Genetic Disorders of Surfactant Deficiency and Neonatal Lung Disease

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Abstract: Pulmonary surfactant is a heterogeneous combination of lipids and proteins, which prevents alveolar collapse at the end of expiration cycle by decreasing the alveolar surface tension at the air-liquid interface. At birth, the expression of surfactant is very important for normal lung function and it is strictly correlated to gestational age. The best known genetic mutations associated with the onset of respiratory distress in preterm and full-term newborns and with interstitial lung disease later in childhood are those involving the phospholipid transporter (ABCA3) or surfactant proteins C and B (SP-C and SP-B) genes. In particular, mutations in the SP-B gene induce respiratory distress in neonatal period, while alterations on gene encoding for SP-C are commonly associated with diffuse lung disease in children or in adults. Both clinical phenotypes are present, if genetic mutations interest even the phospholipid transporter ABCA3 ambiguity in the sentence. Interstitial lung disease in children (chILD) is defined as a mixed category of mainly chronic and rare respiratory disorders with increased mortality and morbidity. Although genetic alterations are mainly responsible for the onset of these diseases, however, there are also other pathogenic factors that contribute to increase the severity of clinical presentation. In this review, we analyze all clinical features of these rare pulmonary diseases in neonatal and in pediatric age.

Keywords: ABCA3, genetics, granulocyte macrophage colony-stimulating factor (GM-CSF), neonatal lung disease, pediatric interstitial lung disease, surfactant proteins C and B, surfactant proteins.

1. INTRODUCTION

Pulmonary surfactant is a heterogeneous combination of lipids and proteins, which prevents alveolar collapse at the end of expiration cycle by decreasing the alveolar surface tension at the air-liquid interface. The main components are phospholipids, in particular, phosphatidylglycerol (PG) and phosphatidylcholine (PC) secreted in late gestation by type II alveolar cells, and four surfactant proteins (SPs) represented by SP-A, SP-B, SP-C, and SP-D. They originate from type II alveolar cells and have a key role in the synthesis of pulmonary surfactant.

In general, SP-A and SP-D are hydrophilic proteins included in the collectin family, which favor both epithelium homeostasis and innate and adaptive immunity of the lung. SP-B and SP-C are small hydrophobic apolipoproteins, synthesized by pulmonary alveolar type II cells and secreted into the alveolar lumen; they play an important role to maintain normal biophysical functions packing surfactant phospholipids membrane in the lamellar bodies, which are specialized intracellular lysosomally derived storage organelles [1]. Once secreted, the complete surfactant extends over the lung parenchyma, in particular in alveolar walls, decreasing surface tension at the air-liquid interface and avoiding pulmonary collapse at the end of every expiration. ABCA3 is a membrane protein belonging to a group of Adenosine Triphosphate (ATP)-binding cassette proteins expressed in the lung. It is found into the limiting membrane of lamellar bodies and plays an important role for lamellar bodies biosynthesis by importing surfactant phospholipids, including PC and PG, into the lamellar bodies. The main functions of surfactant are: 1) decreasing alveolar surface tension at the air-liquid interface thus avoiding pulmonary collapse at the end of every expiratory cycle; 2) preventing the dissemination of pathogens into the lung; 3) modulating both innate and adaptive immune responses [2]. At birth, the expression of these proteins is strictly correlated to gestational age resulting crucial for normal lung function. In fact, in preterm newborn the onset of acute Respiratory Distress Syndrome (RDS) is primarily due to the insufficiency of pulmonary surfactant production. Surfactant dysfunctions diseases, including genetic disorders disrupting normal surfactant metabolism, have been identified currently as one of the most important causes of respiratory failure in the neonatal or pediatric period [3].
fact, other respiratory diseases, as bronchial asthma or cystic fibrosis, represent a common cause of respiratory failure in pediatric patients. In particular, patients with asthma are predisposed to developing exacerbations, leading to respiratory failure and the natural history of cystic fibrosis consisting of early and persistent infections and progressive airways obstruction ultimate in respiratory failure [4, 5].

Even if rare, these particular clinical conditions related to surfactant deficiency, ranging from acute respiratory distress syndrome in preterm and full-term newborn to interstitial and diffuse lung disease in children and adults, represent a high risk of morbidity and mortality. In the pediatric population, respiratory disorders as pediatric acute respiratory distress syndrome or interstitial lung disease, are commonly associated with genetic mutations involving surfactant proteins (mainly SP-B, SP-C and ABCA3) as described by several studies. Usually, they are characterized by impaired alveolar surface tension and pulmonary gas exchanges, with different clinical presentation or histological features. To date, the term Interstitial Lung Disease (ILD) in children (chILD) includes a rare group of respiratory disorders characterized by different pathogenesis and high mortality and morbidity [6].

2. SURFACTANT BIOGENESIS

The components of pulmonary surfactant are commonly represented by lipids (90% of weight) and proteins (10% of weight) which cover the alveolar surface. The lipid portion, mainly PC or dipalmitoyl-PC, determines pulmonary surfactant properties which are responsible for the maintenance of normal surface tension. Other phospholipids are represented at low quantity by PG, sphingomyelin, cholesterol, phosphatidylinositol, phosphatidylethanolamine and phosphatidylserine (Fig. 1). The composition of surfactant proteins (SPs) is represented by four proteins (SP-A, SP-B, SP-C, and SP-D), which are crucial in the definitive structure of surfactant favoring its successive function and metabolism in lung homeostasis (Fig. 2). SP-B is a hydrophobic apolipoprotein, formed by 79 amino acids, and it is encoded by one gene located on chromosome 2 (2p12-p11.2). It interacts superficially binding to phospholipid bilayers contributing to the package into the lamellar bodies of surfactant phospholipids. It plays an important role in reducing surface tension at the air-liquid interface and it has even some antimicrobial activities. It contributes to form tubular myelin, a particular substance composed of lipid-rich film localized into the alveolar surface, which acts during gas exchanges [1-7]. SP-C is another small hydrophobic apolipoprotein (35-aminoacids), encoded by a gene located on chromosome 8 (8p21). It is inserted into the phospholipid bilayer and its function is to maintain surfactant covering at the alveolar surface, and to increase surfactant phospholipid packaging into alveolar type II cells. Both SP-B and SP-C are generated by alveolar type II cells as precursor proteins, in particular, proSP-B and proSP-C, and then processed through proteolytic cleavages of their amino and carboxyl termini. By facilitating the inclusion of the surfactant phospholipids layer to the air-liquid-interface, pulmonary surface tension remains within the normal range. SP-A and SP-D are hydrophilic proteins, both members of the collectin family embracing 10% of the surfactant composition. Once secreted by lamellar bodies in the cytoplasm they are responsible for tubular myelin generation with phospholipidic components. They are expressed particularly in type II epithelial cells after lung maturation, and also in Clara and tracheal gland cells. They play a main role in the innate immune system against various infections, such as bacterial, fungal and viral pathogens including Mycobacterium tuberculosis, Salmonella,
Studies demonstrated their important role in lipid homeostasis reducing pulmonary inflammatory processes [8, 9].

ATP binding cassette number A3 (ABCA3) is a protein composed of 1704 amino acids containing more than 10 specific regions with 2 ATP-binding sites situated in pulmonary cells. Specifically, ABCA3 is synthesized and successively transported to the endoplasmatic endothelium and cytoplasm to package into the lamellar bodies. During gestational period, the expression of ABCA3 and other surfactant proteins change according to lung maturation, and when gestation is almost complete (within 37 weeks) late gestation alveolar type II cells are able to produce all surfactant proteins. During the last 3 months of gestation, lung modifies the components of the surfactant phospholipids portion, thereby increasing the amount of PG and PC and decreasing the amount of sphingomyelin. Genetic alterations and remodeling of surfactant homeostasis and biophysical properties produce surfactant deficiency responsible for some respiratory diseases including respiratory distress syndrome, lung proteinosis, interstitial lung diseases and chronic lung diseases in children and adult population [10].

3. GENETIC SP-B DISORDERS

SFTB is a gene located on chromosome 2 and contains 11 exons and 10 introns. Genetic SP-B disorders are represented by an autosomal recessive pattern involving both alleles in SFTB gene. More than 40 different mutations in the SFTB have been identified. Patients with heterozygous mutations in SFTPB are usually asymptomatic. In 1994, Nogee et al. showed the presence of a mutation-type frameshift, including the substitution of a nucleotide sequence, GAA, with a single nucleotide, C, on codon 121(121ins2) in exon 4, resulted in the absence of pro-SP-B and mature SP-B [11]. The most common genetic alterations consist of nonsense or missense mutations with a specific deletion involving exons 7 and 8 was reported in the last studies. All these genetic mutations result in the absence or dysfunctioning of SP-B and clinically determine acute and progressive respiratory insufficiency in full-term newborns, occasionally evolving in respiratory failure and death usually from 3 to 6 months of age [12]. In 1993, a case of genetic SP-B deficiency was firstly reported in a newborn with normal gestational age who developed acute respiratory distress after birth. The respiratory symptoms were progressive and radiographic findings showed pulmonary interstitial infiltrates as a consequence of surfactant deficiency. This patient had a fatal progressive respiratory failure after 5 months of birth, despite aggressive treatment by using corticosteroids, antibiotics, mechanical ventilation and external surfactant replacement. According to the lung biopsy findings, the suspected diagnosis was Congenital Alveolar Proteinosis (CAP). The family history remarked the presence of another infant with fatal respiratory distress at birth. Then, lung transplantation is currently the only effective treatment option for genetic SP-B disorders [13]. Generally, the histological diagnosis of CAP is associated with SP-B deficiency and consists of an increase of different substances as eosinophilic cells and lipidic material in the alveolar spaces. Rarely, this mutation causes infantile Desquamative Interstitial Pneumonitis (DIP). These histological characteristics are similar to acquired pulmonary alveolar proteinosis observed in older children or adults. This rare disorder is caused by mutation in subunits of the Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) receptor resulting in damage of macrophage function which interferes with catabolism or degradation of secreted surfactant. Other histopathologic features concerning SP-B mutations are the presence of hyperplastic alveolar epithelia with prominent type II cells and thickening of the alveolar walls and fibroblast growth. These features represent the main differences from GM-CSF anomalies which are characterized by a normal alveolar structure [14].

4. GENETIC SP-C DISORDERS

SFTPC is a gene located on chromosome 8 responsible for SP-C synthesis, a hydrophobic protein.
The most common genetic alterations of SFTPC gene are a missense mutation, involving the carboxyl terminus of proSP-C. It is a complex illness with an inherited autosomal dominant pattern with variable penetrance and variable expressivity [15]. SFTPC mutations are generally characterized by an exchange regarding specific amino acids (threonine and leucine) situated at site 73 (I73T) which are presented in more than 25% of pediatric population with a genetic surfactant disorder [16].

Some studies have demonstrated that higher severity of symptoms associated with SFTPC genetic disorders is related to different age of children. In fact, respiratory symptoms caused by SP-C deficiency can develop immediately after birth as neonatal respiratory distress or later in life [17].

In 2001, the first case of these genetic disorders was described in a full-term newborn with progressive respiratory distress after 6 weeks of birth. He presented respiratory insufficiency with tachypnea and progressive cyanosis. Radiographs suggested surfactant deficiency with increased interstitial findings.

Lung biopsy showed the presence of hyperplastic epithelial type II cells, myofibroblasts and lymphocytic cells in the interstitium of pulmonary wall. The family history also revealed an idiopathic pulmonary fibrosis of the patient’s mother, confirmed by histological exam demonstrating alveolar impairment. Genetic studies in both child and mother showed a mutation consisting of an exchange regarding adenine and guanine at a specific site of intron 4 inherited as autosomal dominant trait [18].

Some studies demonstrated even the association between SFTPC mutations and respiratory failure in full-term newborn and rarely diffuse lung disease in adults [19, 20].

Other studies performed on pediatric population with genetic SFTPC disorders with a radiographic diagnosis of interstitial pneumonia found that respiratory distress can be a variable and some viral infections including Respiratory Syncytial Virus (RSV), influenza A and influenza B, may represent an increased risk to develop more severe respiratory disorders [21]. The prognosis is extremely variable and in some cases, lung transplantation is needed [22, 23] According to the case reports described in literature, a variable diffuse alveolar damage, interstitial thickening with mild lymphocytic inflammation, granular alveolar proteinosis material with a cholesterol clefts, and hyperplastic type II cells represent the most common histological findings in children with respiratory disorders; otherwise, pulmonary fibrosis is the only typical histological pattern in adults [24]. In literature, asymptomatic children or adults with SPTC genetic mutations have been rarely described [25].

5. GENETIC SP-A AND SP-D DISORDERS

Some studies demonstrated that the deletion of SP-A and SP-D causes both an increased risk to develop bacterial and viral infections and alterations of surfactant function and lung homeostasis, expressed as defects in the re-uptake of secreted phospholipids by alveolar type II cells [8]. Other studies in vitro demonstrated the capacity of these surfactant proteins to interact with gram-negative and gram-positive bacteria through linking lipopolysaccharide and peptidoglycan, derived from Staphylococcus aureus, Streptococcus pneumoniae, Mycobacterium avium, Mycobacterium tuberculosis and Mycoplasma pneumoniae, resulting in growth bacterial inhibition. Even if specific genetic mutations have not been demonstrated, different polymorphisms involving SFTPA and SFTPD have been identified to be responsible for the increased risk of respiratory distress in full-term newborns, or bronchopulmonary dysplasia in premature patients, and finally of bronchiolitis due to respiratory syncytial virus infection in the first months of life. At the same time, SP-A and SP-D can even favor phagocytosis and agglutination by host cells avoiding specific fungal infections, including Histoplasma Capsulatum, Blastomyces dermatitidis, Cryptococcus neoformans, Pneumocystis carinii and Candida albicans. Furthermore, since SP-A and SP-D are located into the mucus film and alveolar walls they can even prevent viral infections through viral neutralization and consecutive agglutination and phagocytosis [26].

6. GENETIC ABCA3 DISORDERS

The transporting protein ABCA3 is encoded by a gene situated on chromosome 16 (16p13.3) [27, 28]. The main functions are binding and transporting ions, proteins, and surfactant phospholipids from the cytosol to the lamellar bodies, for the crucial activity of lung homeostasis [29]. More than 150 distinct mutations in the ABCA3 gene have been identified. The inheritance pattern is autosomal recessive and the more typical clinical phenotype is characterized by a respiratory failure with a variable severity in full-term newborn [30, 31]. In 2004, Shulein et al. analyzed 21 infants with severe neonatal respiratory distress indicating surfactant deficiency. They sequenced each of the most important coding exons of the ABCA3 in blood DNA by high-resolution light and electron microscopy. Histological findings included hyperplasia of alveolar type II cells, accumulation of alveolar macrophages in distal air spaces and interstitial thickening. Genetic analysis showed several missense polymorphism variants involving ABCA3 gene in all patients. In two of them, a single mutation was identified in one allele, but the clinical presentation was similar to other patients [32, 33] Furthermore, some typical modifications in lamellar bodies structure associated with a deficiency of surfactant B protein have been described by electron microscopy. The most common consequence due to genetic mutation of ABCA3 is the relative deficiency or inactivity of surfactant proteins and phospholipids activity [34]. In other studies performed in infants with severe respiratory distress and with chronic pulmonary interstitial disease, a common mutation characterized by a substitution of valine for glutamic acid in codon 292 (E292V) has been found [35, 36]. In some patients with a histological diagnosis of pulmonary alveolar proteinosis, if this genetic mutation is associated with heterozygous pattern clinical spectrum is less severe [37].
According to clinical reports, infants with RDS present more commonly E292V mutation, indicating that this specific genetic alteration represents a higher risk factor to develop RDS [38].

In a multicenter study, infants with diffuse lung disease underwent lung biopsy for genetic and histopathologic analysis. The authors found genetic mutations involving ABCA3 without other surfactant proteins defects associated mainly to pulmonary pattern of PAP [39, 40]. All patients died, suggesting that these ABCA3 alterations cause a marked and fatal reduction of surfactant, demonstrated by electron microscopy in alveolar cells due to increased surface tension. In these cases, lung transplantation is the only useful treatment option [32].

7. GENETIC NKX2-1 DISORDERS

NKX2-1, located on chromosome 14q13, is a gene composed of 3 exons and 2 introns codifying homeobox protein NKX2-1 [41]. This protein represents a transcriptional factor and it is important during early embryonic development for brain, lung and thyroid functions. In particular, in the lungs NKX2-1 plays a critical role in regulating transcription genes and the production of surfactant proteins [42]. More than 100 specific mutations involving NKX2-1 gene have been identified as a cause of brain-lung-thyroid syndrome, which included a group of clinical conditions affecting brain, lungs and thyroid gland [43, 44]. The most common features are benign hereditary chorea characterized by uncontrolled movements involving especially muscles of face and limbs, severe respiratory distress and hypothyroidism [45]. The inheritance pattern is autosomal dominant. The first case of lung disease resulting from NKX2-1 genetic alterations was reported in 1998 in a child with onset in neonatal age and later a progressive thyroid disease [46]. It has been demonstrated that NKX2.1 mutations determine a highly variable phenotype ranging from involvement of a single organ to an association of two or even three organs (lung, thyroid, brain). Monti et al. showed a novel nonsense mutation of the NKX2.1 gene (Y130X) consisting of a single base exchange in intron 2 (c. 463 + 41C > T), causing only neurological symptoms and congenital hypothyroidism without respiratory distress in a full-term newborn [41]. On the contrary, Salerno et al. described a G142X nonsense mutation in young patient with respiratory distress and congenital hypothyroidism without neurological impairment [47].

8. GRANULOCYTE MACROPHAGE COLONY-STIMULATING FACTOR (GM-CSF) RECEPTORS DEFECTS

GM-CSF belongs to a hematopoietic cell growth factor family and it plays a crucial role particularly on innate immunity, acting on many cell lineages proliferation such as neutrophils, macrophage precursors, dendritic cells, erythrocyte progenitors and megakaryocytes.

To date, the association of PAP and genetic defects of GM-CSF is rarely described and the natural outcome of this disorder remains unknown [48].

Mutations in genes encoding both subunits of GM-CSF receptor (CSF2RA and CSF2RB) lead to the PAP pattern because it alters the correct surfactant clearance by causing an ineffective function of the receptor [49].

PAP is a rare pulmonary disease characterized by alveolar accumulation of surfactant. The most common genetic defects include those involving surfactant proteins and GM-CSF receptor. The pathogenesis can be also secondary to toxic inhalation or hematological disease or autoimmune impairment. Autoimmune alveolar proteinosis represent 90% of cases. There are no typical clinical and radiographic signs, though high resolution computed tomography shows a typical “crazy paving” pattern [50].

The definitive diagnosis is established by broncho-alveolar lavage, which represents even an effective therapeutic procedure. Novel therapies targeting alveolar macrophages as a recombinant GM-CSF therapy or anti GM-CSF antibodies are being studied and represent the goal of near future therapy.

Genetic studies concerning CSF2RB associated with PAP showed a missense mutation in homozygous pattern in four infants with typical clinical symptoms and signs of PAP [51, 52].

9. RESPIRATORY DISTRESS SYNDROME (RDS)

RDS is an important cause of respiratory failure in preterm or full-term newborns and it may be associated with abnormalities of surfactant [53]. In older children, Pediatric Acute Respiratory Distress Syndrome (PARDS) represents a cause of deaths in critically ill children admitted to the pediatric or neonatal intensive care unit. The incidence of PARDS is different than adults and it is relatively rare, in fact it is often under-diagnosed due to the absence of specific guidelines. Clinical features are variable and are characterized by acute or chronic pulmonary inflammation with diffuse alveolar damage and increased vascular permeability, with a progressive hypoxic respiratory failure. Chest radiographs show infiltrates due to diffuse atelectasis and destruction of the alveolar wall [54]. The pathophysiology of RDS in pediatric population is complex and multifactorial. Many pathological conditions contribute to develop respiratory distress by direct damage of lungs including pneumonia, pulmonary contusion or inhalation of gastric contents or other clinical conditions, such as sepsis, blood transfusions and severe trauma [55]. In preterm newborns, RDS is caused by a deficit in surfactant production, suggesting that other factors such as infectious diseases, genetic abnormalities or environmental factors may influence lung development, surfactant biogenesis and effective function of pulmonary system (Table 1) [56].

10. INTERSTITIAL LUNG DISEASE IN CHILDREN (chILD)

Interstitial lung disease in pediatric population, also known as chILD, represents a particular group of respiratory diseases, usually sporadic but with a high risk of mortality and morbidity. Clinical features are represented by lung
injury with diffuse pulmonary infiltrates and a restrictive respiratory function pattern with a progressive reduction of gas exchanges [57]. Lung injury is named “interstitial” because it involves airway interstitial space. To date, chILD guidelines are similar to ILD in adult patients although some differences exist regarding outcome and treatment [58]. In fact, in adults, the incidence of desquamative interstitial pneumonia is usually related to smoke abuse, while in children genetic disorders are more common, and often associated to poor prognosis, if SP-C and ABCA3 mutations are present. To date, it is known that the most common and severe interstitial disease in adults is idiopathic pulmonary fibrosis; differently, other ILD forms are unique to pediatric age [59]. Therefore, there are new appropriate guidelines and classification systems for several childhood disorders recently written by multi-institutional ChILD Research Cooperative in children aged from 0 to 2 years because signs and symptoms are often atypical and the diagnosis may be delayed [60]. Different clinical conditions of diffuse lung disease presenting features similar to chILD are cystic fibrosis, immunodeficiencies, recurrent pneumonia and congenital heart disease. In fact, in all of these diseases, respiratory symptoms as chronic cough, exercise intolerance, progressive tachypnea and consequently hypoxic respiratory insufficiency and radiographs signs of diffuse lung infiltrates are present [61]. At least 3 of these characteristics are required. The diagnostic tests depend on the severity of the case. In a stable child, bronchoscopy with successive histological and genetic exams will be diagnostic, and sometimes can replace lung biopsy. However, in ill patients, this invasive procedure should be performed soon to confirm the diagnosis. Chest Computed Tomography (CT) and radiographs play a crucial role to establish extension and distribution of lung parenchymal abnormalities. Typical radiographic features are represented by ground glass opacity, diffuse consolidation, septal thickening and cystic formations. Bronchoalveolar Lavage (BAL) is indicated for infections and represents an important diagnostic procedure to identify diffuse alveolar disease by histological researches with BAL cellularity as the presence of hemosiderin-laden and hemorrhage process or lipid-laden macrophages as food aspiration [62]. According to the guidelines, the gold standard for chILD diagnosis remains lung biopsy.

Pulmonary transplantation is considered as a unique opportunity for the end-stage of lung disease and its outcome is comparable to lung transplants for other conditions including cystic fibrosis, vascular or cardiac diseases. For these reasons, the prognosis of ILD is highly variable as demonstrated by clinical studies in literature [63]. A retrospective study from 2000 to 2015 in term and preterm newborns presenting severe respiratory distress syndrome and also in patients with ILD, evaluated more than 300 molecular exams concerning all surfactant proteins genetic alterations (ABCA3, SP-B and SP-C). They studied all parents and siblings of affected infants by sequencing ABCA3, SFTPC and SFTPB on genomic DNA and then correlated these features with histopathological and immunohistochemical findings. They found genetically significant mutations in SFTPB, SFTPC and ABCA3 in 71% of subjects who clinically presented a severe and unexplained RDS and also in 50% of children with radiographic and histological diagnosis of ILD [64]. Furthermore, a higher mortality was associated with homozygous alterations in SFTPB and ABCA3.

According to studies published last year, surfactant dysfunctions were identified in a significant number of full term or preterm newborns and also in children who developed a severe unexplained respiratory failure [65]. Even if therapeutic protocol is only supportive and therapeutic strategies are generally not definitive, the specific genetic analysis can be useful to define a correct and definitive diagnosis (Table 1) and can represent the goal of future strategies.

11. DIAGNOSTIC APPROACH

The evaluation of clinical features, such as hypoxemia (in waking or sleeping), pulmonary hypertension, a slowdown in growth, immunodeficiency and positive family history for similar pulmonary diseases is useful to establish the severity of disease. Genetic tests should be performed in clinical suspicion of these particular respiratory diseases, as described in this review, to prevent a more invasive procedure as pulmonary biopsy and are performed by specialized or accredited labs as shown in Table 2.

As described, the main genes involved in surfactant production disorders include SP-B, SP-C, ABCA3 and NNX2.1. In particular, in case of a full-term newborn suffering from acute respiratory distress rapidly progressive and unexplained by other causes and not responding to conventional therapies, it is highly recommended to perform genetic analysis to exclude SFTB or ABCA3 mutations.

Table 1. Classification of main genetic surfactant disorders in pediatric population according to the age.

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<td>• SPTFB genetic mutations</td>
<td>• SPFTB genetic mutations with PAP histologic pattern</td>
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<tr>
<td>• ABCA3 (E292V mutation)</td>
<td>• SPFTC genetic mutations (I73T mutation) associated with CPI dominant histologic pattern and rarely with DIP and NSIP</td>
</tr>
<tr>
<td>• NKX2.1/TTF1</td>
<td>• ABCA3 genetic mutations (other variants) associated with PAP variant dominant pattern and also with CPI, DIP, NSIP</td>
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<td>• GM-CSF receptor: (CSF2RB mutation)</td>
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Abbreviations: SPTFB: surfactant proteins B gene; SPTFC: surfactant proteins C gene; PAP = pulmonary alveolar proteinosis; CPI = chronic pneumonitis of infancy; DIP = desquamative cell interstitial pneumonia; NSIP = nonspecific interstitial pneumonia; ABCA3 = ATP binding cassette number A3; NKX2.1/TTF1 = NKX2.1 / Tiroid Transcriptor Factor; GM-CSF: granulocyte macrophage colony-stimulating factor
Similarly, in an older child with interstitial lung disease and positive family history for chronic respiratory distress or infant respiratory failure, genetic analysis for SFTPC or ABCA3 is useful for correct diagnosis [66, 67]. In a recent retrospective study, analyzing children with respiratory distress in a period of 5 years, authors showed that only in 7.5% of patients a definitive diagnosis was found. Moreover, the high costs associated with these investigations and the long execution time are limitations, especially in rapidly worsening cases. In addition to the more common mutations involving surfactant proteins, new genetic alterations associated with other ILD in pediatric population have been identified, as mutations of FOXF1 gene, particularly deletions, are associated with alveolar capillary dysplasia with misalignment of veins lung, which should be suspected in a newborn with severe pulmonary hypertension not responding to conventional therapies [68]. Other rare x-linked mutations, involving filamin A, are responsible for diffuse lung disease associated with brain alterations on Magnetic Resonance as periventricular nodular heterotopia. Many studies described a mutation in STAT3 to be responsible for autoimmunity disorders or lymphocytic interstitial pneumonia associated with genetic alterations on LRBA, a gene encoding for an important protein for T reg cells. These mutations are frequent in variable common immunodeficiency. Over the last few years, the identification of new mutations involving genes responsible for surfactant production or metabolism has increased the frequency of surfactant dysfunction or other lung interstitial diseases diagnosis, avoiding sometimes invasive procedures as lung biopsy [69, 70].

The radiological features permit to establish the severity of lung disease, but not the outcome or therapy of disease. Early Echocardiographic evaluation is useful to exclude congenital heart disease that can simulate lung failure and to identify precociously the development of pulmonary hypertension. Respiratory function tests in infant or young children are not specific for the diagnosis and performing them is even particularly complex. Even in older children, there are no standardized reference models although a restrictive pattern has been found to be frequent with a reduction of both forced vital capacity and forced expiratory volume in 1 second, with a normal or increased Tiffeneau index. Usually, a restrictive pattern is more frequently associated with chILD, although obstructive patterns may be present [68]. To improve the diagnostic flow chart, both imaging findings and laboratory tests are useful. In a retrospective study conducted in 48 children with diffuse lung disease (DLD) X-ray was abnormal in all patients with the presence of interstitial infiltrates in 75% of cases and alveolar opacity only in 8%, of them confirming a poor sensitivity [71].

Differently, high resolution chest CT provides more details to identify diffuse lung disease and it is even useful to guide the site of lung biopsy. CT patterns may be highly suggestive of genetic surfactant alterations and include different features such as geographic hyperlucency, multiple cysts, septal thickening, ground glass opacity and lung consolidations or nodules. CT is even used to provide prognostic information during patient follow-up. Finally, when performing CT, it is important to follow a pediatric protocol in order to minimize radiation exposure. The quality

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<td>IRCCS Ospedale Pediatrico Bambino Gesù -. Rome, Italy</td>
<td>NGS sequencing (except WES)</td>
<td>SFPTB, SFTPC, ABCA3</td>
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<td>Bioscientia Institut für Medizinische Diagnostik GmbH, Rheinland-Pfalz, INGELHEIM AM RHEIN Germany</td>
<td>Sanger sequencing</td>
<td>SFTPB, ABCA3</td>
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<tr>
<td>CHU Paris Est - Hôpital d'Enfants Armand-Trousseau, Île De France Paris, France</td>
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<td>SFPTA, SFTPD, SFPTB, SFTPC, ABCA3</td>
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<tr>
<td>Great Ormond Street Hospital for Children, York House, London, United Kingdom</td>
<td>NGS sequencing (except WES)</td>
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<td>CHU Henri Mondor Créteil, France</td>
<td>Sanger sequencing, MLPA based techniques</td>
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<td>Medizinische Hochschule Hannover, Germany</td>
<td>Sanger sequencing</td>
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Table 2. Main European accredited labs for molecular diagnosis about surfactant metabolism. https://www.orpha.net/consor/cgi-bin/ClinicalLabs_Search_Simple.php (Accessed on 30.05.19).
of CT images is crucial to perform a correct diagnosis. In order to prevent artefacts related to anesthesia ventilated controlled high resolution, CT may represent a technique that increases the alveolar recruitment, permitting to identify air trapping and lung ground glass images [72].

Laboratory investigations are helpful to exclude comorbidities such as infectious or autoimmune disease. In children or infants with respiratory distress suspecting diffuse lung disease, bronchoscopy and BAL play a key role to exclude pulmonary infections, hemorrhages and lung aspirations and for the assessment of airway anatomy and physiology.

The indications and the correct timing to perform a pulmonary biopsy in a patient with a clinical history of suspected interstitial lung disease are still controversial [73].

12. TREATMENT

Management is often considered only supportive as supplemental oxygen to correct chronic hypoxemia, or nutrition support and antibiotic treatment to prevent recurrent infections. Unfortunately, guidelines for chILD treatment are not yet available even if pharmacological therapy may be helpful in particular cases.

Pharmacological therapies consist of anti-inflammatory drugs, although the efficacy of this treatment has never been confirmed by clinical trials. In fact, the standard dose of prednisone generally used in the clinical practice is from 1 to 2 mg/kg per day, while methylprednisolone is usually given from 10 to 30 mg/kg per day for 3 consecutive days. The specific doses and the time of all treatments are related to severity and clinical response [74]. According to the clinical cases reported in literature, there are some specific conditions responding to corticosteroids treatment including nonspecific interstitial pneumonia NSIP, idiopathic pulmonary hemosiderosis and desquamative interstitial pneumonia. As alternative, hydroxychloroquine is considered in case of steroid resistance or in association with anti-inflammatory drugs in severe disease. Recently, Braun et al. described 85 case reports present in literature between 1984 and 2013, about children with ILD treated with hydroxychloroquine and corticosteroids [75].

Other pharmacological options include antibiotics as macrolides and immunosuppressive therapy including azathioprine, cyclophosphamide, cyclosporine and methotrexate [76]. In the recent years, lung transplantation seemed to represent a valid option in children with severe disease and might be offered as a chance for end-stage ILD [77]. Treatment response and success depend on the reduction of respiratory symptoms, lung function improvement and gain of quality life. The purpose of the future treatment is to find personalized healthcare and specific therapy implementing phenotypic databases and genetic analysis according to clinical phenotypes [78, 79].

Specific guidelines for these particular pulmonary diseases are needed as well as more effective pharmacological strategies to personalize disease management.

CONCLUSION

Surfactant dysfunctions diseases, including genetic disorders disrupting normal surfactant metabolism, have been identified currently as one of the most important causes of respiratory failure in the neonatal (RDS) or pediatric period (PARDS). chILD represents a group of pulmonary disorders caused mainly by surfactant dysfunction that in the near future years will have new diagnostic and therapeutic approaches. Genetic factors are important contributors to chILD pathogenesis and surfactant metabolism alterations. Because of underlying mechanisms, the development of next generation sequencing capacities together with novel bioinformatics approaches may represent an important progress for the management of these conditions. Finally, we expect that new identified molecular defects will help to predict clinical course and to establish individual therapies [80].

LIST OF ABBREVIATIONS

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ABCA3</td>
<td>ATP Binding Cassette Number A3</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
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<tr>
<td>chILD</td>
<td>Interstitial Lung Disease in Children</td>
</tr>
<tr>
<td>CPI</td>
<td>Chronic Pneumonitis of Infancy</td>
</tr>
<tr>
<td>DIP</td>
<td>Desquamative cell Interstitial Pneumonia</td>
</tr>
<tr>
<td>DLD</td>
<td>Diffuse Lung Disease</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Granulocyte Macrophage Colony-stimulating Factor</td>
</tr>
<tr>
<td>ILD</td>
<td>Interstitial Lung Disease</td>
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<tr>
<td>NKX2.1/TTF1</td>
<td>NKX2.1 / Tiroid Transcriptor Factor 1</td>
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<tr>
<td>NSIP</td>
<td>Nonspecific Interstitial Pneumonia</td>
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<tr>
<td>PAP</td>
<td>Pulmonary Alveolar Proteinosis</td>
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<tr>
<td>PC</td>
<td>Phosphatidylcholine</td>
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<tr>
<td>PG</td>
<td>Phosphatidyglycerol</td>
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<tr>
<td>RDS</td>
<td>Respiratory Distress Syndrome</td>
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<td>SP-A</td>
<td>Surfactant Proteins A</td>
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<tr>
<td>SP-B</td>
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CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.
Surfactant Genetic Disorders


