Identification of Early Interstitial Lung Disease in an Individual With Genetic Variations in *ABCA3* and *SFTPC*

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A man with usual interstitial pneumonia (age of onset 58 years) was previously found to have an Ile73Thr (I73T) surfactant protein C (*SFTPC*) mutation. Genomic DNA from the individual and two daughters (aged 39 and 43 years) was sequenced for the I73T mutation and variations in ATP-binding cassette A3 (*ABCA3*). All three had the I73T *SFTPC* mutation. The father and one daughter (aged 39 years) also had a transversion encoding an Asp123Asn (D123N) substitution in *ABCA3*. The daughters were evaluated by pulmonary function testing and high-resolution CT (HRCT). Neither daughter had evidence of disease, except for focal subpleural septal thickening on HRCT scan in one daughter (aged 39 years). This daughter underwent bronchoscopy with transbronchial biopsies revealing interstitial fibrotic remodeling. These findings demonstrate that subclinical fibrotic changes may be present in family members of patients with *SFTPC* mutation-associated interstitial lung disease and suggest that *ABCA3* variants could affect disease pathogenesis.

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Abbreviations: *ABCA3* = adenosine triphosphate binding cassette A3 gene; AEC = alveolar epithelial cell; BIP = immunoglobulin-binding protein; ER = endoplasmic reticulum; HRCT = high-resolution CT; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis; PFT = pulmonary function test; RML = right middle lobe; *SFTPC* = surfactant protein C gene; UIP = usual interstitial pneumonia; UPR = unfolded protein response; XBP-1 = x-box binding protein 1

Recent discovery of genetic mutations associated with pediatric and adult interstitial pneumonias and functional correlation of these mutations have provided clues to potential pathogenic mechanisms in idiopathic pulmonary fibrosis (IPF). Mutations within the gene encoding for surfactant protein C (*SFTPC*) have been associated with pediatric and adult-onset interstitial lung disease (ILD).^{1,2} In 2002, our group described a mutation in the carboxy-terminal region of *SFTPC* in a large familial interstitial

pneumonia family, including 11 adults with usual interstitial pneumonia (UIP).² This heterozygous mutation resulted in substitution of glutamine for leucine at amino acid 188 (L188Q) of the precursor protein for surfactant protein C. *SFTPC* mutations have been associated with protein misfolding and altered post-translational processing, causing protein accumulation in the endoplasmic reticulum (ER), leading to ER stress and activation of the unfolded protein response (UPR).^{3,4} Interestingly, we and others have shown that ER stress and UPR activation are present in sporadic IPF, even in the absence of an *SFTPC* mutation.^{5,6}

The possibility of disease modifiers has been suggested because the age of onset and severity of ILD is variable, ranging from onset of interstitial pneumonia in infancy to development of pulmonary fibrosis later in life. Mutations in ATP-binding cassette A3 (*ABCA3*) have been implicated as potential disease modifiers in pediatric ILD.^{7,8} *ABCA3* encodes a 1704-amino acid protein that is highly expressed in the lung, namely in lamellar bodies within type 2 alveolar epithelial cells (AECs). *ABCA3* is responsible for lipid transport into lamellar bodies, which are critical storage organelles for pulmonary surfactant. Deficiency of *ABCA3* is associated with fatal neonatal respiratory distress syndrome⁹ and heterozygous mutations have been demonstrated to worsen *SFTPC*-associated ILD in children.⁷

CASE REPORT

We previously reported a study in which we screened for *SFTPC* mutations in DNA from sporadic cases of UIP (89 patients) and nonspecific interstitial pneumonia (46 patients). In this study, only one individual out of 135 had a mutation in *SFTPC*, a threonine-to-isoleucine substitution in codon 73 (I73T).¹⁰ The heterozygous I73T mutation may be the most common *SFTPC* mutation as it has been reported in several children with ILD.¹¹ This 61-year-old man had been diagnosed with IPF based on clinical presentation that included dyspnea, restriction on pulmonary function tests (PFTs), and a surgical biopsy that revealed UIP. He had been adopted as a child and did not know any biologic relatives except for his three daughters and their children. Given identification of a mutation associated with ILD, his daughters were offered a research evaluation, approved by the Vanderbilt University Institutional Review Board, to screen for the mutation and occult lung disease. Two daughters (aged 39 and 43 years) participated, and a third daughter (aged 41 years) declined. A family pedigree is shown in Figure 1.

Because of a report implicating heterozygous *ABCA3* mutations as potential modifiers for *SFTPC* mutation-associated pediatric ILD,⁷ we evaluated family members for both the I73T *SFTPC* mutation and variations in

FIGURE 1. Pedigree demonstrating carrier status of the Ile73Thr *SFTPC* mutation and Asp123Asn *ABCA3* variation. The proband (I-1) carried both variations and died of idiopathic pulmonary fibrosis (IPF) at age 62 years; he had previously had a surgical lung biopsy that revealed usual interstitial pneumonia (UIP). He had three daughters, two of whom were screened for both mutations and for evidence of early interstitial lung disease (ILD). One daughter carried only the *SFTPC* mutation (II-1), whereas the other daughter carried both mutations (II-2). Both daughters who participated in this study were completely asymptomatic and had normal physical examinations and pulmonary function tests (PFTs). The daughter who carried both mutations had early ILD detected by high-resolution CT (HRCT) scan and on transbronchial biopsies. *ABCA3* = adenosine triphosphate binding cassette A3 gene; *SFTPC* = surfactant protein C gene.

ABCA3. Genomic DNA from the proband and both daughters were used as templates for polymerase chain reaction amplification of *SFTPC* and *ABCA3* exons and exon-intron

boundaries. DNA sequencing of the polymerase chain reaction products confirmed the presence of a heterozygous thymine-to-cytosine transition in exon 3 of *SFTPC* in the father's DNA that results in an isoleucine-to-threonine substitution of amino acid residue 73 (I73T). This mutation was also present in the DNA of both daughters. Additionally,

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FIGURE 2. HRCT image of the chest. HRCT scanning of the chest was performed using prone positioning. One asymptomatic daughter (aged 39 years) had evidence of interstitial thickening in the lower lobes in a few areas suggestive of early interstitial pneumonia (arrows). Overall, these findings were mild and most of the lung fields appeared normal. Standard positioning image shown for orientation. These mild abnormalities did not resolve with prone positioning. See Figure 1 legend for expansion of abbreviation.

FIGURE 3. Hematoxylin and eosin-stained lung tissue obtained at transbronchial biopsy from an asymptomatic individual who was heterozygous for both the Ile73Thr *SFTPC* mutation and Asp123Asn *ABCA3* variation. Multiple foci of interstitial fibrosis are present; associated inflammation is variable. Anthracotic pigment (arrows) marks respiratory bronchioles. Original magnification $\times 25$ (A). Alveolar tissue involved by interstitial fibrosis (fine arrows); focus of dense scar with associated lymphoplasmacytic inflammation (heavy arrows). Original magnification $\times 62.5$ (B). Linear alveolar septal fibrosis with lymphocytic infiltrate (fine arrows); single paucicellular collagen deposit (open arrow). Original magnification $\times 62.5$ (C). A single fibroblastic focus is present. Original magnification $\times 62.5$ (D). See Figure 1 legend for expansion of abbreviations.

DNA from the father and one daughter (aged 39 years) were also heterozygous for an exon 3 guanine-to-adenosine transition of *ABCA3* predicted to convert residue 123 from aspartate to asparagine. This amino acid (Asp123) is highly conserved down through *Drosophila*. Thus, this D123N *ABCA3* variation may represent a mutation. One daughter (aged 43 years) did not have the D123N *ABCA3* variant. These findings are summarized in Figure 1.

In both daughters, screening for early ILD was performed. For both, no history of respiratory symptoms was reported and physical examination was normal. Both had an active lifestyle including regular exercise, normal PFTs without restriction or decrement in diffusing capacity for carbon monoxide, and exercise oximetry that revealed no oxygen desaturation. In the older daughter (aged 43 years), high-resolution CT (HRCT) scan of the chest was normal. In the younger daughter (aged 39 years), who carried both the *SFTPC* mutation and the *ABCA3* variation, HRCT scan demonstrated early interstitial thickening in a few peripheral areas in the lower lobes, findings possibly suggestive of early ILD (Fig 2). Given these HRCT scan findings, a bronchoscopy, with BAL and transbronchial biopsies, was performed. BAL was performed in the right middle lobe (RML), with predominance of monocytes/macrophages on lavage fluid cell count and differential. Biopsies were performed in the RML and right lower lobe.

Evaluation of hematoxylin and eosin-stained sections from the biopsies revealed multiple foci where alveolar tissue was involved by interstitial fibrosis, including areas with dense scar and associated lymphoplasmacytic inflammation (Fig 3). A single fibroblastic focus was noted on one section. These histologic findings were observed in biopsies from the right lower lobe where mild HRCT scan abnormalities were detected as well as from the RML where the HRCT scan was completely normal. Electron microscopy images from the biopsies revealed bands of collagen deposition in the lung interstitium and type 2 AECs that had poorly developed lamellar bodies, fewer mature lamellar bodies, and the accumulation of numerous small cytoplasmic vesicles (Fig 4).

Given evidence of ER stress and UPR activation in the lungs of patients with IPF,⁵ we assessed the affected daughter's lung tissue for markers associated with ER stress and the UPR, including immunoglobulin-binding protein (BiP) and x-box binding protein-1 (XBP-1). Prominent immunoperoxidase staining for both BiP and XBP-1 was detected in AECs in areas of fibrotic remodeling as well as alveoli with preserved alveolar structure, consistent with widespread ER stress and UPR activation (Fig 5) in the lungs of this asymptomatic individual. ER stress markers were expressed in the hyperplastic AECs lining areas of fibrosis in the father's lung tissue in the same pattern we had observed in sporadic IPF.⁵ In that same

FIGURE 4. Electron microscopic images of lung tissue obtained at transbronchial biopsy from an asymptomatic individual heterozygous for both the Ile73Thr *SFTPC* mutation and Asp123Asn *ABCA3* variation. Bands of collagen were prominent in the lung interstitium (asterisks). Type 2 alveolar epithelial cells demonstrated the presence of poorly developed lamellar bodies (large arrows), which had a more solid core rather than the typical lamellar whorls seen with mature lamellar bodies and the accumulation of numerous small vesicles in the cytoplasm (small arrows). See Figure 1 legend for expansion of abbreviations.

publication, we showed that these ER stress markers are minimally detectable in normal lungs.

DISCUSSION

In this report, we describe the presence of two heterozygous gene variations, I73T *SFTPC* and D123N *ABCA3*, within a single family. In addition, we found evidence of early ILD in an asymptomatic family member. HRCT scan

of this daughter, who like her father carried both variations, demonstrated subtle interstitial changes within the lower lobes of the lungs, suggesting the presence of subclinical ILD. Transbronchial biopsies also demonstrated findings consistent with early ILD. Interestingly, despite the absence of interstitial changes by HRCT scan within the RML, transbronchial biopsies confirmed presence of ILD within this lobe, suggesting that pathologically recognizable fibrosis can occur before it is evident radiographically.

From this family, we can infer an association between mutations in *SFTPC* and *ABCA3* and adult-onset ILD. Given that surfactant protein C and *ABCA3* are involved in the secretory pathway in the type 2 AEC, we suspect that these two genetic variations may lead to type 2 AEC dysfunction. The I73T *SFTPC* mutation has been linked to FIP and is considered the primary contributor to disease pathogenesis in this family. Previously, Bullard and Nogee⁷ analyzed children with I73T *SFTPC* mutation-associated ILD and found heterozygous *ABCA3* mutations in three of four individuals—two with E292V (glutamic acid-to-valine) and one with L212M (leucine-to-methionine). Although it is not known from our studies how the D123N *ABCA3* variation affects *ABCA3* function, given prior pediatric reports and the fact that Asp123 is conserved across species, we speculate that this variation could affect surfactant processing and contribute to development of ILD in this family. Although this study is limited by the small number of individuals in this family, it is interesting to note that the daughter (aged 43 years) who only had the I73T *SFTPC* mutation did not have HRCT scan evidence of ILD, whereas the daughter (aged 39 years) with both gene variants did have evidence of early ILD. We hope that future studies will explore the molecular relationship between *SFTPC* and *ABCA3* variations.

Previous evaluations of carboxy-terminal *SFTPC* mutations have demonstrated that misfolded surfactant protein C causes ER stress and UPR activation.^{3,4} However, *in vitro* studies suggest that expression of I73T *SFTPC* is not sufficient to cause ER stress.⁴ Our previous work,⁵ which has been confirmed by Korfei et al,⁶ revealed that ER stress and UPR activation are commonly found in familial and

FIGURE 5. Immunohistochemistry (IHC) for components of the unfolded protein response (UPR) from lung tissue obtained at transbronchial biopsy from an asymptomatic individual heterozygous for both the Ile73Thr *SFTPC* mutation and Asp123Asn *ABCA3* variation (same individual in Fig 2-4). IHC for immunoglobulin-binding protein (BiP) revealed positive staining in alveolar epithelial cells in areas of affected lung and in normal appearing alveolar tissue adjacent to affected lung (A). IHC for x-box binding protein 1 revealed positive staining in epithelial cells lining areas of affected lung (B). Arrows point to immunostain-positive cells. Original magnification $\times 100$. See Figure 1 legend for expansion of abbreviations.

sporadic cases of IPF, but these studies have been in well-established disease. Here, we found evidence of ER stress in an individual with subclinical disease, consistent with the idea that activation of these pathways is a cause rather than the result of fibrotic remodeling in IPF. It is important to note that despite the evidence of fibrotic remodeling on HRCT scan and transbronchial biopsies, we do not know whether disease will advance in this asymptomatic individual. In summary, this report demonstrates that subclinical fibrotic changes may be present in family members of patients with familial interstitial lung disease and provides further evidence for the role of AEC dysfunction in IPF pathogenesis.

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REFERENCES

1. Nogee LM, Dunbar AE III, Wert SE, Askin F, Hamvas A, Whitsett JA. A mutation in the surfactant protein C gene associated with familial interstitial lung disease. *N Engl J Med*. 2001;344(8):573-579.
2. Thomas AQ, Lane K, Phillips J III, et al. Heterozygosity for a surfactant protein C gene mutation associated with usual interstitial pneumonitis and cellular nonspecific interstitial pneumonitis in one kindred. *Am J Respir Crit Care Med*. 2002;165(9):1322-1328.
3. Mulugeta S, Maguire JA, Newitt JL, Russo SJ, Kotorashvili A, Beers MF. Misfolded BRICHOS SP-C mutant proteins induce apoptosis via caspase-4- and cytochrome c-related mechanisms. *Am J Physiol Lung Cell Mol Physiol*. 2007;293(3):L720-L729.
4. Mulugeta S, Nguyen V, Russo SJ, Muniswamy M, Beers MF. A surfactant protein C precursor protein BRICHOS domain mutation causes endoplasmic reticulum stress, proteasome dysfunction, and caspase 3 activation. *Am J Respir Cell Mol Biol*. 2005;32(6):521-530.
5. Lawson WE, Crossno PF, Polosukhin VV, et al. Endoplasmic reticulum stress in alveolar epithelial cells is prominent in IPF: association with altered surfactant protein processing and herpesvirus infection. *Am J Physiol Lung Cell Mol Physiol*. 2008;294(6):L1119-L1126.
6. Korfei M, Ruppert C, Mahavadi P, et al. Epithelial endoplasmic reticulum stress and apoptosis in sporadic idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2008;178(8):838-846.
7. Bullard JE, Nogee LM. Heterozygosity for ABCA3 mutations modifies the severity of lung disease associated with a surfactant protein C gene (SFTPC) mutation. *Pediatr Res*. 2007;62(2):176-179.
8. Bullard JE, Wert SE, Whitsett JA, Dean M, Nogee LM. ABCA3 mutations associated with pediatric interstitial lung disease. *Am J Respir Crit Care Med*. 2005;172(8):1026-1031.
9. Shulenin S, Nogee LM, Annilo T, Wert SE, Whitsett JA, Dean M. ABCA3 gene mutations in newborns with fatal surfactant deficiency. *N Engl J Med*. 2004;350(13):1296-1303.
10. Lawson WE, Grant SW, Ambrosini V, et al. Genetic mutations in surfactant protein C are a rare cause of sporadic cases of IPF. *Thorax*. 2004;59(11):977-980.
11. Nogee LM, Dunbar AE III, Wert S, Askin F, Hamvas A, Whitsett JA. Mutations in the surfactant protein C gene associated with interstitial lung disease. *Chest*. 2002;121(3 suppl):20S-21S.

Right Ventricular Involvement and Pulmonary Hypertension in an Elderly Woman With Tako-Tsubo Cardiomyopathy

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We describe a case of tako-tsubo cardiomyopathy in an elderly woman with a permanent pacemaker admitted to the ED with chest pain and dyspnea. Coronary angiography revealed normal coronary arteries. Typical left ventricular apical ballooning was demonstrated on contrast ventriculography. Left and right ventricular ballooning with pulmonary artery systolic hypertension was detected by transthoracic echocardiography. Velocity vector imaging and strain analysis showed a typical pattern of regional myocardial apical right ventricular contraction characterized by paradoxical positive longitudinal systolic strain. Biventricular involvement was associated with hemodynamic instability, signs of pulmonary vascular congestion, and bilateral basal pleural effusion. The patient's clinical condition gradually improved, and she was discharged after prolonged hospitalization. PredischARGE echocardiography showed substantial recovery of left and right systolic function along with normalization of pulmonary artery systolic pressure. Right ventricular function should be carefully evaluated in patients with tako-tsubo cardiomyopathy, especially in the acute phase.

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Abbreviations: E = peak of early diastolic mitral flow; E' = peak of early diastolic myocardial velocity; EF = ejection fraction; LV = left ventricular; PASP = pulmonary arterial systolic pressure; RV = right ventricular; TTC = tako-tsubo cardiomyopathy; TTE = transthoracic echocardiogram; VVI = velocity vector imaging

An 81-year-old woman was admitted to the ED with chest pain and dyspnea that began 2 h earlier after the unexpected death of a family member. Past medical history consisted of systemic arterial hypertension, permanent atrial fibrillation, and brady-tachy syndrome treated with ventricular inhibited rate responsive pacemaker implantation in 2006. On physical examination, BP was 95/65 mm Hg,

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