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Genetic Disorders of Surfactant Dysfunction

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Abstract

Mutations in the genes encoding the surfactant proteins B and C (SP-B and SP-C) and the phospholipid transporter, ABCA3, are associated with respiratory distress and interstitial lung disease in the pediatric population. Expression of these proteins is regulated developmentally, increasing with gestational age, and is critical for pulmonary surfactant function at birth. Pulmonary surfactant is a unique mixture of lipids and proteins that reduces surface tension at the air-liquid interface, preventing collapse of the lung at the end of expiration. SP-B and ABCA3 are required for the normal organization and packaging of surfactant phospholipids into specialized secretory organelles, known as *lamellar bodies*, while both SP-B and SP-C are important for adsorption of secreted surfactant phospholipids to the alveolar surface. In general, mutations in the SP-B gene *SFTPB* are associated with fatal respiratory distress in the neonatal period, and mutations in the SP-C gene *SFTPC* are more commonly associated with interstitial lung disease in older infants, children, and adults. Mutations in the *ABCA3* gene are associated with both phenotypes. Despite this general classification, there is considerable overlap in the clinical and histologic characteristics of these genetic disorders. In this review, similarities and differences in the presentation of these disorders with an emphasis on their histochemical and ultrastructural features will be described, along with a brief discussion of surfactant metabolism. Mechanisms involved in the pathogenesis of lung disease caused by mutations in these genes will also be discussed.

Keywords

ABCA3; alveolar proteinosis; interstitial lung disease; respiratory distress syndrome; surfactant protein B; surfactant protein C

INTRODUCTION

Genetic disorders disrupting normal surfactant metabolism (also known as *pulmonary surfactant metabolism dysfunctions* or *surfactant dysfunction disorders*) have been recognized recently as underlying causes of respiratory disease in the neonatal and pediatric populations. Although rare, these disorders cause significant mortality and morbidity, including acute respiratory distress and failure in full-term neonates, and interstitial lung disease (ILD) in older

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NOTE

A mutation in the SP-A gene, *SFTPA*, associated with familial pulmonary fibrosis, was published after submission of this manuscript [201].

infants, children, and adults [1-13]. The genes involved in these disorders are critical for surfactant production and function in the lung, and include the surfactant protein B gene (*SFTPB*; Online Mendelian Inheritance in Man [OMIM] number 178640), the surfactant protein C gene (*SFTPC*; OMIM number 178620), and the ABCA3 gene (*ABCA3*; OMIM number 601615). The surfactant proteins B and C (SP-B and SP-C) are small hydrophobic proteins that are synthesized in pulmonary alveolar type II cells, packaged into lamellar bodies, and secreted into the alveolar lumen, where they function to stabilize and enhance spreading of surfactant phospholipids along the alveolar surface [13-22]. Lamellar bodies are specialized, intracellular, lysosomally derived storage organelles for surfactant lipids and proteins, which are composed of multiple, tightly packed, concentrically arranged phospholipid membranes. Once secreted, this mixture of proteins and lipids spreads rapidly along the alveolar surface, reducing surface tension at the air-liquid interface and preventing collapse of the lung at the end of expiration. ABCA3 is a large, integral membrane protein that is highly expressed in the lung and has been localized primarily to the outer, or limiting, membrane of the lamellar body [23,24]. ABCA3 is a member of a large family of adenosine triphosphate (ATP)-binding cassette proteins that actively transport a variety of substances across biological membranes, including lipids [25-29]. ABCA3 most likely functions to import surfactant phospholipids, such as phosphatidylcholine (PC) and phosphatidylglycerol (PG), from the cytosol into the lamellar body [24,30-33] and is thought, therefore, to be important for lamellar body biogenesis.

The lung disorders caused by mutations in these genes exhibit considerable overlap in their clinical and histologic presentation, primarily due to impaired surfactant function and gas exchange in the lung. Although genetic analysis for specific mutations in these genes is the definitive diagnostic test for these disorders, differences in genetic transmission, family and clinical histories, expression of the surfactant proteins, and lamellar body ultrastructure may be helpful in distinguishing these genetic disorders.

SURFACTANT METABOLISM

Pulmonary surfactant is a complex mixture of lipid (90% by weight) and protein (10% by weight) that lines the alveolar surface of the lungs and prevents atelectasis at the end of expiration [15,18,21,22]. Surfactant lipids are composed primarily of PC (or lecithin) and disaturated or dipalmitoyl PC with lesser amounts of PG, phosphatidylinositol, phosphatidylethanolamine, phosphatidylserine, sphingomyelin, and cholesterol. PC/dipalmitoyl PC is the most abundant surfactant phospholipid in the lung and is responsible for the surface tension-reducing properties of pulmonary surfactant. SP-B is a 79-amino-acid protein that is encoded by a single gene on chromosome 2 (2p12-p11.2). SP-B binds to phospholipid bilayers and has both membranolytic and fusogenic properties that may contribute to the organization and packaging of surfactant phospholipid membranes in the lamellar body [19-22,34-36]. SP-C is a small, 35-amino-acid, integral membrane protein that is encoded by a single gene located on chromosome 8 (8p21). Although SP-C is not involved in the packaging of phospholipids into the lamellar body, it is embedded in the phospholipid bilayer and plays an important role in the formation and maintenance of the surfactant monolayer at the external alveolar surface [17,19-21,37,38]. SP-C also enhances the uptake of surfactant phospholipids into isolated alveolar type II cells and, as such, may be involved in surfactant catabolism [39].

SP-B and SP-C are synthesized in alveolar type II cells as large precursor proteins (proSP-B and proSP-C) that are cleaved by proteolytic enzymes at both their amino and carboxyl termini to yield smaller, extremely hydrophobic peptides [40-42] (Fig. 1A,B). SP-B and SP-C are synthesized in the endoplasmic reticulum (ER) and transported through the Golgi apparatus to multivesicular bodies, during which proteolytic processing of their precursor proteins is

initiated [43-53]. The multivesicular body then fuses with the lamellar body, where final processing and packaging of the mature proteins into surfactant phospholipid membranes occurs (Fig. 1C). Secretion into the alveolar lumen takes place by exocytosis, after fusion of the limiting membrane of the lamellar body with the plasma membrane at the cell surface [54-56]. Once secreted, the lamellar body unwinds to produce tubular myelin, a lattice-like structure that is converted into a lipid-rich film that spreads along the alveolar surface at the air-liquid interface [21,22]. Both SP-B and SP-C facilitate the adsorption of the surfactant phospholipid film to the air-liquid interface, where they contribute to the maintenance of the surfactant surface tension-reducing properties, while SP-B also participates in the formation of tubular myelin [21,22,35]. Secreted surfactant phospholipids and proteins are then taken up by endocytosis and recycled by the alveolar type II cell or catabolized by alveolar macrophages [57].

ABCA3 is a 1704-amino-acid protein that contains 12 membrane-spanning regions with 2 ATP-binding domains located in the cytoplasm [58,59]. ABCA3 is most likely synthesized and glycosylated in the ER and then transported through the Golgi apparatus to the multivesicular body and the outer membrane of the lamellar body [30-33,60]. Although ABCA3 is incorporated into the plasma membrane during fusion and exocytosis of the lamellar body, it is internalized and recycled back through the Golgi apparatus and multivesicular body to the lamellar body [61,62].

Surfactant phospholipid and protein expression is regulated developmentally, increasing with advancing gestational age, and is critical for normal respiratory function at birth. The expression of SP-B and SP-C [63,64], as well as of ABCA3 [24,65] and other proteins involved in surfactant lipid production and lamellar body biogenesis, increases in late gestation in association with other aspects of lung maturation. During the last third of gestation, immature, glycogen-rich, alveolar type II cells begin to mature. Glycogen disappears as surfactant production increases, and lamellar bodies form in the cytoplasm and are secreted into the alveolar lumen [66]. The composition of surfactant phospholipids also changes during late gestation, with increasing amounts of PG and PC (lecithin) and decreasing amounts of sphingomyelin, resulting in an increase in the lecithin-to-sphingomyelin ratio, a well-known indicator of lung maturity [67-69]. Respiratory distress syndrome (RDS) associated with prematurity (gestational age <37 weeks) is caused by a deficit in surfactant production and results in diffuse atelectasis, destruction of the alveolar epithelium, formation of hyaline membranes lining the small airways, inflammation, and death, if left untreated. Severe RDS in infants born at >36 weeks of gestation, at a time when the surfactant system should be functional, suggests other reasons for lung disease, including infectious, genetic, and/or developmental mechanisms that impair normal lung morphogenesis or surfactant production and metabolism.

In addition to SP-B and SP-C, 2 larger, hydrophilic proteins, SP-A and SP-D, are highly expressed in the fetal and postnatal lung, as well as in other tissues [70-74]. SP-A (*SFTPA*; OMIM number 178630; and *SFTPA1*; OMIM number 178642) and SP-D (*SFTPD*; OMIM number 178635) are collectins that play an important role in host-defense of the lung through their ability to opsonize and enhance killing of various microorganisms, including bacterial, viral, and fungal pathogens [75-78]. Although no mutations in these genes have been identified to date, polymorphisms in both *SFTPA* and *SFTPD* have been associated with susceptibility to RDS and bronchopulmonary dysplasia in premature infants [79,80], as well as with susceptibility to respiratory syncytial virus infections in young infants [81-83]. In mice, deletion of SP-A caused increased susceptibility to bacterial and viral infections with little effect on surfactant function or homeostasis, although tubular myelin was lacking [84-87]. On the other hand, SP-D–deficient mice exhibited defects in the uptake and recycling of secreted surfactant phospholipids by alveolar type II cells, resulting in increased phospholipid pool

sizes; the development of large, foamy, lipid-laden macrophages; and progressive emphysema [88-94], as well as increased susceptibility to viral and bacterial pathogens [95].

GENETIC SP-B DISORDERS (SURFACTANT METABOLISM DYSFUNCTION, PULMONARY, 1, SMDP1, OMIM NO. 265120)

Index case

Hereditary SP-B deficiency was first described in 1993 in a full-term infant with diffuse lung disease and radiographs suggesting surfactant deficiency [96]. This infant died at 5 months of age from progressive respiratory failure that developed shortly after birth and did not respond to mechanical ventilation, corticosteroids, surfactant replacement, or extracorporeal membrane oxygenation. Lung biopsy showed changes consistent with congenital alveolar proteinosis. The family history was notable in that a previous child also died from neonatal lung disease. A frameshift mutation, caused by a net 2-base pair insertion (121ins2) in exon 4 of the *SFTPB* gene was subsequently detected on both alleles in this child and in another sibling [97]. This mutation caused a premature stop codon in exon 6, which was associated with an unstable transcript that precluded expression of mature *SFTPB* (encoded in exons 6 and 7), resulting in the complete absence of SP-B messenger RNA and protein in the lung of this patient (Fig. 2A). Incomplete processing of proSP-C was also observed, with formation of a 6- to 9-kd intermediate form of proSP-C containing part of the amino terminus [97,98].

Genetics and clinical presentation

Hereditary SP-B deficiency is inherited as an autosomal recessive disorder with mutations required on both alleles in order to cause disease. Parents and siblings who are heterozygous for mutations in *SFTPB* are usually asymptomatic [99]. More than 40 distinct mutations in the SP-B gene have been identified to date [100-110]. Two thirds of the mutant alleles have been accounted for by the 121ins2 mutation in exon 4, while the remaining one third include nonsense, missense, frameshift, and splice-site mutations, as well as insertions and deletions throughout the gene (Fig. 2B). A large deletion spanning exons 7 and 8 has also been reported [111,112]. In general, these mutations result in the complete absence, or loss of function, of SP-B, causing acute respiratory distress in full-term infants at birth, which is progressive and usually fatal by 3 to 6 months of age [3,107]. Clinical and radiographic findings are consistent with those seen in immature preterm infants with RDS resulting from insufficient surfactant stores [113]. Phospholipid content is also abnormal with decreased phospholipid-to-protein ratios, elevated phosphatidylinositol and decreased PG in tissue, and bronchoalveolar lavage fluid (BALF) when compared with controls [114-116]. Accordingly, surfactant isolated from the lungs of SP-B–deficient infants is less effective in lowering surface tension [115,116]. Although transient or modest improvement may be seen with surfactant replacement and/or corticosteroid therapy, lung transplantation is currently the only effective treatment option [117-120]. Several children with partial defects in SP-B production have been seen with severe chronic lung disease in infancy and have survived beyond the neonatal period, but these cases are rare [101,106,121]. Although the population frequency of genetic SP-B deficiency is currently unknown, clinical estimates suggest an incidence of 1 in 1 million live births in the United States [122]. The allele frequency of the 121ins2 mutation is approximately 1 per 1000 to 3000 individuals [122-124].

Histopathology and ultrastructural features

SP-B deficiency is associated primarily with histopathology diagnoses of congenital alveolar proteinosis and, less frequently, with infantile desquamative interstitial pneumonitis (DIP). Accordingly, the most characteristic, histologic feature of genetic SP-B deficiency is the accumulation of granular, eosinophilic, periodic acid-Schiff–positive, lipoproteinaceous

material in the alveolar spaces, which often contains desquamated alveolar type II cells and foamy alveolar macrophages (Fig. 3A). These findings are similar, but not identical, to those observed in adult patients with acquired pulmonary alveolar proteinosis (PAP), an autoimmune disorder caused by endogenous production of antibodies directed against granulocyte-macrophage colony-stimulating factor (GM-CSF) ([125-127], and in children with PAP caused by mutations in the common beta or alpha subunit of the GM-CSF receptor [128-130] (Fig. 3B). Both disorders disrupt GM-CSF signaling in the macrophage, which impairs macrophage function and interferes with catabolism or degradation of secreted surfactant. In contrast to the large amounts of alveolar proteinosis material found in association with impaired GM-CSF signaling, the amount of alveolar proteinosis observed in SP-B deficiency may be quite variable from one patient to the next, and may even be absent in some patients who exhibit histologic features consistent with infantile DIP (Fig. 3C). Additional histopathologic findings in SP-B deficiency include hyperplastic alveolar epithelia with prominent type II cells and thickening of the alveolar walls, which is characterized by fibroblast proliferation with little to no inflammatory cell infiltrates (Fig. 3D). These features also tend to distinguish SP-B deficiency from GM-CSF abnormalities in which there is generally good preservation of the alveolar architecture (Fig. 3B). Although hyperplastic alveolar epithelium interspersed with thickened interstitial septa are features consistent with impaired alveolar formation, these findings may reflect nonspecific changes in the tissue, which may be caused by injury from prolonged mechanical ventilation and oxygen therapy rather than by abnormal lung development. In support of this interpretation, the lungs of SP-B–deficient mice show normal lung development at birth [131]. Ultrastructural findings in both human and mouse genetic SP-B deficiency demonstrate that lamellar body formation is also perturbed. No normal, mature, well-organized lamellar bodies or secreted tubular myelin are found in either case. Instead, many large, disorganized, or irregular multivesicular structures are detected by electron microscopy in the alveolar type II cells of both species [111,131-135] (Fig. 3E,F).

Immunohistochemistry and pathogenesis

Immunohistochemical features include (1) markedly reduced or absent immunostaining for proSP-B and the mature SP-B peptide and (2) accumulation of SP-A and proSP-C immunopositive material in the alveolar lumen [97,107] (Fig. 4). Although pathogenesis of this disorder is caused by impaired surfactant function from the loss of SP-B, accumulation of partially processed proSP-C in the alveolar lumen may also contribute to lung disease, as this peptide has reduced surface activity [136]. Normally, processing of the 21-kd proSP-C peptide to the smaller, hydrophobic, 4-kd mature SP-C peptide occurs by successive proteolytic cleavage of the carboxyl terminus and then the amino terminus to yield intermediate forms of 18- to 16-kd and 6- to 7-kd peptides (Fig. 1B). In SP-B deficiency, a 6-kd form of proSP-C, containing 12 amino acids of the amino terminus and the active hydrophobic domain, is found in the alveolar lumen by immunoblot and by immunohistochemistry examination [96,97, 107] (Fig. 4D). The presence of increased amounts of this partially processed intermediate peptide suggests that SP-B is required for normal processing and/or trafficking of proSP-C. Alternately, disruption of lamellar body formation may account for the incomplete processing of proSP-C to the mature SP-C peptide, which normally occurs during transport from the multivesicular body to the lamellar body. In some cases, full-length or partially processed proSP-B may also be detected by immunoblot and immunohistochemistry examination [107]. Both proSP-B and mature SP-B are absent in patients with nonsense and frameshift mutations, while detectable proSP-B expression is associated with missense mutations and mutations causing in-frame deletions or insertions [107]. However, in these latter cases, the mutation usually prevents efficient processing of proSP-B to the mature SP-B peptide, resulting in a reduction or absence of SP-B.

To date, there is no explanation for the increased amounts of SP-A that accumulate in the alveolar lumen, although SP-A synthesis and secretion may be influenced differentially by the therapies used to support infants with these conditions [137-141]. Likewise, little is known about alveolar macrophage function in this disorder, so that abnormalities in surfactant composition or function (i.e., surface tension-lowering ability) may impair the catabolic and/or recycling mechanisms that normally operate in the lung to maintain surfactant homeostasis. For example, macrophage phagocytosis was shown to be impaired in the SP-B–deficient mouse [142], which might affect the uptake of surfactant lipids and proteins into the cell for degradation.

GENETIC SP-C DISORDERS (SURFACTANT METABOLISM DYSFUNCTION, PULMONARY, 2, SMDP2, OMIM NO. 610913)

Index cases

Lung disease due to mutation in the *SFTPC* gene was first described in 2001 in a full-term infant and mother with respiratory insufficiency [143]. The infant developed respiratory symptoms at 6 weeks of age, consisting of tachypnea and cyanosis while breathing room air. Radiography of the chest showed hyperinflation with increased interstitial markings. The infant's lung biopsy showed changes consistent with nonspecific interstitial pneumonitis (NSIP), exhibiting well-preserved but thickened alveolar septa, hyperplastic alveolar type II cells, an interstitial infiltrate composed of mature lymphocytes, and scattered myofibroblasts in the alveolar wall. Some noninflated alveoli were filled with desquamated cells, the majority of which were alveolar macrophages. Lung tissue from the patient's mother had areas of diffuse fibrosis and honeycombing, with patchy areas of mild interstitial lymphocytic infiltration, accumulation of alveolar macrophages, and regions of superimposed alveolar damage consistent with idiopathic pulmonary fibrosis. A heterozygous mutation involving a substitution of adenine for guanine at the first base of intron 4 (IVS4+1G>A, also known as c. 460+1G>A) was subsequently identified on one allele of the *SFTPC* gene in both the infant and mother, which is consistent with an autosomal dominant form of inheritance. This mutation abolished the normal donor splice site, which caused skipping of exon 4 (designated as the Δexon 4 mutation) and resulted in deletion of 37 amino acids in the carboxyl terminus of the precursor protein [143] (Fig. 5A). Immunohistochemistry revealed that the full-length, 21-kd proSP-C was present in reduced amounts in the lung tissue of both the infant and the mother, while immunoblotting demonstrated the presence of a smaller, less abundant form of proSP-C (18 kd), as well as the absence of mature SP-C in both tissue and BALF. SP-B, on the other hand, was present at normal levels. The family history was of interest in that the child's mother was examined at 1 year of age and found to have DIP, and the maternal grandfather died of chronic lung disease of unknown etiology.

Genetics and clinical presentation

More than 40 distinct mutations in the *SFTPC* gene have been identified, with the majority of these mapping to the carboxyl terminus of proSP-C [4,143-155]. These mutations consist primarily of missense mutations, although frameshift and splice-site mutations, as well as small insertions and deletions, have been identified [4,11,144] (Fig. 5B). Most of these mutations are found in the carboxyl terminus (exons 3, 4, and 5) of the precursor protein and are thought to cause misfolding of proSP-C and to preclude processing of the precursor protein to the mature peptide [156-159]. A substitution of threonine for isoleucine in codon 73 (I73T) is the most common *SFTPC* mutation [145,149-151] and is found in >25% of patients with genetic SP-C disorders. In contrast to genetic SP-B deficiency, lung disease caused by *SFTPC* mutations is inherited as an autosomal dominant trait with variable penetrance and severity (45%), or as sporadic disease caused by a de novo mutation on one allele (55%) [4,144,153].

Although *SFTPC* mutations are associated primarily with chronic ILD in infants and older children, onset of respiratory symptoms in patients with *SFTPC* mutations can be highly variable [146,147,151]. Approximately 10% to 15% of patients with a *SFTPC* mutation develop respiratory symptoms within the 1st month of life, while another 40% develop symptoms as infants between 1 and 6 months of life, with the average age of onset being 2 to 3 months [4,8,13,153,160]. Presentation in full-term infants in the newborn period is associated with signs and symptoms typical of RDS and may be fatal in the neonatal period [153,155]. Presentation in older infants is associated with symptoms of diffuse lung disease, including tachypnea, retractions, hypoxemia, digital clubbing, and failure to thrive. In a multicenter study of children ≤2 years old with diffuse lung disease, the mean age at biopsy for those with *SFTPC* mutations was 8.9 ± 3 months (range, 2 to 22 months) [160]. Although all of the children were alive at follow-up (10 to 61 months), significant ongoing pulmonary morbidity was found in this group. In another report from the St. Louis Children's/Washington University transplant program, the median age of onset for children with *SFTPC* mutations was 1 month, ranging from birth to 14 months [11,120]. Status at follow-up (2to15 years) in this study included 5 patients who had improved and were free of oxygen support, 1 receiving supplemental oxygen, 1 receiving mechanical ventilation, 2 who had received transplants, and 1 who died awaiting transplant [11,120].

Onset of respiratory symptoms has also been reported in adults, in whom it is associated with histopathologic diagnoses of NSIP and usual interstitial pneumonitis (UIP). In a large family with a heterozygous mutation in exon 5 of the SP-C gene (L188Q), onset of lung disease was variable and included children with NSIP and adults with UIP [146,147]. Age at diagnosis ranged from 4 months to 57 years. Lung disease in a family with the common I73T mutation was also associated with both early (1 year old) and late (71 years old) onset of lung disease [151]. Asymptomatic individuals with *SFTPC* mutations have also been identified [146,147]. Several instances of respiratory infection, including pneumonia caused by respiratory syncytial virus (RSV), influenza A, and influenza B, have been reported in patients with *SFTPC* mutations prior to the onset of pulmonary disease [146,147], which suggests that viral infection may precipitate the onset of respiratory symptoms in this disorder. This hypothesis is supported by in vitro studies in which respiratory syncytial virus infection of cells that were transfected with the Δexon 4 mutation resulted in accumulation of the mutant proSP-C peptide and extensive cell death [161]. Progression of the disease can also be quite variable. Some patients require transplantation, while others survive with persistent respiratory insufficiency requiring supplemental oxygen, or improve and graduate to room air [11,120]. This variability suggests that there are additional genetic and/or environmental factors that modify the onset and progression of lung disease in these patients. This is supported by studies in SP-C–deficient mice, which show a variable phenotype depending on genetic background, age, and exposure to pathogens or environmental toxins [162-165]. Although *SFTPC* mutations have been found in diverse racial and ethnic groups, the incidence and prevalence of lung disease caused by these mutations, as well as the population frequency of disease-causing *SFTPC* variants, is currently unknown. Recent studies indicate that *SFTPC* mutations are a rare cause of adult ILD, with the majority of patients presenting in the pediatric age group [4,8,153,160]. In 2 recent studies of adults with idiopathic pulmonary fibrosis or NSIP, only 1 patient was identified with a *SFTPC* mutation (I73T mutation) [166,167].

Histopathology and ultrastructural features

In general, histopathologic evaluation of lung disease associated with *SFTPC* mutations reveals diffuse alveolar damage of varying severity, interstitial thickening with mild lymphocytic inflammation and muscularization of the alveolar septa, foamy alveolar macrophages, variable amounts of granular alveolar proteinosis material with a few cholesterol clefts, and regenerating alveolar epithelium lined by hyperplastic type II cells (Fig. 6). These features are

associated with histopathologic diagnoses indicative of interstitial pneumonitis, including neonatal PAP [145,148-150,152], infantile DIP [148], chronic pneumonitis of infancy (CPI) [148,151,160], and NSIP [143,145,149,152], although the most common histopathologic diagnosis in infants is CPI [151,153,160]. In adults with *SFTPC* mutations and chronic ILD, the most common histopathologic diagnosis is pulmonary fibrosis [146,147,151].

Ultrastructural analysis of lamellar body formation in this disorder is limited, although mixtures of normal lamellar bodies with electron-dense vesicles, intracellular membranous aggregates, or disorganized lamellar bodies have been reported in association with several of the *SFTPC* mutations [145,146,148,150]. Ultrastructural analysis of tissue from the index patient with the Δexon 4 mutation [143], however, revealed large, well-organized lamellar bodies in the alveolar type II cells (Fig. 6). Occasionally, larger composite bodies containing 2 or more smaller lamellar bodies were also found (Fig. 6). These findings are supported by analysis of the SP-C–deficient mice, which exhibited large, intact lamellar bodies, as well as tubular myelin [162,163].

Immunohistochemistry and pathogenesis

In general, immunohistochemical analysis reveals robust staining for all of the surfactant proteins (SP-A, proSP-B, SP-B, and SP-D), including proSP-C, which is restricted to alveolar type II cells [145,146,148,151]. Two different patterns of proSP-C staining have been observed: (1) diffuse staining of the cytoplasm and (2) accumulation of immunoreactive material in a perinuclear compartment, a pattern typically observed with misfolded proteins (Fig. 7). Diffuse staining of the cytoplasm may be associated with abnormalities in trafficking of the mutant propeptide with accumulation in the ER (P30L mutation) or in the early endosomal compartment (I73T and E66K mutations) [145,149-152,159], while perinuclear staining may be associated with impaired degradation, accumulation, and/or aggregation of incompletely processed proSP-C (Δexon 4, L188Q, and 91-93del9 mutations) [143,146,148, 157-159,168-170]. These observations have led to the hypothesis that accumulation of mutant proSP-C peptide is toxic to the cell, resulting in chronic cell injury, ER stress, and cell death [161,168-172], which then leads to chronic interstitial inflammation and fibrosis in the lung.

Decreased or absent levels of mature SP-C are found in patients with the Δexon 4 and 91-93del9 mutations, suggesting that routing and processing of the nonmutated protein from the normal allele is perturbed in these mutations [143,148]. Because proSP-C is normally routed through the ER as a homodimer, association between mutant and wild type proSP-C in the ER may lead to subsequent misrouting, accumulation, and/or degradation of both propeptides [20, 156,157,159,170]. SP-C is also involved in reuptake and catabolism of secreted surfactant, as well as in the activated alveolar macrophage response to infection [42,165]. Therefore, loss of mature SP-C might also contribute to the onset of lung disease in patients with *SFTPC* mutations who are exposed to airborne pathogens. In other mutations (E66K and I73T), both mature SP-C and proSP-C were detected in the patient's BALF [145,152], suggesting that these peptides are secreted. In these cases, however, phospholipid composition, content, and function were altered in association with histologic observations of alveolar proteinosis and increased SP-A and SP-B content in the BALF [145,152]. Abnormalities in surfactant composition and function, along with alveolar proteinosis and increased levels of SP-A, were also observed in association with the 91-93del9 mutation [148], although no mature SP-B or SP-C was detected in the BALF. These observations suggest that surfactant composition and function may also play a role in the pathogenesis of lung disease in these patients.

GENETIC ABCA3 DISORDERS (SURFACTANT METABOLISM DYSFUNCTION, PULMONARY, 3, SMDP3, OMIM NO. 610921)

Index cases

Lung disease caused by mutations in the *ABCA3* gene was first described in 2004 in a group of racially and ethnically diverse full-term infants with severe respiratory distress, resulting in death in early infancy [173]. All had family histories of lung disease, clinical or radiographic findings consistent with surfactant deficiency, and histologic findings consistent with infantile DIP and/or neonatal PAP. A variety of mutations consisting of homozygous, missense, nonsense, and frameshift mutations, as well as heterozygous insertion and splice-site mutations, were identified in the *ABCA3* genes of these patients. Small, markedly abnormal lamellar bodies with densely packed membranes and eccentrically placed, dense inclusion bodies were observed by electron microscopy in the alveolar type II cells of lung tissue from 4 patients with nonsense, splice-site, and missense mutations in the *ABCA3* gene [173,174].

Genetics and clinical presentation

More than 150 distinct mutations have been identified in the *ABCA3* gene [7,8,13,116, 173-184], making this the largest class of mutations that cause genetic abnormalities in surfactant metabolism. *ABCA3* is a large gene located on chromosome 16 (16p13.3) and contains 30 coding exons [58,59]. Mutations in the *ABCA3* gene are distributed throughout its length and consist of missense, nonsense, frameshift, and splice-site mutations, as well as insertions and deletions (Fig. 8A). Lung disease caused by *ABCA3* mutations is inherited as an autosomal recessive disorder, requiring mutations on both alleles. The majority of cases reported to date have been associated with surfactant deficiency, respiratory distress, and failure in the neonatal period or in infancy [160,173,175,178,180-182]. In a multicenter study of children ≤2 years old with diffuse lung disease, the mean age at biopsy for children with *ABCA3* mutations was 1.3 ± 0.5 months (range, 0.2 to 3 months) [160]. The histopathologic findings in this group of children were associated predominantly with neonatal PAP. In contrast to the children in this study with *SFTPC* mutations, the children with *ABCA3* mutations had a 100% mortality rate with an average age at death of 1.9 ± 0.7 months (range, 4 to 4.5 months) [160]. Surfactant isolated from the lungs of children with fatal *ABCA3* mutations was ineffective in lowering surface tension [116], suggesting that surfactant deficiency was the cause of lung disease in these patients. Phospholipid analyses revealed abnormal phospholipid profiles with decreased PC and PG [116]. Because clinical presentation, mortality rate, and histopathology for the *ABCA3* mutations are often similar to those for genetic SP-B deficiency, lung transplantation has been offered as a treatment for those infants with severe, early-onset lung disease [11,116,120,177].

A common mutation involving a substitution of valine for glutamic acid in codon 292 (E292V), located in the first cytosolic loop of the ABCA3 protein, has been identified in older children with chronic ILD who have survived into their teens without lung transplant [176,177]. Onset of symptoms in the majority of these patients was at birth, in the neonatal period, or in infancy (<1 year), with histopathologic diagnoses of PAP or DIP [176], and was associated with compound heterozygous inheritance of the E292V mutation on at least 1 allele. Milder neonatal disease with presentation of nonspecific symptoms and findings, including cough, tachypnea, hypoxemia in room air, clubbing, and failure to thrive, were reported in some of these children, while other children did not have respiratory symptoms or findings until later in childhood [176,177]. Recently, the E292V carrier frequency was reported to be 3- to 5-fold higher than those for the common mutations in *SFTPB* (121ins2) or *SFTPC* (I73T), which were rare (<0.4%) [185]. In this study, the E292V mutation was overrepresented in newborns with RDS, suggesting that the E292V mutation might be associated with a greater genetic risk for RDS. Mutations in *ABCA3* have also been identified in adults with chronic ILD, as well as in an

adolescent patient who presented at age 15 with a 6-month history of exercise intolerance, chest discomfort, and histologic features of UIP [184]. *ABCA3* may also act as a disease-modifying gene in lung disease caused by the *SFTPC* mutations, as patients who were heterozygous for both an *ABCA3* mutation and the *SFTPC* I73T mutation had more severe lung disease than family members with only the I73T mutation [186].

Histopathology and ultrastructural features

In general, *ABCA3* disorders are associated with histopathology diagnoses of infantile DIP and neonatal PAP (Fig. 9), with onset of symptoms in infancy. In lung biopsies from older infants and children, however, UIP and NSIP with superimposed lipoid pneumonia have also been reported [177,184]. Multiple, small lamellar bodies with densely packed phospholipid membranes and eccentrically placed electron-dense cores have been detected in the type II cells of the majority of these patients [173-176,179-182,187] (Fig. 9), although more normal-appearing lamellar bodies have been reported in several patients [116,175,177].

Immunohistochemistry and pathogenesis

In general, immunostaining for the surfactant proteins, SP-A, SP-B, proSP-B, proSP-C, and SP-D is readily detected [180-182], although a subset of cases exhibits poor immunostaining for the mature SP-B peptide [175,186] (Fig. 10). Unlike SP-B deficiency, proSP-C staining is restricted to the cytoplasm of alveolar type II cells and is not found in the airspaces. Absence of immunostaining for mature SP-B, reduced amounts of SP-B, and/or impaired processing of proSP-B to the mature SP-B peptide have been observed in some patients with *ABCA3* mutations [175,186], suggesting that normal lamellar body formation is required for processing of SP-B. Although immunostaining for proSP-C is generally robust, little information is available for the status of mature SP-C in patients with *ABCA3* mutations except for one series of patients with fatal respiratory distress in whom mature SP-C could not be detected [175]. Both ABCA3 and mature SP-B were also reduced or absent in this group of patients. This observation suggests that processing of proSP-C to the mature SP-C peptide might also be impaired by disruption of lamellar body formation. This hypothesis is supported by recent findings in transgenic mouse models of ABCA3 deficiency, wherein processing of both proSP-B and proSP-C was defective [188-190]. These observations suggest that mutations in the *ABCA3* gene cause severe surfactant deficiency as a result of secondary effects on SP-B and SP-C processing, as well as on surfactant phospholipid packaging and secretion.

Depending on location of the mutation in the gene, lung disease in genetic ABCA3 disorders may be caused by a number of different mechanisms, including loss of expression, decreased expression, abnormal intracellular trafficking of the protein to the lamellar body, abnormal packing of phospholipids, and/or defects in functional activity (i.e., ATP hydrolysis). Accordingly, immunolocalization of ABCA3 in HEK293 (kidney) cells or A549 (lung) cells vary with the type of mutant *ABCA3* gene that is transfected into these cells. Mutations that interfere with trafficking of ABCA3 to the lysosomal compartment of these cells in vitro exhibited robust staining for ABCA3 in the ER, while mutations that interfered with ATP-dependent phosphocholine transport were associated with immunolabeling of a lysosomal-like, post-ER/Golgi compartment [191,192]. Interestingly, *ABCA3* mutations associated with fatal ABCA3 disease were correlated with trafficking defects, while mutations associated with less severe pulmonary disease, such as the E292V mutation, were correlated with defects in phosphocholine transport [191,192] (Fig. 8B).

DIAGNOSTIC CONSIDERATIONS

Because there is considerable overlap in the clinical and histologic presentation of these disorders, genetic analysis is essential for establishing a specific diagnosis in patients with

suspected surfactant disorders. Full sequence-based analysis of all three genes is now available through Clinical Laboratory Improvement Amendments-certified laboratories. (Laboratories offering clinical testing in the United States include Ambry Genetics Corp., Aliso Viejo, CA, and Johns Hopkins Hospital, Baltimore, MD.) If tissue is available for patients whose lungs were biopsied for clinical indications, or who died prior to the availability of genetic studies, additional immunohistochemical and/or ultrastructural evaluation may be useful in determining a probable genetic basis for their lung disease. In these cases, immunohistochemical and ultrastructural examination of lung tissue may provide valuable clues to the diagnosis, as well as insights into the pathophysiology of these disorders.

Immunohistochemistry

As can be appreciated from the previous descriptions, there is considerable overlap in the clinical presentation and histopathology of these patients, especially for lung disease due to the *SFTPB* and *ABCA3* mutations, which often present in the neonatal period and can be fatal. In these cases, immunohistochemistry can be of benefit in distinguishing *SFTPB* mutations from *ABCA3* or *SFTPC* mutations. Fortunately, antibodies to the surfactant proteins have been generated in the research laboratory and are now available commercially. Several polyclonal and monoclonal antibodies have been generated to mature SP-B, as well as to specific epitopes of the proSP-B and proSP-C peptides, including their amino and carboxyl termini. Use of these monospecific antibodies allows immunodetection of the partially processed and/or misfolded forms of these propeptides. Immunohistochemistry is especially useful in determining a diagnosis of genetic SP-B deficiency, because distinct immunohistochemical patterns for mature SP-B and proSP-C expression have been described for the *SFTPB* mutations. In these cases, there is often complete loss, or absence, of immunostaining for mature SP-B, which cannot be recovered with epitope or heat-induced antigen retrieval (HIER) techniques. In addition, immunolocalization of the secreted, partially processed proSP-C peptide in the alveolar lumen (using a monospecific polyclonal antibody to the amino terminus of proSP-C) has been detected in all cases of genetic SP-B deficiency examined in the research laboratory by immunohistochemistry [107].

Absent or weak staining for mature SP-B has been observed in some *ABCA3* mutations. This pattern differs from SP-B deficiency in that immunostaining for the mature SP-B peptide can be recovered by use of HIER, while immunostaining for proSP-B is robust and can be readily detected in the absence of HIER in *ABCA3*-related disease. The genetic, clinical, and/or pathologic significance of these differences in immunodetection of mature SP-B is currently unknown, and further studies are needed to see whether this observation has diagnostic value or can be correlated with genotype. In contrast to SP-B deficiency, immunolocalization of proSP-C in patients with *ABCA3* mutations is restricted to the cytosol of the alveolar type II cell, where it is normally detected, and is not found in the alveolar lumen. Likewise, immunolocalization of proSP-C is restricted to the alveolar type II cell in patients with PAP caused by impaired GM-CSF signaling [129,175]. Therefore, immunodetection of incompletely processed proSP-C in the alveolar lumen may be considered as a useful diagnostic marker for genetic SP-B deficiency.

No consistent immunohistochemical patterns for the detection of proSP-C or mature SP-C have been established for patients with *SFTPC* mutations, although perinuclear staining for proSP-C suggests an *SFTPC* mutation. In general, immunostaining for proSP-C, using a monospecific polyclonal antibody generated to the amino terminus of proSP-C, is robust and restricted to the alveolar type II cell. Markedly reduced proSP-C was observed, however, in patients with the Δexon 4 mutation [143], as well as in familial lung disease in which no *SFTPC* mutations could be identified [193]. Although Brasch and colleagues [145] reported immunodetection of proSP-C in the alveolar lumen of a patient with the I73T mutation using a monospecific

polyclonal antibody to the carboxyl terminus of proSP-C, this antibody has not been used widely to investigate secretion of the misfolded protein in other *SFTPC* mutations. Likewise, little information is available for the status of mature SP-C in patients with *SFTPC* mutations, although some mature SP-C may be secreted in the I73T mutation [145].

In general, immunohistochemistry for SP-A and SP-D is robust in all of these disorders and tends to reflect the amount of proteinosis material in the sample. This material is often immunopositive for proSP-B when detected with a monospecific antibody to the carboxyl terminus of the propeptide. Normally the first cleavage steps in processing of proSP-B involve removal of the amino terminus to yield a 23-kDA intermediate form of the peptide. Subsequent cleavage steps occur in the multivesicular body to yield the 8-kd, mature SP-B peptide. In lung samples from patients with *SFTPC* and *ABCA3* mutations, immunodetection of a partially processed proSP-B peptide (23 kd) containing part of the carboxyl terminus can be found in the alveolar lumen [145,175]. The detection of partially processed intermediate forms of proSP-B in the lung and/or in the BALF, however, is more likely to be a general indicator of lung injury and is not specific for these genetic disorders [194,195]. Finally, because glucocorticoids, hyperoxia, and ventilation can impact synthesis, secretion, and activity of the surfactant phospholipids and proteins, quantitative interpretations of staining intensity should be avoided. In this regard, it is important to note that the effects of these therapeutic interventions are complex, affecting synthesis, secretion, and function of the surfactant proteins differentially, depending on the animal species or model, ventilation technique, oxygen level, and duration of exposure, as well as causing cell injury, cell death, and induction of proliferative responses in the tissue.

Ultrastructural features

The ultrastructural findings associated with lamellar body formation in the *SFTPB* and *ABCA3* mutations are very distinctive. Therefore, ultrastructural studies may be helpful in distinguishing these 2 disorders from each other, and the preparation of tissue for electron microscopy should be considered in full-term infants with fatal respiratory distress or in children with diffuse lung disease (196). As previously described, loss of SP-B disrupts formation of the well-organized concentric rings of phospholipid membranes that normally are observed in the lamellar body by electron microscopy. Instead, large, disorganized multivesicular bodies are observed in the cytosol of alveolar type II cells, as well as in the alveolar lumen. In contrast, small, markedly abnormal lamellar bodies with densely packed phospholipids membranes and electron-dense inclusions have been observed by electron microscopy in the alveolar type II cells from patients with a variety of *ABCA3* mutations. Currently, no distinctive ultrastructural abnormalities have been described in association with the *SFTPC* mutations, although irregular or disorganized lamellar bodies have been reported. On the other hand, normal lamellar bodies have been observed in human tissue samples from patients with the Δexon 4 and P30L mutations in the *SFTPC* gene. Abnormal lamellar bodies are also found in certain lysosomal storage diseases, such as Chediak-Higashi and Hermansky-Pudlak syndromes (197–200). Although these lamellar bodies tend to have normally arranged, concentric, phospholipid membranes, they are abnormally large. This “giant” lamellar body phenotype reflects abnormal accumulation of phospholipid in the cell, which is caused by a variety of defects in vesicle formation, trafficking, secretion, and/or catabolism of pulmonary surfactant, and is quite distinctive from the abnormal lamellar bodies found in the *SFTPB* and *ABCA3* mutations.

CONCLUSIONS

Although rare, genetic disorders in surfactant production and function in the lung have now been shown to cause significant primary lung disease in full-term infants and older children.

Given their critical roles in surfactant function and metabolism, common variants in these genes (polymorphisms) may also be important in the risk for developing or modifying the course of other common lung diseases. In evaluating patients who present with symptoms of surfactant deficiency or dysfunction, it is important to consider multiple factors, including family history, age of onset, clinical presentation, and disease progression, as well as the histopathologic findings (Table 1). If lung biopsy is available, then immunohistochemical detection of partially processed proSP-C in the alveolar lumen, along with the absence of immunostaining for mature SP-B, may be pathognomonic for genetic SP-B deficiency, while ultrastructural detection of aberrant lamellar body formation may be diagnostic for mutations in the SP-B and/or *ABCA3* genes. Therefore, both immunohistochemistry and ultrastructural analysis may be useful in making a preliminary diagnosis in these patients when the clinical presentation and histopathology overlap.

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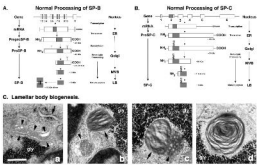

Figure 1.

Proposed structure, posttranslational processing, and trafficking of surfactant proteins B and C (SP-B and SP-C) in alveolar type II cells. **A.** Normal processing of SP-B. Human SP-B is encoded by a single gene, *SFTPB*, on chromosome 2, spanning about 10 kilobases (kb), and contains 10 introns (straight lines) and 11 exons (boxes), of which the last is untranslated. The mature SP-B peptide is encoded in exons 6 and 7 (shaded boxes). *SFTPB* is transcribed into a 2-kb mRNA that is translated in the endoplasmic reticulum (ER) to yield a 40-kd, 381-amino acid preproprotein. After cotranslational cleavage of its signal peptide, proSP-B undergoes glycosylation to yield a 42-kd intermediate form of the proprotein. Proteolytic cleavage of the NH_2 and COOH termini of proSP-B occurs during trafficking of the proprotein through the Golgi apparatus and the multivesicular body (MVB), generating 23- to 26-kd and then 9-kd intermediate forms of the proprotein, before yielding the 8-kd, 79-amino acid, mature peptide that forms homodimers of ~18 kd in the mature lamellar body (LB). The shaded region of the proprotein represents the mature SP-B peptide. (Adapted from data in references 34–37, 43–45, and 48–53.) **B.** Normal processing of SP-C. Human SP-C is encoded by a single gene, *SFTPC*, on chromosome 8, spanning about 3.5 kb, and contains 5 introns (straight lines) and 6 exons (boxes), of which the last is untranslated. The mature SP-C peptide is encoded in exon 2 (shaded box). *SFTPC* is transcribed into a 0.9-kb messenger RNA that is translated in the ER to yield a 21-kd, 191- to 197-amino acid proprotein (proSP-C). ProSP-C then undergoes palmitoylation (PP) of cysteine residues within the mature peptide domain to yield a 24-kd intermediate. Proteolytic cleavage of the COOH and NH_2 termini of proSP-C occurs during trafficking of the proprotein through the Golgi apparatus and the MVB, generating 16-kd and then 7- to 6-kd intermediate forms of the proprotein, before yielding the 4-kd, 35-amino acid, mature peptide that is found in the mature LB. The shaded region of the proprotein represents the mature SP-C peptide. (Adapted from data in references 38–42, 46, and 47.) **C.** Cellular components required for biosynthesis and processing of SP-B and SP-C. **a.** SP-B and SP-C are transcribed on ribosomes associated with the ER (arrows) and transported to the Golgi apparatus (arrowheads) for proteolytic processing of the larger precursor proteins to smaller, intermediate forms of the proproteins. **b.** Endosomal vesicles containing proSP-B and proSP-C bud off the Golgi apparatus and accumulate in MVBs (arrowheads). An LB with prominent phospholipid lamellae is seen nearby (arrow). **c.** The partially processed proproteins are delivered to LBs via fusion of the MVB (arrowhead) with the LB (arrow) to form a composite body, wherein final processing of the proproteins to mature SP-B and SP-C is thought to occur. **d.** The mature LB is composed of both surfactant phospholipids and the fully processed, mature SP-B and SP-C peptides that are now tightly associated with the phospholipid bilayers. Images were acquired by electron microscopy of newly forming LBs in immature alveolar type II cells found in fetal mouse lung tissue. gly, glycogen. Scale bar = 500 nm.

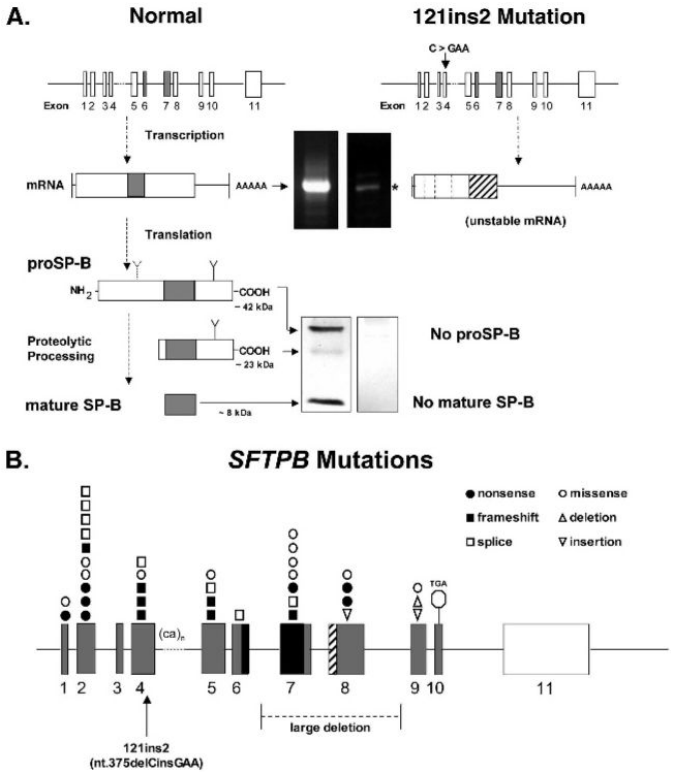

Figure 2.

Structural models of the human surfactant protein B (SP-B) gene and protein illustrating abnormal processing of SP-B and the location of mutations found throughout the gene. **A.** Comparison of normal and abnormal transcription, synthesis, and processing of SP-B caused by the 121ins2 mutation. Aberrant transcription of *SFTPB* caused by the 121ins2 mutation results in formation of an unstable messenger RNA (mRNA) that is rapidly degraded and barely detected by polymerase chain reaction (PCR) analysis (faint white band on black at *, upper right panel) compared with the presence of normal mRNA observed in a control patient (white band on black at arrow, upper left panel). This results in the complete absence of both proprotein SP-B (proSP-B) and the mature SP-B peptide, as assessed by immunoblot of protein isolated from lung tissue of an affected patient (no black bands, lower right panel). In contrast, the presence of 42-kd and 23-kd forms of proSP-B, as well as the 8-kd mature SP-B peptide (black bands on white at arrows, lower left panel) can be detected in protein isolated from lung tissue of a control patient. **B.** Structural model of *SFTPB* illustrating the location of mutations found throughout the gene. The mature peptide is encoded in exons 6 and 7 (black boxes); exon 11 (white box) is not translated. Mutations in *SFTPB* include a variety of nonsense, missense, frameshift, and splice-site mutations, as well as insertions and deletions. The common mutation, 121ins2, is located in exon 4 (arrow). A large deletion, encompassing exons 7 and 8, has been reported recently. TGA, translational stop sequence located in exon 10.

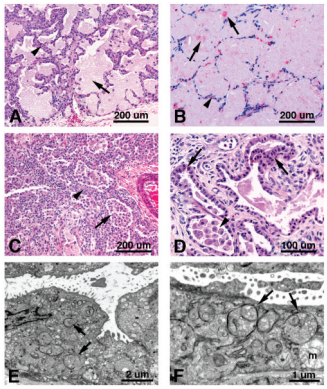

Figure 3.

Histopathology and ultrastructural features of disorders caused by mutations in the human surfactant protein B (SP-B) gene. **A.** Autopsy tissue from a 23-day-old child who was homozygous for the 121ins2 mutation. Alveolar proteinosis with foamy, eosinophilic, lipoproteinaceous material filling the alveoli (arrow) is typically found in the lung of patients with SP-B mutations. Thickened alveolar septa (arrowheads) are also a prominent feature of this disorder. **B.** Biopsy tissue from a 6-year-old child with a granulocyte-macrophage colony-stimulating factor receptor alpha chain mutation [129]. In contrast to SP-B deficiency, the large amount of alveolar proteinosis material found in this genetic disorder is denser and contains larger globules of eosinophilic material (arrows), which fills and expands the alveoli. There is also good preservation of the alveolar septa (arrowhead). **C.** Explant tissue from a 13-month-old child who was a compound heterozygote for the 121ins2 and C100G mutations [107], demonstrating infantile desquamative interstitial pneumonitis (DIP) with accumulation of foamy alveolar macrophages in the alveoli (arrow) and little to no alveolar proteinosis. The alveolar epithelia are hyperplastic, and the thickened alveolar septa (arrowhead) contain lymphocytic infiltrates in this sample. **D.** Autopsy tissue from a 2-month-old child who was a compound heterozygote for the 121ins2 and c.282-2delA mutations [107]. Higher magnification of hyperplastic alveolar epithelia with prominent type II cells (arrows) and accumulation of foamy macrophages in the alveolar lumen is shown. Note the prominent interstitial widening composed of loose connective tissue and disruption of the normal alveolar capillary architecture, which precludes normal gas exchange and lends an immature appearance to the lung. (Hematoxylin and eosin-stained paraffin sections.) **E.** In lieu of normal lamellar bodies, electron microscopic analysis demonstrates the presence of many large, membrane-bound structures containing smaller membranous vesicles (arrows) and, occasionally, several concentric layers of phospholipid lamellae in the type II cells of a child with the 121ins2 mutation. **F.** Higher magnification of these aberrant structures (arrows), which are similar in appearance to the multivesicular and composite bodies found during lamellar body biogenesis. m, disrupted mitochondria.

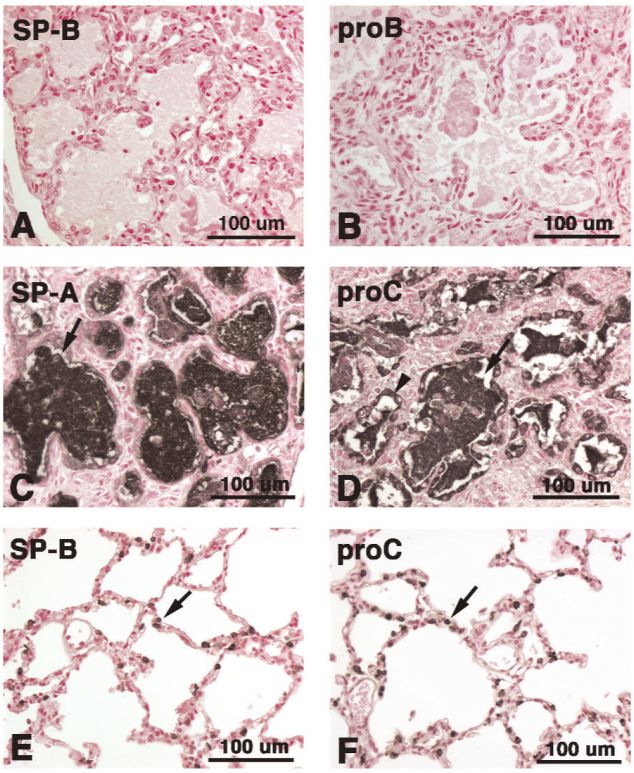

Figure 4.

Immunohistochemical staining for the surfactant proteins in lung tissue from subjects with mutations in the human surfactant protein B (SP-B) gene. Autopsy tissue from a child who was homozygous for the 121ins2 mutation is shown in panels A through D; biopsy tissue from a control sample is shown in panels E and F. No immunostaining for mature SP-B (**A**) or proprotein SP-B (proSP-B; **B)** is found in the 121ins2 mutation. On the other hand, the alveolar proteinosis material (arrow) found in this mutation stains intensely for SP-A **(C)** and for proSP-C **(D)**, which are detected in both alveolar type II cells (black reaction product at arrowhead) and in the alveolar lumen (black reaction product at arrow). In contrast, immunostaining for mature SP-B **(E)** and proSP-C **(F)** is restricted to alveolar type II cells (arrows) and is not detected in the alveolar lumen in biopsy tissue from a control lung. Immunohistochemistry was performed using polyclonal antibodies to (1) full-length SP-A, (2) the mature SP-B peptide, (3) the carboxyl terminus of proSP-B, and (4) the amino terminus of proSP-C. A color version of this figure is available online.

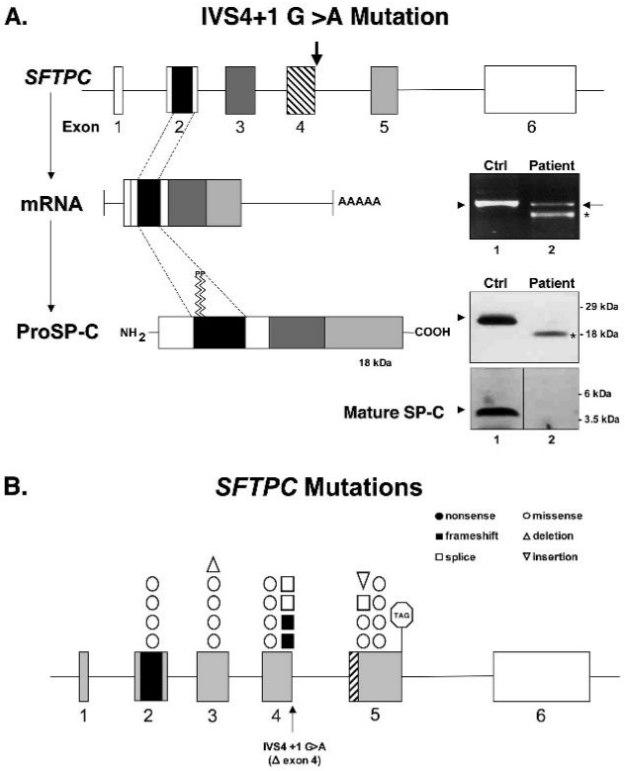

Figure 5.

Structural models of the human surfactant protein C (SP-C) gene and protein illustrating abnormal processing of SP-C and the location of mutations found throughout the gene. **A.** Abnormal processing of SP-C caused by the IVS4+1G>A mutation. The mutation, IVS4 +1G>A (also known as c.460+1G>A or Δexon 4), is located at the junction of exon 4 and its adjacent intron (arrow), and is found on only one allele of the affected patient. This causes exon 4 to be skipped, resulting in a truncated form of the messenger RNA for this allele, which contains sequences for exons 1 through 3 and exon 5. This can be observed by reverse transcriptase polymerase chain reaction analysis of the patient's RNA (lower band at *, lane 2, upper panel). A normal-sized band (upper band at arrow, lane 2, upper panel) indicating that the nonmutated allele is found in the affected patient, as well as in a control (Ctrl) patient (upper band at arrowhead, lane 1, upper panel). This results in translation of an aberrant 18-kd form of the proprotein (lower band at *, lane 2, middle panel depicting an immunoblot of the protein) compared with the 21-kd proprotein observed in the control patient (upper band at arrowhead, lane 1, middle panel). No mature peptide is found by immunoblot in the affected patient (lane 2, right lower panel of protein blot) when compared with the control patient (band at arrowhead, lane 1, left lower panel). **B.** Structural model of *SFTPC* illustrating the location of mutations found throughout the gene. Mutations in *SFTPC* include a variety of nonsense, missense, frameshift, and splice-site mutations, as well as insertions and deletions. The IVS4+1G>A mutation (also known as c.460+1G>A or the Δexon 4 mutation) is located at the junction of exon 4 and its adjacent intron (arrow). The mature peptide is encoded in exon 2 (black boxes); exon 6 (white box) is not translated. TAG, translational stop sequence located in exon 10.

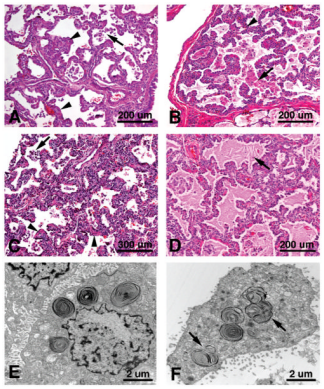

Figure 6.

Histopathology and ultrastructural features of genetic disorders caused by mutations in the human surfactant protein C (SP-C) gene. **A.** Nonspecific interstitial pneumonitis (NSIP), with thickened alveolar septa (arrowhead) and a few alveolar macrophages, is seen in a biopsy from a 6-week–old child, heterozygous for the Δexon 4 mutation (also known as IVS4+1 G>A or c.460+1G>A) [143]. **B.** Chronic pneumonitis of infancy (CPI) with alveolar septal thickening (arrowheads) and accumulation of large, foamy macrophages and granular, eosinophilic, alveolar proteinosis material in the alveoli (arrow) is seen in a biopsy from a 9-month–old child, heterozygous for the P115L mutation [144]. **C.** Another example of CPI, with diffuse alveolar septal thickening, alveolar type II cell hyperplasia, and accumulation of macrophages (arrow) in the alveolar lumen, is seen in a biopsy from a 1-year–old child, heterozygous for the common I73T mutation [151]. Muscularization of the alveolar septa and ducts (arrowhead) and inflammatory cell infiltrates are found in the adjacent thickened interstitial structures. **D.** Accumulation of larger amounts of foamy alveolar proteinosis material with cholesterol clefts (arrow) is found in explanted tissue from a 9-month-old child, heterozygous for the 91-93del9 mutation [148]. (Hematoxylin and eosin-stained paraffin sections.) **E.** Electron microscopic analysis demonstrates the presence of large, well-organized lamellar bodies (arrows) found in the type II cells of a 6-week-old child heterozygous for the Δexon 4 mutation. **F.** Large composite bodies containing multiple lamellar body–like structures and membrane-bound vesicles were also found in this sample (arrows).

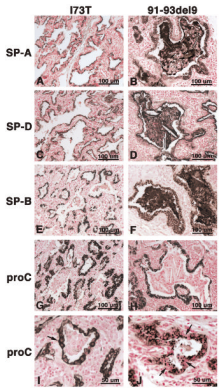

Figure 7.

Immunohistochemical staining for the surfactant proteins in lung tissue from subjects with mutations in the human surfactant protein C (SP-C) gene. Immunohistochemistry for the common I73T mutation [151] is shown in column one (**A, C, E, G**, and **I**) and for the 91-93del9 mutation [148] in column two (**B, D, F, H**, and **J**). Immunostaining for SP-A **(A, B)**, SP-D, **(C, D)**, mature SP-B **(E, F)**, and proprotein SP-C (proSP-C; **G** through **J**) is robust in both mutations (black reaction product). The alveolar proteinosis material and macrophages found in the 91-93del9 mutation are immunopositive for SP-A **(B)**, SP-D **(D)**, and mature SP-B **(F)**, but not for proSP-C **(H)**, which is restricted to the alveolar type II cells. Two different immunostaining patterns are detected for proSP-C. Diffuse staining of the alveolar type II cell cytoplasm is seen in the I73T mutation **(I)**, while a more perinuclear staining pattern is seen in the 91-93del9 mutation **(J)**. Immunohistochemistry was performed using polyclonal antibodies to (1) full-length SP-A, (2) full-length SP-D, (3) the mature SP-B peptide, (4) the carboxyl terminus of proSP-B, and (5) the amino terminus of proSP-C. A color version of this figure is available online.

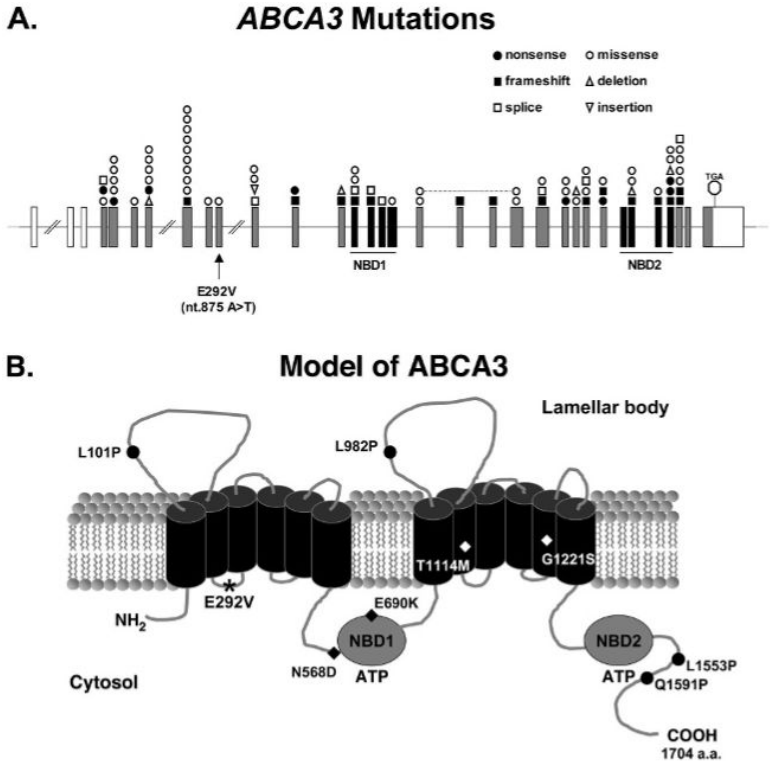

Figure 8.

Structural models of the human *ABCA3* gene and protein illustrating the location of mutations found throughout the gene and protein. **A.** Structural model of the *ABCA3* gene. ABCA3 is encoded by a single gene, *ABCA3*, on chromosome 16 (16p13.3), and contains 33 exons (boxes). The adenosine triphosphate (ATP)-binding domain or nucleotide-binding domain (NBD) is encoded in exons 14–17 (NBD1) and exons 27–30 (NBD2) (black boxes). Mutations in *ABCA3* include a variety of nonsense, missense, frameshift, and splice-site mutations, as well as insertions and deletions. The common mutation, E292V, is located in exon 9 (arrow). TGA, translational stop sequence located in exon 33. **B.** Structural model of the ABCA3 protein. ABCA3 is a 1704-amino acid, integral membrane protein located at the limiting membrane of the lamellar body (LB). It contains 2 homologous repeats, each consisting of 6 putative transmembrane helices and an ATP-binding domain. ABCA3 is thought to be oriented in the lipid bilayer such that its ATP-binding domains (NBD1, NBD2) are located in the cytoplasm. Phospholipids are then actively transported from the cytosol to the interior of the LB through the membrane channel (black cylinders) that is formed in the phospholipid bilayer. The location of mutations that have been shown to impair ABCA3 function [191,192], either by interfering with ATP hydrolysis (N568D, E690K, T1114M, G1221S) or with trafficking of the protein from the endoplasmic reticulum (ER) to the LB (L101P, L982P, L1553P, Q1591P), are illustrated. *, location of the E292V mutation. (Adapted from Matsumura and colleagues [191,192].)

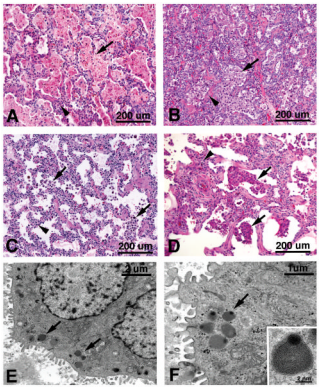

Figure 9.

Histopathology and ultrastructural features of disorders caused by mutations in the human *ABCA3* gene. **A.** Alveolar proteinosis, admixed with alveolar macrophages, and thickened alveolar septa (arrowhead) are seen in a biopsy from a neonate with fatal lung disease who was homozygous for the c.4909+1G>A splicing mutation [173]. **B.** Infantile desquamative interstitial pneumonitis (DIP) with accumulation of foamy macrophages in the alveoli is seen in an autopsy from 33-day-old child who was a compound heterozygote for the c.1474insT-D953N and c.5012insA mutations. The alveolar epithelium is hyperplastic, and muscularization of the alveolar septa is found in the adjacent thickened interstitial structures (arrowhead). **C.** Thickened alveolar septa (arrowhead) and accumulation of alveolar macrophages (arrow) are seen in explanted tissue from a 21-year-old adult with a diagnosis of DIP and who is a compound heterozygote for the E292V and N1076K mutations [176]. **D.** Lung remodeling with thickened alveolar septa and macrophage accumulation are seen in a biopsy from a 2-year-old child with prolonged survival (>12 years old) who is a compound heterozygote for the E292V and c.1742-9G>A mutations [176]. (Hematoxylin and eosin-stained paraffin sections.) **E.** Electron microscopic analysis demonstrates the presence of small, abnormal, lamellarlike bodies (arrows) with eccentrically placed electron-dense inclusions in a biopsy from a neonate who was homozygous for the c.4909+1G>A mutation (see panel A). **F.** Higher magnification of small, lamellarlike bodies (arrow) with tightly packed phospholipid membranes (inset) found in the type II cells of this sample.

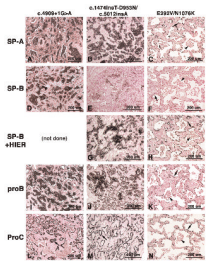

Figure 10.

Immunohistochemical staining for the surfactant proteins in lung tissue from subjects with mutations in the human *ABCA3* gene. Immunohistochemistry from a neonate, homozygous for the c.4909+1G>A mutation [173], is shown in column one (**A, D, I**, and **L**); from a 33-day-old, compound heterozygote for the c.1474insT-D953N and c.5012insA mutations, is shown in column two (**B, E, G, J**, and **M**); and from a 21-year-old adult, compound heterozygote for the E292V and N1076K mutations [176], is shown in column 3 (**C, F, H, K**, and **N**). Immunostaining for surfactant protein A (SP-A; **A, B**, and **C**), proprotein SP-B (proSP-B; **I, J**, and **K**), and proSP-C **(L, M**, and **N)** is robust in all three mutations (black reaction product). Immunostaining for mature SP-B is readily detected in the c.4909+1G>A mutation (**D**), but is weak or not detected in the other two mutations (**E, F**). Immunostaining for mature surfactant protein B (SP-B) is recovered after use of heat-induced epitope retrieval (**G, H**). The alveolar proteinosis material and macrophages found in the first 2 mutations are immunopositive for SP-A **(A, B)**, SP-B **(D, G)**, and proSP-B **(I, J)**. Immunostaining for proSP-C, however, is restricted to alveolar type II cells (arrows) in all three mutations (**L, M**, and **N**). Immunostaining for the surfactant proteins is detected primarily in the alveolar type II cells (arrows) in the E292V/N1076K mutation (**C, F, H, K**, and **N**). There is little to no secreted, immunopositive, proteinosis material found in this sample, and no immunostaining is detected in the alveolar macrophages (arrowheads). Immunohistochemistry was performed using polyclonal antibodies to (1) full-length SP-A, (2) the mature SP-B peptide, (3) the carboxyl terminus of proSP-B, and (4) the amino terminus of proSP-C. A color version of this figure is available online.

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Table 1

Comparison of the genetic surfactant disorders

	SFTPB	***SFTPC***	***ABCA3***
Protein	SP-B	SP-C	ABCA3
Inheritance	Autosomal recessive	Autosomal dominant or sporadic	Autosomal recessive
Mechanism	Loss of function	Dominant negative or toxic gain of function	Loss of function
Age of onset	Neonatal	Infancy to adult	Neonatal > childhood
Clinical syndrome	RDS	ILD >> RDS	RDS or ILD
Outcome	Fatal without transplant	Variable	Severe variable
Histopathology	PAP, DIP	CPI, PAP, DIP, NSIP, UIP	PAP, DIP NSIP, UIP
Lamellar bodies (EM)	Abnormal	Variable	Variable
Dominant phenotype	Large MVBs	Normal	Small, dense LBs
Immunohistochemistry			
SP-B	Absent	Present	Variable
proSP-B	Variable	Present	Present
	Mutation dependent		
proSP-C	Present	Variable	Present
	+ Type II cells	+ Type II cells only	+ Type II cells only
	+ Alveolar lumen		
SP-A	Present	Present	Present
SP-D	Present	Present	Present

SP-B indicates surfactant protein B; SP-A, surfactant protein A; RDS, respiratory distress syndrome; ILD, interstitial lung disease; PAP, pulmonary alveolar proteinosis; DIP, desquamative interstitial pneumonitis; CPI, chronic pneumonitis of infancy; NSIP, nonspecific interstitial pneumonitis; UIP, usual interstitial pneumonitis; EM, electron microscopy; MVB, multivesicular body; LB, lamellar body; pro, proprotein; SP-D, surfactant protein D; >, greater than.