The genetics of interstitial lung diseases due to surfactant dysfunction disorders in children

Matthias Griese

Pediatric Pneumology, Childrens’ Hospital of the Ludwig-Maximilians-University, Munich

Corresponding author:
Prof. Dr. M. Griese
Dr. von Haunersches Kinderspital
Lindwurmstr. 4
D-80337 Munich
Phone ++49-89-5160-7870
Fax ++49-89-5160-7871
E-mail: matthias.griese@med.uni-muenchen.de

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Diffuse lung diseases in children comprise a large and heterogeneous group of respiratory disorders. Some entities are characteristic for neonates and infants, whereas others occur at all ages. Although in the majority of the diseases, especially in those characteristic for infants, a genetic cause is suspected, this has been shown only for a small fraction of the disorders related to surfactant dysfunction. From a practical point of view it is important to provide an algorithm which allows a rapid and relatively safe assessment of these diseases.

Clinical presentation

The conditions present mostly during times of particular stress of the respiratory system. This is

(A) After birth or during infections, or

(B) Outside these times, insidiously and challenged particularly with exercise.

Presentation during the neonatal period (A) is rather homogenous as respiratory distress syndrome of unknown cause in a mature neonate. Some neonates with milder lung diseases present like wet lung syndrome (transient tachypnoea), improving after some days with the need of chronic oxygen supply and tachypnoea and retractions. During a viral infection symptoms are worsening. (B) Those children with insidious deterioration manifest beyond the neonatal period. Their lung disease is mostly restrictive and the chest radiographs may have diffuse infiltrates. Affected children usually present with dry cough, dyspnoea, tachypnoea and hypoxemia. The resting respiratory rate is elevated, crackles may be heard in the basal segments of the lungs, finger clubbing and growth failure occurs.

Pulmonary surfactant components with relevance to diagnosis of surfactant disorders

The alveolus of the human lung is kept open for gas exchange during expiration. Many components of the surfactant system are important for this, including the surfactant (surface active agent) film which contains among other components many phospholipids, neutral lipids, the surfactant specific proteins SP-A, SP-B, SP-C and SP-D (1). Surfactant is synthesized and secreted by alveolar type II epithelial cells. After the synthesis in the endoplasmic reticulum,
surfactant components are transferred to the Golgi apparatus where they are being modified. Further transport steps via multivesicular bodies facilitate the assembly of surfactant in the composite body, which is the immediate precursor of the lamellar body. The phospholipid transporter ABCA3 is located in the membrane of the lamellar bodies and responsible for the translocation of the lipids into the lamellar bodies. The assembled lamellar bodies are secreted into the alveoli by regulated exocytosis (2;3).

Up to date, for routine diagnosis of all these components, three are of relevance for the diagnosis of diffuse lung diseases: SP-B, SP-C and ABCA3. Analysis can be done on the genetic level, looking for mutations in the 3 genes encoding the proteins or directly the protein levels.

**Diagnostics**

**Histology:** the presence and amount of SP-B and SP-C in the lungs can be assessed on tissue slices after staining with anti-SP-B or anti-SP-C antibodies. Lung biopsy specimens are obtained by open or thoracoscopic lung biopsy; the time point for this procedure must be selected very carefully, to be of help for the infant. In neonates the procedures are often done to determine the ultimate prognosis and the direction of further care. It is important not to perform the procedure too late, because than the harm due to the procedure may be critical for the course of the disease. On the other hand, the clinical course should be somewhat progressive despite all efforts.

**Biochemistry:** Alveolar SP-B and SP-C amounts can be quantified in bronchoalveolar lavage or tracheal aspirate samples. Great care must be taken not to sample the alveolar space after exogenous surfactant application, as all natural components contain SP-B and SP-C which cannot be differentiated from endogenous material. Very low or absent proteins indicate primary or secondary problems, associated with disturbances of the surfactant system. The frequency of such findings in neonates is relatively low (4). Of specific diagnostic interest in
SP-B deficiency is the presence of aberrant pro-SP-C processing intermediates in the alveolar space in some, but not all forms of SP-B deficiency. Low SP-C and SP-B may be find in patients with ABCA3 deficiency.

**Genetics:** Definite and specific diagnosis of SP-B deficiency is obtained from sequencing the involved genes. Human SP-B is encoded on the short arm of chromosome 2 and spans about 10 kilobases. The SP-B gene contains 11 exons, with the 11th exon being untranslated, and is transcribed into a ~2000 base pair mRNA. Current costs of analysis are around 600 Euro in Germany. The SFTPC gene is smaller and costs for analysis are about Euro 250. The gene for the ABCA3 transporter is much larger and thus more costly to sequence.

**Surfactant protein B deficiency in human lung disease**

SP-B deficiency was the first reported genetic cause of lethal RDS in infants(5). Genetic analysis revealed that the affected infant was homozygous for a frameshift mutation in codon 121 (termed 121ins2 mutation) in the SP-B gene that caused the lack of SP-B(6;7). The 121ins2 loss-of-function mutation is suggested to account for up to two-thirds of the mutant alleles identified in the SP-B locus. At least 27 loss of function mutations were found in the SP-B gene that resulted in neonatal RDS. Since most of these mutations are unique to the index family the sensitivity of genetic counselling is limited. Although SP-B-deficient neonates usually present with respiratory failure within the first 24 to 48 hours of life, the clinical course can sometimes be more prolonged since affected infants may show initially mild symptoms and do not require ventilation or further medical support for some time. Since the lung disease is rapidly progressive lung transplantation is suggested as the only effective treatment(8).

There is evidence that SP-B gene mutations may also result in milder pulmonary phenotypes. In 4 patients in the literature milder courses have been described. A male neonate with the mutation (G135S) presented at the first hours of life with respiratory distress and required mechanical ventilation. The patient developed persistent pulmonary hypertension and was
treated with continuous oxygen therapy. The patient developed chronic lung disease and was alive at 3 years of age requiring oxygen supplementation(9). An infant with a 121ins2/R236C compound heterozygous mutation developed tachypnea shortly after birth and bilateral pneumonitis on chest radiograms. The infant received ECMO, could then be weaned and remained oxygen dependent for 9 months. The patient was lung transplanted, but unfortunately died at the age of 9.5 months of unexplained sudden respiratory and cardiac arrest. Two unrelated children were homozygous for a mutation in exon 5 (479G→T). The first patient was mature, developed RDS at 8 h of age, developed a spontaneous pneumothorax at 18 h of age. After extubation he remained tachypneic and required further oxygen supplementation. He underwent bilateral lung transplantation at 4 months of age. The second patient with this mutation also developed RDS shortly after birth, but did not require mechanical ventilation. At 6 months of age she was discharged with home oxygen therapy and has been stable for several years with a persistent oxygen requirement (10).

**Surfactant protein C deficiency in human lung disease**

There is increasing body of evidence linking SP-C deficiency to various types of acute and chronic lung disease in humans(11;12). While SP-B deficiency generally results in fatal neonatal lung disease, abnormal expressions of SP-C have been shown to present as a much more variable phenotype. SP-C mutations can cause interstitial lung disease in neonates and children. Different disease-causing mutations with sporadic or autosomal dominant inheritance have been described. Similar as in animal models where a strong dependence on the genetic background of the animals as found, the phenotype of patients bearing SP-C mutations has a large interindividual variance. Mutations in the distal region of proSP-C (F94-I197; BRICHOS), e.g. hSP-CΔexon4[49] and hSP-CL188Q[50], form intracellular toxic aggregates. These mutations may result in a more severe pulmonary phenotype, mostly associated with death in the neonatal period. In contrast the non-BRICHOS mutants, hSP-CE66K or hSP-
CI73T, lead to aberrant proSP-C accumulation which can be found in bronchoalveolar fluid and may result in a milder clinical outcome (13).

**ABC-A3 transporter mutations human lung disease**

The ATP-binding cassette (ABC) transporters are multidomain integral membrane proteins that utilise the energy of ATP hydrolysis to translocate solutes across cellular membranes. ABCA3 is highly expressed in the lung at the limiting membranes of lamellar bodies in type II epithelial cells.

Mutations in the ABCA3 gene have been identified as an important cause of acute RDS in newborn infants (14). In 16 of 21 patients (76 percent) in whom SP-C and SP-B mutations were excluded, various kinds of mutations were found including nonsense, frameshift and splice sites mutations. The ABCA3 gene alterations were inherited in an autosomal recessive manner.

In addition other mutations were identified as causes for underlying chronic interstitial lung diseases in children living beyond the neonatal period.

The histological findings were typical for desquamative interstitial lung disease and neonatal alveolar proteinosis. Important findings were abnormally small lamellar bodies identified by electron microscopy. A defective ABCA3-dependent transport of lamellar-body associated lipid components will result in abnormal alveolar surfactant composition, i.e. reduced concentrations of SP-C.

**Summary**

It is important to know that the three proteins, surfactant protein B (SP-B), surfactant protein C (SP-C) and ABCA3 are the critical components that need to be assessed during the diagnosis of diffuse lung diseases suspected to be related to inborn disorders of the pulmonary surfactant system. Further information can be found at [www.ped-pneumology.de](http://www.ped-pneumology.de).
Reference List


(2) Rooney SA. Function of type II cell lamellar inclusions in surfactant production. 1976;147-52.


