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Genetic Disorders of Surfactant Proteins

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Abstract
Inherited disorders of pulmonary surfactant-associated proteins are rare but provide important insights into unique mechanisms of surfactant dysfunction. Recessive loss-of-function mutations in the surfactant protein-B and the ATP-binding cassette family member A3 (ABCA3) genes present as lethal surfactant deficiency in the newborn, whereas other recessive mutations in ABCA3 and dominant mutations in the surfactant protein-C gene result in interstitial lung disease in older infants and children. The molecular basis and the genetic and tissue-based approaches to the evaluation of children suspected of having one of these disorders are discussed.
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Introduction

Pulmonary surfactant, a mixture of phospholipids and proteins synthesized, packaged and secreted by alveolar type II cells, lowers surface tension and prevents atelectasis at end-expiration. The phospholipid components constitute approximately 90% by weight of pulmonary surfactant, of which 70–80% is phosphatidylcholine (PC). Pulmonary surfactant is unique in its abundance of dipalmitoylated PC which is the primary surface active component [1]. The protein components constitute approximately 10% by weight of pulmonary surfactant, half of which consists of four surfactant-associated proteins, surfactant proteins A, B, C, and D (SP-A, SP-B, SP-C, and SP-D). SP-A and SP-D participate in host defense in the lung whereas SP-B and SP-C contribute to the surface tension-lowering activity of the pulmonary surfactant [2, 3].

A complex and tightly regulated cycle of synthesis, processing, transport, secretion, degradation, re-uptake or clearance and re-processing involves both the phospholipid and protein components of pulmonary surfactant [1, 4, 5]. Surfactant phospholipids and proteins are synthesized within type II cells, packaged into specialized organelles, lamellar bodies, which are extruded into the alveolar lumen by exocytosis. The lamellar bodies unravel to form tubular myelin, a lattice structure upon which phospholipids adsorb and create the interface between air and liquid [6]. This monolayer of phospholipid and protein is then degraded and recycled into the type II cell or phagocytosed by macrophages. The kinetics of this metabolic cycle have not been fully elucidated for humans, but limited studies of human newborns using stable isotope-labeled precursors suggest that surfactant turnover is more rapid in term infants without respiratory dysfunction than in preterm infants with respiratory distress syndrome (RDS) [7–9].

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In 1959, Mary Ellen Avery and Jere Mead [10] established that RDS in preterm newborns was due to a developmentally regulated deficiency in pulmonary surfactant. Antenatal corticosteroids and surfactant replacement have decreased the incidence and severity of RDS and have thereby unmasked other causes that are not responsive to surfactant replacement [11, 12]. The identification in 1993 of an inherited deficiency of surfactant protein-B (SP-B) in a term newborn with lethal respiratory distress was the original demonstration of a specific genetic mechanism for acute inherited RDS in newborns [13, 14]. Since that initial report, mutations in the genes encoding surfactant protein-C (SP-C) and the ATP-binding cassette protein member A3 (ABCA3) have also emerged as prominent causes of respiratory disease in infants and children, each of which represent distinct mechanisms of disease, thereby providing insight into the complexities of pulmonary surfactant synthetic processes [15–20].

Clinical Presentation

A family history of lung disease or a history of consanguinity increases the suspicion of a genetic mechanism. Two types of clinical presentation bring infants and children with inherited disorders of surfactant metabolism to attention. In the first and most dramatic, term newborns develop severe respiratory failure that requires significant ventilatory support shortly after birth, is minimally or transiently responsive to surfactant replacement, may require extracorporeal membrane oxygenation (ECMO), and fails to improve after the first week of life. Pulmonary hypertension which may be responsive to nitric oxide may accompany the clinical syndrome. The chest radiographic appearance mimics that of RDS in premature infants with diffuse haziness and air bronchograms. This acute presentation is typical for infants with loss-of-function mutations in *SFTPB* or *ABCA3* but has also been seen in approximately 10% of infants with mutations in *SFTPC* [18, 21–30].

A more chronic clinical presentation has been seen in children with mutations in *SFTPC* as well as *ABCA3* [17, 31–37]. Some present in the newborn period with milder respiratory dysfunction that is ascribed to transient tachypnea of the newborn or congenital pneumonia, while those presenting beyond the newborn period develop gradual onset of respiratory insufficiency, hypoxemia, failure to thrive and interstitial lung disease on chest radiographs. While some cases have had a history of an associated viral illness or chronic aspiration, these features have not consistently been elicited, suggesting that other genetic or environmental modifiers influence the presentation of disease.

Histopathology and Ultrastructure

Diverse descriptions of the histopathology associated with surfactant metabolic disorders have been reported, regardless of the underlying molecular disruption, including alveolar proteinosis, interstitial pneumonitis, or chronic pneumonitis of infancy [13, 19, 20, 38]. A few histopathologic features common to the currently known disorders of surfactant metabolism, however, suggest a disorder of alveolar homeostasis that warrants more directed evaluation. These features include alveolar type II cell hyperplasia, interstitial thickening and/or fibrosis, and areas of alveolar proteinosis [39].

Ultrastructural hallmarks using electron microscopy provide an important branch point in directing a more focused genetic evaluation. Disorganized or unrecognizable lamellar bodies and an accumulation of abnormal-appearing multivesicular bodies suggest SP-B deficiency [40], while small lamellar bodies with electron-dense inclusions suggest ABCA3 deficiency [18, 41]. In patients with mutations in *SFTPC*, disorganized lamellar bodies may occasionally be seen, along with aggregates of small vesicles with electron-dense cores [33, 34, 36].

Molecular Genetics and Epidemiology

Surfactant Protein B

SP-B deficiency is an autosomal recessive disorder that occurs in approximately 1 per million live births and has been recognized in diverse racial and ethnic groups [42]. The SP-B gene *(SFTPB)* is approximately 10 kilobases (kb) in length, has been localized to human chromosome 2 and encodes a 381 amino acid proprotein that is glycosylated and undergoes a series of proteolytic cleavages to yield the 79 amino acid hydrophobic mature SP-B protein [19, 43–45]. Over 30 recessive loss-of-function mutations in *SFTPB* that result in partial to complete absence of SP-B protein have been identified [17, 21–25, 46–49]. The most common mutation, a GAA substitution for C in codon 121, the '121ins2' mutation, is associated with approximately 70% of the cases of SP-B deficiency and has a carrier frequency of 1 per 1,000 individuals [50]. To

date, no spontaneous mutations have been identified; all have been inherited.

The absence of SP-B leads to abnormal surfactant composition that consists of a decrease in the proportion of phosphatidylglycerol and the presence of a 6–12 kDa incompletely processed proSP-C peptide that inhibits surfactant function in vitro [13, 51–53]. The absence of SP-B and the presence of this unprocessed proSP-C likely cause significant surfactant dysfunction that contributes to the clinical syndrome.

The threshold of mature SP-B expression required for normal surfactant function in humans is unknown. Murine models that conditionally express SP-B have demonstrated that respiratory failure develops when SP-B expression is approximately 25% of normal values [54, 55]. On the other hand, heterozygous siblings and parents of SP-B-deficient infants whose SP-B expression is presumably 50% of normal have normal pulmonary function, and have not exhibited any evidence of respiratory illness [56]. Thus, there appears to be a limited margin of SP-B expression that permits normal surfactant function and any environmental or developmental condition that disrupts SP-B expression may result in significant respiratory dysfunction in genetically susceptible individuals.

Surfactant Protein C

The frequency of disease due to dominant mutations in *SFTPC* in the population is unknown. The 3.5-kb SP-C gene *(SFTPC)*, localized to human chromosome 8, encodes a 191 or 197 amino acid proprotein that is palmitoylated and undergoes a series of proteolytic cleavages to yield the 35 amino acid hydrophobic mature SP-C protein [2, 19, 57]. Over 35 dominantly expressed mutations in *SFTPC* have been identified in association with acute and chronic lung disease in patients ranging in age from newborn to adult [17, 26, 31–34, 36]. Approximately 55% of these mutations arise spontaneously and result in sporadic disease while the remainder are inherited. The most common mutation is a threonine substitution for isoleucine in codon 73 (I73T) and is associated with 25% of the cases of SP-C-associated disease. The I73T mutation has been identified in both sporadic as well as inherited disease [35].

Mutations in *SFTPC* result in production of misfolded proSP-C that accumulates within cellular secretory pathways (endoplasmic reticulum and Golgi) in the alveolar type II cell resulting in activation of cell stress responses and subsequent cellular injury and apoptosis [57–59]. A complete review of SP-C disorders and protein conformational disease can be found in Beers and Mulugeta [57]. In limited studies, surfactant composition and function appear to be mutation-dependent, although the exact mechanisms of these alterations are unknown, and it is therefore unclear whether the presence of proSP-C, the absence of mature SP-B or the absence of mature SP-C impairs surfactant function, and furthermore it is unclear if this altered surfactant function contributes to the pathogenesis of symptomatic disease [33, 34].

In contrast to SP-B deficiency, the clinical presentations and outcomes of lung disease associated with *SFTPC* mutations are significantly more variable, even within families known to carry a mutation, with some patients ultimately succumbing or requiring lung transplantation, some persisting with respiratory insufficiency and others improving to the point that they no longer require supplemental oxygen [28, 32, 33, 35]. Still other individuals are asymptomatic. This variability in severity and course of the disease is not mutation-specific and also precludes accurate assessment of prognosis for an individual.

ABCA3

The frequency of disease due to recessive mutations in *ABCA3* in the population is unknown although initial studies suggest that ABCA3 deficiency may be the most common of these disorders of surfactant homeostasis [18, 29, 30]. ABCA3 is one of a large family of ATP-binding cassette (ABC) transporters that has been localized to the limiting membrane of lamellar bodies in alveolar type II cells [60, 61]. The 80-kb ABCA3 gene *(ABCA3)* is located on chromosome 16 and encodes a 1704 amino acid protein that is most highly expressed in lung tissue, but is also expressed in the heart, brain, pancreas, kidney and platelets [62–64]. Over 70 recessive mutations in *ABCA3* have been identified in association with lethal RDS in newborns and with chronic respiratory insufficiency in children. One variant, a substitution of valine for glutamic acid in codon 292 (E292V), has been found in association with another *ABCA3* mutation in children with chronic respiratory insufficiency, and was found in approximately 7% of a cohort of infants ≥33 weeks' gestation with RDS, suggesting that this variant may contribute to the risk or severity of respiratory disease in susceptible individuals [37, 65].

While the exact role of ABCA3 in surfactant metabolism remains unknown, surfactant isolated from bronchoalveolar lavage fluid of ABCA3-deficient infants had significantly reduced amounts of PC and a markedly

reduced function, suggesting that ABCA3 mediates PC transport into lamellar bodies [29]. Furthermore, absence of ABCA3 function may affect SP-B and SP-C trafficking [30]. Little is known about the normal cell biology and intracellular processing of ABCA3, but different classes of mutations that result in absence, misrouting or altered function of ABCA3 protein are likely to lead to differences in clinical expression of disease [66, 67].

Evaluation for Inherited Disorders of Surfactant Metabolism

Because there is significant overlap in the clinical presentation of these inherited disorders of surfactant metabolism, a combined genetic and tissue-based approach to diagnosis is warranted. Consultation with selected centers experienced in evaluation of these infants is also important to efficiently develop an approach for management and discussions with families. Term newborns with unexplained RDS and who require mechanical ventilation for more than 5–7 days without apparent sign of improvement should be considered for evaluation, sooner if ECMO is required or if there is a family history of lung disease. This evaluation should be done promptly so that lung transplantation can be considered if one of these disorders is present. Older children with lung disease without a clearly defined mechanism should also be evaluated, since SP-C-associated disease and ABCA3 deficiency may present at older ages with chronic respiratory insufficiency.

The initial approach to evaluation centers on genetic diagnosis using DNA extracted from whole blood or buccal swabs, much of which can be done in clinical genetic laboratories. Concurrent analyses for the 121ins2, I73T and E292V mutations in *SFTPB, SFTPC* and *ABCA3* respectively provide the initial screens which must be confirmed with direct sequencing if present. In the absence of identification of one of the common mutations, the more labor intensive amplification and direct sequencing of the entire gene is necessary. The small sizes of *SFTPB* and *SFTPC* permit sequencing to be accomplished in a timely fashion for clinical decision-making, but sequencing the significantly larger *ABCA3* is expensive and time-consuming, and results may not be available in sufficient time for clinical guidance. Thus, while sequencing studies are underway, a lung biopsy, while significantly more invasive, should be seriously considered because it can distinguish a disorder of surfactant metabolism from other structural causes of lung disease such as alveolar capillary dysplasia. At the time of biopsy, tissue should be processed for electron microscopy to evaluate lamellar body morphology, and tissue should also be frozen in liquid nitrogen to be preserved for future molecular and proteomic analyses as new mechanisms of lung disease are identified [68].

The availability of molecular diagnosis also permits genetic counseling for affected families in order to discuss the risks for future pregnancies and to convey the risks for other family members. In families in which a mutation has been previously identified, antenatal diagnosis can be established by molecular assays of DNA from chorionic villus biopsy or amniocytes and permits advanced planning of a therapeutic regimen.

Treatment of Inherited Disorders of Surfactant Metabolism

No specific treatment for any of these disorders is currently available. SP-B-deficient infants are extremely ill and conventional neonatal intensive care interventions can maintain extrapulmonary organ function for a limited time (weeks to months). Replacement of SP-B with commercially available surfactants provides only transient improvement in gas exchange and is largely ineffective [69]. No systematic trial of surfactant replacement has been attempted for children with mutations in *SFTPC* or *ABCA3*. Hydroxychloroquine and corticosteroids may provide some improvement in patients with interstitial lung disease due to mutations in *SFTPC* or *ABCA3* but have not been systematically evaluated [70].

Lung transplantation has been successful in the short term for infants and children with refractory and progressive respiratory failure due to mutations in *SFTPB, SFTPC* or *ABCA3*. The significant challenges of transferring a critically ill child to a pediatric lung transplant center, a pretransplant mortality rate of up to 30% and a 40–50% 5-year survival rate have prompted approximately half the families of affected children to decline transplantation and choose compassionate care [38, 71]. Of the infants undergoing transplantation for disorders of surfactant metabolism, the pulmonary and developmental outcomes have been similar to those of children who have undergone lung transplantation for other reasons, suggesting that these surfactant-associated genes do not have clinically detectable extrapulmonary function.

Table 1. Comparison of surfactant deficiency syndromes

	SP-B deficiency	SP-C disease	ABCA3 deficiency
Age of onset	Birth	Birth–adulthood	Birth–childhood
Inheritance	Recessive	Dominant/sporadic	Recessive
Mechanism	Loss of function	Gain of function or dominant negative	Loss of function
Natural history	Lethal	Variable	Often lethal, may be chronic
Diagnosis			
Biochemical (tracheal aspirate)	Absence of SP-B and presence of aberrant proSP-C	None	None
Genetic (DNA)	Sequence *SFTPB*	Sequence *SFTPC*	Sequence *ABCA3*
Ultrastructural (lung biopsy – EM)	Disorganized lamellar bodies	May have cytoplasmic dense aggregates	Small, dense lamellar bodies
Treatment	Lung transplantation or compassionate care	Supportive care, lung transplantation if progressing	Consider lung transplantation

Future Directions

Disease due to mutations in *SFTPB*, *SFTPC* or *ABCA3* represents three distinct mechanisms that lead to pulmonary epithelial cell and surfactant dysfunction. A comparison of these disorders is summarized in table 1. The complexities of the surfactant metabolic cycle suggest that additional inherited disorders representing unique mechanisms of disease will be identified. Furthermore, these disorders suggest candidate genes in which variants may contribute to the pathogenesis and variable outcomes of respiratory disease in newborns.

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