major influence on development of disease.

Radiological and histological findings

Mutations in surfactant genes cause a variety of pulmonary phenotypes that are most strongly associated with age, ranging from neonatal RDS (*ABCA3*, *SFTPB* and *NKX2-1*) to children with ILD (*ABCA3*, *SFTPC* and *NKX2-1*) and adults with ILD and development of pulmonary fibrosis or lung cancer (*SFTPC*, *SFTPA1*, *SFTPA2* and *NKX2-1*).

Adult patients with surfactant-related mutations develop pulmonary fibrosis; however, the radiological pattern is usually inconsistent with usual interstitial pneumonia (UIP) and is often described as nonclassifiable interstitial pneumonitis. Features described for patients with an *SFTPC* mutation include bilateral reticular abnormalities, septal thickening, traction bronchiectasis or bronchiolectasis, ground-glass opacities (GGOs) and infiltrates [40, 43, 57, 63, 65, 66]. HRCT images commonly show parenchymal lucencies in the form of one or more scattered cystic lesions, most often in both lungs and varying in size between 0.5 cm and large bullae, or emphysematous changes [40, 43, 57, 63, 65, 66]. For children with *SFTPC* mutations, where HRCT shows GGOs, increasing signs of fibrosis and cyst formation are observed with increasing age [61]. Follow-up of such cystic changes in our adult patients shows that the clearly walled cysts are not static, but may collapse in time (figures 3a and 3b) or, conversely, develop into large bullae-like structures (figures 3c and 3d). These structural changes in adults are likely caused by mechanic forces that arise when the lungs shrink from progressive fibrosis.

In contrast with radiological findings, the histological findings in adult patients with *SFTPC* mutations most commonly meet the criteria for a UIP classification and fibroblast foci have been detected even in mildly affected patients [40, 43]. The UIP pattern in adults is often superimposed by other inflammatory interstitial pneumonitis patterns, such as DIP or cellular nonspecific interstitial pneumonia (NSIP) [39, 40, 43, 63, 66]. Young children with *SFTPC* mutations present with histological patterns like cellular NSIP, DIP or pulmonary alveolar proteinosis (PAP) [39, 61, 66, 125] and may only develop fibroblast foci when entering adolescence. Furthermore, the proteinaceous material may be concentrated in abundantly present foamy alveolar macrophages [120]. However, even more diverse histological findings have been observed in adults, such as lesions suggestive of chronic hypersensitivity pneumonitis (HP) with airway inflammation and granuloma (along with a UIP pattern) [63] and lesions suggestive of sarcoidosis with well-formed noncaseating granuloma (own data). Furthermore, a patient with an *SFTPC* mutation has been found among a large cohort of adults with rheumatoid arthritis-associated ILD (RA-ILD) [56]. This is further proof of the idea that the different forms of pulmonary fibrosis may have highly overlapping disease pathogenesis, with molecular make-up not so much determining disease phenotype as determining disease outcome. For example, in telomere-related pulmonary fibrosis it has been shown that regardless of the disease phenotype the disease has a fatal outcome [126]. This may also apply to surfactant-related mutations in adults but more research is needed.

HRCT of *SFTPA1* and *SFTPA2* mutation carriers is difficult to classify, but most commonly contains septal thickening and GGOs and may be described as NSIP [95, 97, 99]. The few histological descriptions of *SFTPA* mutation carriers most often describe a pattern of UIP, but NSIP, DIP and organising pneumonia (OP) have also been mentioned [93, 95, 97, 99].

While there may be some gene-related radiological characteristics, such as parenchymal lucencies (*i.e.* solitary cysts) in *SFTPC* mutation carriers, histological examinations have yet to reveal specific gene-related features. However, reviewing all evidence, we suggest that the development of fibroblast foci is the common denominator in biopsies of all adult progressive surfactant-related mutation carriers and determines outcome. Development of other features, such as infiltrates or granuloma may be the result of intrinsic or extrinsic factors that differ between patients.

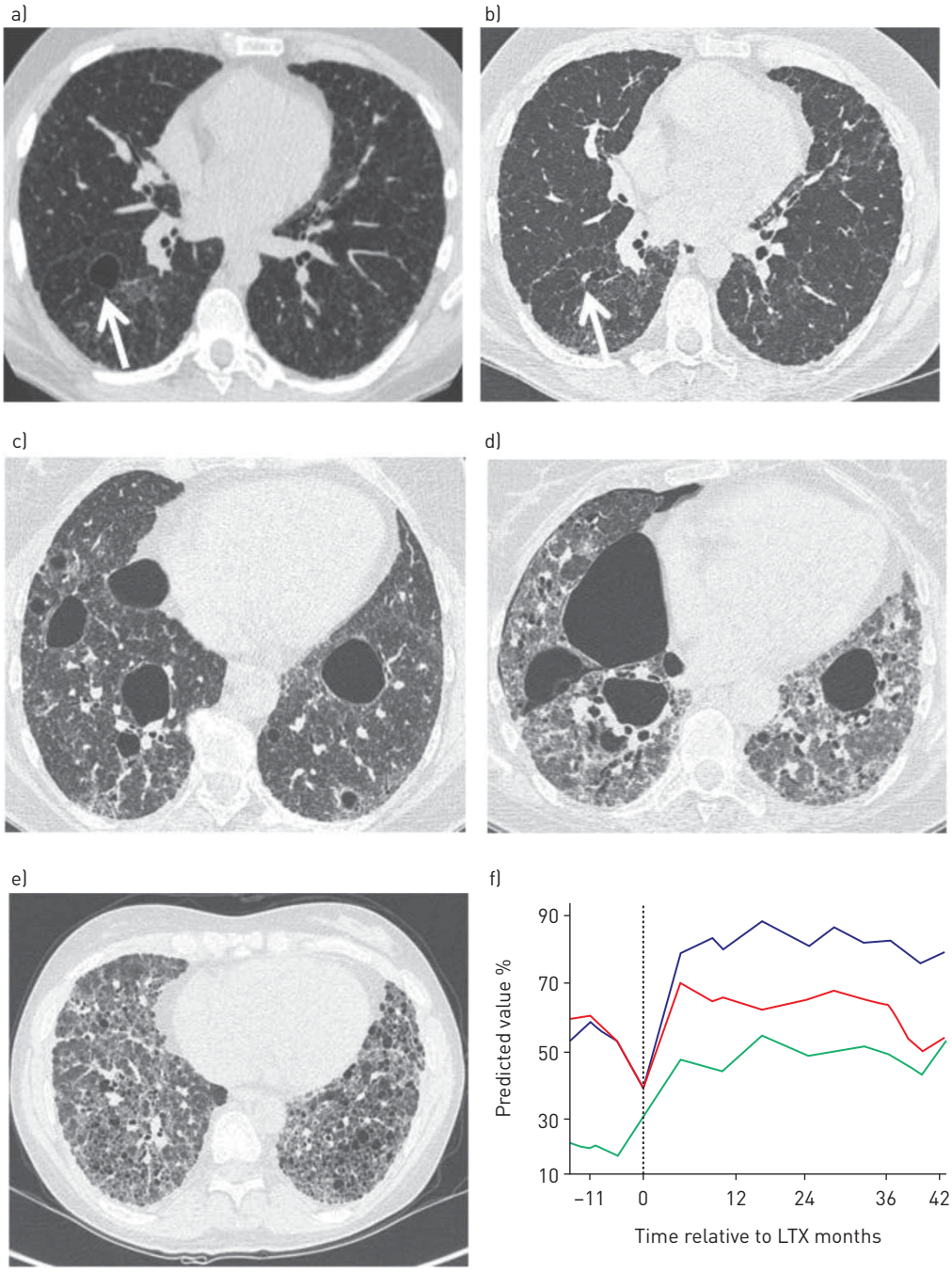

FIGURE 3 Radiology of patients heterozygous for a surfactant protein mutation. High-resolution computed tomography (HRCT) of patient 1 (*SFTPC* M71V) at diagnosis (a) and after 14 years (b) (the arrow points to a clearly walled cyst at diagnosis that collapses during disease evolution. HRCT of patient 2 (*SFTPC* Y113C) at diagnosis (c) and after 2 years (d) (cysts are evident at diagnosis that develop into a bullae-like structure over 2 years. HRCT of patient 3 (*SFTPA2* N171Y) at 1 month prior to bilateral lung transplantation (e) with pre- and post-transplantation pulmonary function tests (f). Blue line: maximal vital capacity; red line: forced expiratory volume in 1 s; green line: diffusing capacity of the lung for carbon monoxide; dotted vertical line: time of lung transplantation (LTX).

Lung cancer

In general, patients with pulmonary fibrosis have an increased risk for development of lung cancer [127]. In families carrying an *SFTPC* mutation, incidental cases of lung cancer have been reported [40]; however, in families carrying either *SFTPA1* or *SFTPA2* mutations, the number of cases with lung cancer exceeds the number of expected cases [90, 95, 96]. Summarising data from *SFTPA* mutation carriers shows that 37% were diagnosed with lung cancer, of which two-thirds had a combination of both lung cancer (most

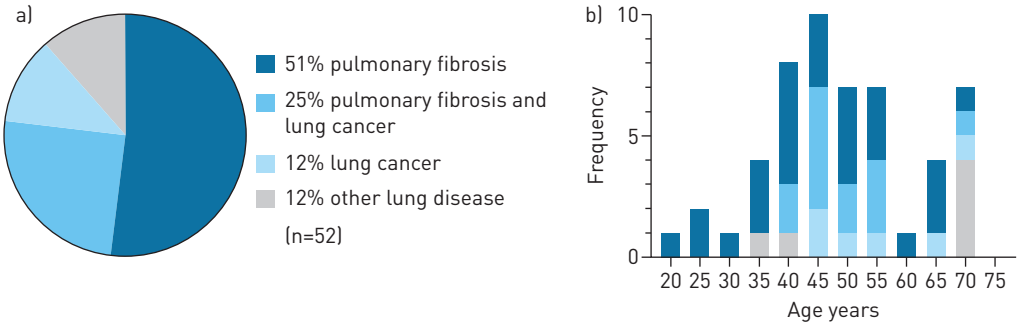

FIGURE 4 Pulmonary phenotype in patients with *SFTPA1* or *SFTPA2* mutations at diagnosis. The cohort consists of all cases reported in the literature combined with cases from the St. Antonius cohort. a) Frequency of pulmonary phenotype in the total cohort. b) Number of patients with pulmonary fibrosis, lung cancer or other lung disease grouped by age (where age is the patient's age at disease diagnosis or the age in the original report).

commonly adenocarcinomas) and pulmonary fibrosis (figure 4a). Importantly, while the age of *SFTPA* patients ranges between 19 and 71 years, lung cancer was only present in patients aged >40 years. Furthermore, even though lung cancer is an aging disease, numbers are not highest in elderly patients (figure 4b). More data is needed to see if these patterns will last and to investigate their cause.

Although the mechanistic link with lung cancer is not understood, several possibilities are worth mentioning. Firstly, recent investigations into the pathogenesis of *SFTPA1* mutations have shown that necroptosis is increased but not apoptosis [99]. For necroptosis, both tumour-promoting and tumour-suppressing effects are reported [128]. In the study of a family with an *SFTPA1* mutation and evidence of necropsis, one uncle with lung cancer was reported; however, his mutation status was unknown and tumourigenesis was not studied [99, 100]. Secondly, the expression of SP-A protein is not limited to AT2 epithelial cells but also occurs in club cells. A recent study in mice has shown that adenocarcinomas may originate from club cells after exposure to smoke [129]. Aberrant processes in club cells may therefore promote tumourigenesis. Thirdly, there is the role of the SP-A protein itself in preventing tumourigenesis. SP-A may suppress tumour development *via* recruitment and activation of natural killer cells and control of tumour-associated macrophage polarisation [130], or *via* epidermal growth factor receptor (EGFR) binding and down-regulation of epidermal growth factor (EGF) signalling [131]. Quantitative or qualitative changes due to *SFTPA* mutations may thus inhibit the protein's tumour suppressing abilities. Further studies are needed to elucidate the direct relationship between *SFTPA* mutations and lung cancer.

NKX2-1 plays a double-edged role in cancer as a lineage-survival oncogene in lung adenocarcinomas and an inhibitor of invasion, metastasis and progression, thereby conferring better prognosis. Somatic *NKX2-1* loss of function mutations have been identified in adenocarcinomas and cause loss of tumour suppressing abilities [132, 133]. A similar process is likely responsible for the increased risk for pulmonary carcinoma in young adults with germline *NKX2-1* mutations [134].

Genetic testing

The contribution of surfactant-related genetic defects in patients suspected of monogenic disease varies between 5% and 25% [16, 35–37, 114–119, 135]. However, the contribution is low in sporadic patients with pulmonary fibrosis, especially when familial illness has been properly questioned [56, 95, 136]. In ILD guidelines, recommendations regarding genetic testing are absent even though testing is highly informative in patients with early-onset and familial disease [112, 137]. As surfactant mutation carriers are difficult to diagnose from radiological imaging alone, genetic testing could aid diagnosis while removing the need for a biopsy. A recent study in children with ILD (age >2 years) has shown that genetic tests contribute to 15% of the diagnoses, slightly better than lung biopsies which contribute to 13.5% [116]. Furthermore, genetic testing aids clinical management with regard to disease prognostication, drug choice and timing, and type of lung transplantation. In addition, the patient's choice for genetic testing is guided by counsellors, which will aid patients in making informed decisions about family planning and other life-changing events.

Although genotype–phenotype patterns exist, clinical characteristics do not always allow for a clear distinction between surfactant-related disease and telomere-related disease. Patients eligible for genetic analysis have pulmonary fibrosis of unknown cause and 1) have a first-degree family member with pulmonary fibrosis; 2) have a first-degree family member with short-telomere syndrome; 3) have features

of short-telomere syndrome; or 4) are below 55 years of age. Together with the clinical genetics department, we built a two-step gene panel for exome sequencing analysis of eligible adult patients with pulmonary fibrosis. The first panel includes all genes published to be involved in adult pulmonary fibrosis and all genes published to be involved in short-telomere syndrome. If negative, the second exploratory panel includes all genes published as involved in paediatric ILD and all genes involved in telomere length maintenance. Results from the exploratory panel are more difficult to interpret but may direct additional clinical testing of subjects (*e.g.* towards telomere length measurement or a bronchoalveolar lavage (BAL) procedure).

Due to the rarity of disease and the predominance of unique family specific mutations, the clinical significance of many genetic findings remains uncertain. Many mutations are categorised as variant of uncertain significance (VUS), meaning that the significance of the mutation to the function or health of the patient is not known. Gathering genotype–phenotype information in a worldwide database is of the utmost importance in better understanding of the impact of mutations on health. The Leiden Open Variation Database (LOVD; https://lovd.nl) is a large community-owned public variant database collecting case-associated genomic variants and phenotypes [138]. LOVD covers all important aspects of a case (*i.e.* data on the individual, the phenotype, longitudinal clinical changes, (combinations of) identified variant (s) and their classification). We have started gathering and curating published data and call on other researchers to supplement it for surfactant-related genes (*e.g.* www.databases.lovd.nl/shared/individuals/SFTPA2).

Therapy

To date no proven effective drug therapies for surfactant-related genetic disease exist. Immunomodulation therapies yield variable results, have considerable side-effects in children [2, 139] and require careful consideration in adults because of the harm observed in patients with IPF [140, 141]. Since disease in adults resembles IPF, the drugs of first choice are the anti-fibrotics pirfenidone and nintedanib. However, efficacy is unknown and a small trial investigating pirfenidone in HPS was stopped due to futility. Furthermore, side-effects leading to dose reduction of anti-fibrotics and treatment discontinuation are common in IPF [142] and challenge optimal timing for start of therapy in familial patients with early disease.

We recently performed a review of drug effects in patients with a surfactant-related mutation, as well as cell or mouse models and concluded that the outcome of drug treatments was highly variable and most likely mutation specific [143]. Studies evaluating the outcome of drugs are hampered by the large number of different mutations with different pathogenic effects. Drug development in cystic fibrosis has profited tremendously from the *CFTR* mutation classification scheme. A first attempt to classify *ABCA3* mutations has divided them into two types: Type I, which cause abnormal intracellular protein localisation, protein misfolding, ER stress and induction of apoptosis; and Type II, which associate with the catalytic domains of the transporter and result in normally localised proteins with a functional deficit in ATP hydrolysis and impaired lipid transfer [1, 144]. However, insufficient understanding of ABCA3 dysfunction and insufficient homology with cystic fibrosis transmembrane conductance regulator (CFTR) hamper development of a clinically useful scheme. Analogous to successful CFTR therapy, the potentiators ivacaftor and genistein may rescue protein functionality. Recently, these potentiators were shown to rescue the ABCA3 phospholipid transport function of three different mutations stably expressed in A549 cells [145].

Gene-based therapies, such as gene replacement or editing, hold promise for the future. Indeed, studies performed in mice and in organoid-like cell systems provide proof of principle for successful gene correction and restored functionality in SP-B deficiency [146–148].

For now, lung transplantation is the most successful option in infants (5-year survival: 55%) and in older children (5-year survival: >75%) [149], as well as in adults with end-stage disease (figures 3e and 3f). In the case of lung transplantation, it is of the utmost importance to recognise patients with *SFTPA* mutations, particularly in countries with a shortage of donor lungs where unilateral transplantation is common. The high probability of developing lung cancer in the native lung justifies bilateral transplantation in cases with *SFTPA* mutation (we have previously reported metastasised lung cancer in a patient with an *SFTPA2* mutation having undergone unilateral lung transplantation [95]). The successful outcome of bilateral lung transplantation in an end-stage patient with an *SFTPA2* mutation is presented in figure 3f.

Conclusion

Mutations in surfactant-related genes cause severe parenchymal lung disease in a significant group of patients of all ages. Genes associate primarily with age but not with sex and genetic testing may aid diagnosis and disease management. Monogenic disease provides the opportunity for detection of early disease and holds the promise of early treatment and prolonged survival. Clinical counselling, offering the option of genetic and pulmonary screening, is therefore recommended for patients and first-degree family

members. Most importantly, there is a need for better understanding of the impact of family specific mutations on health. The rarity of the disease strongly warrants worldwide gathering of genotype and phenotype data to improve clinical management.

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