Inherited disorders of neonatal lung diseases

Murat Yurdakök

Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey

SUMMARY: Yurdakök M. Inherited disorders of neonatal lung diseases. Turk J Pediatr 2004; 46: 105-114.

Although genetic factors are assumed to have a role in the etiology of respiratory distress syndrome (RDS), specific genes underlying this susceptibility are incompletely known. The most promising candidates are the genes coding for the lung-specific protein components of the surfactant. In congenital absence of surfactant protein A in mice, lung mechanics or surfactant homeostasis is normal. However, there is an increased susceptibility to infections. The major surfactant protein A alleles, $6A^2$ and $1A^0$, are the general high-risk RDS alleles, while the allele $6A^3$ carries a decreased risk of RDS. The allele $6A^6$ is also over-represented in infants with bronchopulmonary dysplasia. To date, no human infants who lack surfactant protein A have been identified, and the human respiratory phenotype associated with the $1A^0$ allele has been demonstrated to be variable, Therefore, surfactant protein A polymorphisms are not currently useful for estimation of individual risk of having an affected infant. Surfactant protein B (SP-B) plays an essential role in the structure of tubular myelin. Mutations resulting in an absence of surfactant protein B have been identified. They cause a recessively inherited, progressive respiratory disease. More than 27 loss of function mutations have been identified in the surfactant protein B gene that result in lethal neonatal respiratory failure. Of the several known common variants of the surfactant protein B gene, the most common mutation is 121ins22 that accounts for 60-70% of the mutant cases. Although the frequency of the 121ins2 mutation is rare, the consistent phenotype is exhibited by infants with a homozygous genotype. The clinical presentation in infants homozygous for the 121ins2 mutation is full-term infants who develop respiratory distress within the first 12-24 hours of life. Surfactant replacement therapy fails to reverse this outcome, and without lung transplantation, they expire within the first 1-6 months of life. Surfactant protein B gene mutations may also result in milder phenotypes. These mutations resulting in reduced synthesis of SP-B appear to be family-specific and result in respiratory distress, but sometimes with more gradually progressive or chronic respiratory failure. Surfactant protein C plays a role in the stabilization of surfactant and may also have a role in the intracellular processing of the surfactant complex. Surfactant protein B is important in the intracellular processing and production of surfactant protein C. Although surfactant protein C–deficient mice are viable and survive to adulthood without obvious pulmonary abnormalities, their lung have reduced viscoelasticty. Human respiratory disease in the neonatal period caused by loss-of-function mutations in the surfactant protein C gene has not been identified. However, an autosomal dominant inherited mutation at the surfactant protein C gene causes chronic interstitial lung disease. Surfactant protein D is a member of the collectin family like surfactant protein A, therefore it opsonizes pathogens and enhances their phagocytosis by alveolar macrophages and neutrophils. Unlike surfactant protein A, it does not contribute to lowering surface tension. Surfactant protein D-deficient mice have no respiratory abnormalities at birth, but it causes development of emphysema and predisposition to specific infections. No human infant or child with respiratory distress and mutation in the surfactant protein D gene has been identified.

Key words: surfactant proteins, genetics, respiratory distress, inherited disorders.

Neonatal respiratory distress syndrome (RDS), a major cause of morbidity and mortality in preterm babies, is caused by lung immaturity with a deficiency of surfactant in the alveolar spaces. Babies with surfactant deficiency fail to establish adequate functional residual capacity after birth, and repeated collapse and reexpansion of the lungs can lead to mechanical distruption of airway epithelium and profuse leakage of proteinaceous edema fluid into the air spaces, causing additional impairment of lung mechanics and gas exchange.

Surfactant Synthesis

Surfactant contains 70 to 80% phospholipids, about 10% protein, and about 10% neutral lipids. Surfactant proteins can be divided into the hydrophobic -B and C- and the hydrophilic- A and D. Pulmonary surfactant is synthesized, packed and secreted by alveolar type II cells.

Pulmonary alveolar surface is covered by squamous epithelial cells (type I cells); interspersed along the alveolar epithelium are cuboidal cells (type II cells). Alveoli are unstable structures due in large part to a thin liquid lining layer (hypophase) that generates high surface tension forces as alveolar diameter narrows during expiration. Elevated surface tension can lead to alveolar collapse, making it very difficult to expand the alveolus during the subsequent inspiratory cycle and, ultimately, resulting in RDS. This highly undesirable outcome is prevented by formation of a phospholipid film at the air-liquid interface that dramatically reduces surface tension as the interfacial film is compressed during expiration. The lipid film (pulmonary surfactant) is synthesized in type II epithelial cells and stored in specialized secretory granules, referred to as lamellar bodies.

Lamellar bodies are members of a subclass of lysosome-related organelles referred to as secretory lysosomes. The principal constituents of the lamellar body, surfactant phospholipids, are organized into tightly packed, bilayer membranes in a process that is strongly influenced by the lung-specific, hydrophobic peptide surfactant protein B (SP-B). Newly synthesized SP-B is transported from the Golgi to the lamellar body via multivesicular bodies; in contrast, newly synthesized surfactant phospholipids are transported from the endoplasmic reticulum and incorporated into the internal membranes of the lamellar body. The biochemical composition of the lamellar body is unknown, although the lamellae probably represent lipid and the core material stains for protein and enzyme activities such as acid phosphatase. Although surfactant protein A (SP-A) has been immunolocalized to lamellar bodies, the precise amount or even the location of the surfactant apoprotein molecules within the lamellar bodies is not known.

During exocytosis, the membrane of the lamellar body fuses with the apical plasma membrane of the type II cell. The freshly secreted lamellar body, stripped of its surrounding body, enters the alveolar hypophase liquid. The next series of morphological transformations occur rapidly by unknown mechanisms. Several lamellar bodies together contribute closed tubular sheets of membraneous material to a lung-specific structure called tubular myelin. Tubular myelin is formed by surfactant proteins with phopholipids inserted into the critical places. Surfactant A and B provide the most essential function to the myelin structure. Surfactant protein C plays a role in lipid traffic into and organization of the tubular myelin. High magnification electronmicrographs of tubular myelin membranes reveal a regular spacing of small globular profiles on short stalks which decorate the lipid bilayers. "Lollipop" appearance of the decorations is probably related to the presence of SP-A. Tubular myelin is an unstable form of surfactant and may be specialized to enhance lipid insertion into alveolar interfacial film. SP-A is dissolved from the lipid during that adsorption step. Desorption of surfactant lipids makes the film stable.

Epidemiology

Results of a number of studies, particularly epidemiologic studies, lead us to think that several factors may contribute to the etiology of RDS (i.e., age, sex, and race)[1-3]. Male gender is a risk factor for RDS[4]. Prematurely born infants representing black or African-American populations have a lower incidence of and less severe disease than those of Caucasian ancestry[5].

A small study of twin pairs revealed a trend towards a higher concordance of RDS in monozygotic versus dizygotic pairs. However, the number of patients is not sufficient to make a conclusion, and this finding is not supported by the others. In a Finnish study, no significant difference in the concordance rate between the

mono- and dizygotic twins was found, suggesting a lack of major direct genetic impact on RDS in this population[6]. This finding was supported by the same authors in another study. The authors concluded that the traditional twin concordance study was insufficient to evaluate genetic predisposition to RDS in cases that are confounded by the birth order or multiple pregnancy in itself[7].

Genetic risk for RDS in infancy has been suggested by reports of family clusters of affected infants, and by studies of different ethnic groups and sex[8]. Earlier studies suggested an increased recurrence risk of RDS among the subsequent infants of women with a previously affected infant[9,10]. Relative risk of RDS in the second sibling was 3.3 times higher in women whose first preterm infant had RDS versus in those whose first preterm infant did not have RDS. The recurrence rate of RDS has been shown to be lower in paternal half-sibs compared to maternal half-sibs or full sibs[11]. These findings suggest an important genetic (or other familial) tendency in its origin[12].

However, familial predisposition is not a sufficient reason to assume a genetic etiology, since families may share predisposing environments as well as genes. Numerous genes required for neonatal adaptation are likely to contain sequence variation, many of which may influence the risk of RDS either directly or indirectly via an environmental or a constitutional factor that in turn associates with RDS.

Despite improvement in neonatal survival, long-term respiratory morbidity and mortality have persisted in 5-25% of affected infants. Pulmonary morbidity has been attributed to oxygen toxicity, barotraumas, developmental immaturity, and nutritional deficiencies. However, significant differences in pulmonary outcomes among developmentally similar infants with comparable exposures to oxygen, mechanical ventilation, and nutritional deficiency suggest that genetic factors contribute to pulmonary outcome[13,14].

Although antenatal steroid therapy is used routinely for the prophylaxis of RDS, it is not easy to give an answer to the question as to why some infants are susceptible to RDS despite steroid prophylaxis whereas others are protected from the disease despite severe prematurity ("hypernormals").

Surfactant is a complex of lipid and protein components, and the collective evidence indicates that the different surfactant genes may not be regulated coordinately, therefore the use of various therapies aimed at the acceleration of endogenous surfactant productions should be carefully considered. For example, the expression of the human SP-B gene is enhanced in response to glucocorticoid treatment, whereas the expression of the human SP-A gene is decreased in response to glucocorticoid treatment. Moreover, there is evidence to indicate that the level of decreased expression that occurs in response to glucocorticoid treatment differs between the two human SP-A genes and/or alleles. Such a lack of coordinate regulation poses challenges and raises a number of questions, such as, when is it appropriate during the course of the disease to use a specific treatment and which infant is likely to benefit when various hormonal or other treatments are used?

Although genetic factors are assumed to have a role in the etiology of RDS, the specific genes underlying this susceptibility are incompletely known. The most promising candidates are the genes coding for the lung-specific protein components of the surfactant, especially those coding for SP-A and SP-B[15]. On the whole, the etiology of RDS is considered multifactorial and possibly multigenic.

Surfactant Protein A

Surfactant protein A (SP-A), which is a relatively large and complex protein with 248 amino acids, is important in lamellar body structure and function as well as a collectin in host defense.

Although its contribution to surface tension lowering is less than that of other surfactant proteins, in tubular myelin, it is located in the corners of the myelin and is essential for the formation of myelin lattice with its property of aggregating lipids and probably regulating the insersion of phospholipids into the surfactant monolayer. The association of SP-A with tubular myelin places the collectin in the first line of defense against alveolar pathogens, in a configuration that does not disrupt surface activity. SP-A also plays a modulating role in the recycling of surfactant and increases resistance to surfactant inactivation by plasma proteins.

In congenital absence of SP-A in knockout mice, prematurely delivered pups lack tubular myelin but do not develop respiratory distress, and

lung mechanics or surfactant homeostasis is normal[16-18]. However, there is an increased susceptibility to viral and bacterial infections observed in these animals[19,20].

The major role of SP-A is in pulmonary host defense. SP-A is a member of the collectin subgroup of mammalian C-type lectins that also includes surfactant protein D, mannose-binding protein, and conglutinin. It can bind encapsulated bacteria and potentiates the antibacterial actions of alveolar macrophages, opsonizes viruses and increases phagocytosis of Pneumocystis carinii. However SP-A may also have a role in protecting the lung from over inflammatory response by inhibiting some neutrophil functions[21-23].

The SP-A protein is encoded by two closely linked, highly polymorphic functional genes (SP-A1 and SP-A2) on chromosome 10q22-q23.1. Each of the two functional genes contains four coding exons. The human SP-A locus has also a pseudogene. The gene for the hydrophilic protein, SP-D, has also been mapped to the same chromosome.

The SP-A alleles have been denoted as $6A^n$ for the exons of the SP-A1 gene and $1A^n$ for the SP-A2 gene. Five SP-A1 alleles and six SP-A2 alleles occur at a generally moderate frequency in the populations studied, whereas the rest of the altogether more than 30 alleles described are rare[24-26]. The alleles of SP-A1 and SP-A2 are in linkage disequilibrium, indicating that certain alleles of SP-A1 and SP-A2 (for example, $6A^2$ and $1A^0$) tend to localize to the same chromosome.

In the SP-A1 gene there are five exonic polymorphisms, which correspond to amino acid positions 19, 50, 62, 133 and 219 of the protein. Two of these are silent (62 and 133), while the others result in a non-conservative amino acid substitution (Ala19→Val, Leu50→Val and Arg219→Trp). In the SP-A2 gene, there are four exonic polymorphisms (Thr9→Asn, Pro91→Ala and Lys223→Gln); the polymorphism at position 140 is silent. Nineteen haplotypes have been identified in the SP-A1 gene (designated 6A to $6A^{20}$), and 15 haplotypes have been identified in the SP-A2 gene (designated 1A to $1A^{13}$). Of these haplotypes, the most frequent are the SP-A1 ($6A^2$) and SP-A_2 ($1A^0$) haplotypes. These two haplotypes comprise the following amino acids: SP-A1 ($6A^2$: Val19/Val50/Arg219) and SP-A2 ($1A^0$: Asn9/Ala91/Gln223). In functional studies, these haplotypes correlated with low or moderate mRNA levels.

The various study populations shows that the major SP-A alleles, $6A^2$ and $1A^0$, are the general high-risk RDS alleles[27,28]. The discrepancies or inconsistencies between the studies may be caused by ethnic or racial differences in disease pathogenesis and allele frequency, or by other confounding factors. A population-based study of Finnish premature infants showed that the SP-A alleles $6A^2$ and $1A^0$ were associated with an increased, and the allele $6A^3$ with a decreased, risk of RDS[27,29,30]. The SP-A1 polymorphism $6A^6$ is also over-represented in infants with bronchopulmonary dysplasia[24].

The SP-A alleles associated with an increased risk of RDS have also been associated with low levels of SP-A transcript in human lung tissues[31]. Murine and human studies thus suggest that alleles associated with low concentrations of SP-A may increase the genetic risk of respiratory distress and infection[32]. However, to date, no human infants who lack SPA-A have been identified, and the human respiratory phenotype associated with the SP-A2 $1A^0$ allele has been demonstrated to be variable[32]. Therefore SP-A polymorphisms are not currently useful for estimation of individual risk of having an affected infant. It is, however, still unknown whether the relevant factor is the predisposing role of the high-risk SP-A alleles ($6A^2$ and $1A^0$) or the protective role of the low-risk SP-A alleles ($6A^3$ and $1A^1/1A^2$) or both.

Surfactant Protein B

Surfactant protein B (SP-B) improves surface activity by increasing the surface adsorption and surface stability of surfactant phospholipids. SP-B plays an essential role in the structure of tubular myelin and trafficking of phospholipids into the air-liquid interference. SP-B contains two identical-linked subunits (polypeptide chains), and it is possible that each polypeptide chain can interact with a lipid bilayer; the native protein might therefore be able to crosslink two lipid bilayers. Therefore, it has critical roles in the intracellular processing of the surfactant complex. In the absence of SP-B in mice, the lipid-protein complex secreted by type 2 alveolar cells does not contain mature surfactant protein C (SP-C) and is not surface active[33-35].

The SP-B gene is encoded on chromosome 2 (2p12-p11.2), and it has 11 exons. Exons 1 through 11 encode synthesis of a 381-amino acid preproprotein that is subsequently glycosylated and proteolytically processed before incorporation into pulmonary surfactant. The mature protein with 79 amino acids is encoded in exons 6 and 7.

Mutations resulting in an absence of SP-B have been identified. They cause a recessively inherited, progressive respiratory disease. In contrast to SP-A, genetic disruption of SP-B expression causes neonatal respiratory distress[36,37].

SP-B Deficiency

Surfactant protein B deficiency was the first reported genetic cause of lethal RDS in infants[38]. More than 27 loss of function mutations have been identified in the SP-B gene that result in neonatal respiratory failure that is usually lethal without lung transplantation[39,40]. Of the several known common variants of the SP-B gene, the exon 4 polymorphism influencing the amino acid sequence may have an important functional consequence[27]. The most common mutation, 121ins22, accounts for 60-70% of the mutant cases[40,41]. Affected infants are homozygous for a mutation that involves a 1-bp deletion and 3-bp insertion at codon 121 (a GAA substitution for C in codon 121) in exon 4 of the SP-B gene (121ins2). This mutation results in premature translation stop signal at codon 214 that accounts for the lack of protein[42].

Inherited SP-B deficiency due to the 121ins2 mutation is rare, with an allele frequency in the U.S. of approximately 0.3-1.0/1000 individuals, and a disease frequency of approximately one per million births[39].

Although the frequency of the 121ins2 mutation is rare, the consistent phenotype is exhibited by infants with a homozygous genotype. The clinical presentation in infants homozygous for the 121ins2 mutations is full-term infants who develop respiratory distress within the first 12-24 hours of life. Surfactant replacement therapy fails to reverse this outcome, and without lung transplantation, they expire within the first 1-6 months of life[37,41,43].

The typical histopathological findings include interstitial thickening, abundant type II hyperplasia, and eosinophilic PAS-positive material in the alveolar spaces as seen in alveolar proteinosis. Immunohistochemical staining shows an absence of SP-B, little SP-A in type II cells, but an abundance in the alveolar spaces, and large amounts of SP-C in both type II cells and the alveolar spaces. Electron microscopy demonstrates no tubular myelin, although abnormal multilamellated structures and membranous vesicles are present in the alveoli. Biochemical analysis of the SP-C in these patients shows that it is abnormal with immature form. The mechanisms for abnormal processing of pro-surfactant protein C and enhanced accumulation is unknown. But it seems that SP-B is important in the intracellular processing and production of SP-C[38,42,44,45].

In families with a term infant who dies of unexplained respiratory failure, genetic testing of the parents should be evaluated, since the presence of the detected mutation (e.g. 121ins2) enables prenatal diagnosis in later pregnancies. DNA-based prenatal diagnosis is simple, fast and reliable, and can be performed much earlier in pregnancy than any other method, e.g. the direct measurement of SP-B in amniotic fluid after the second trimester, and tracheal excretion or broncho-alveolar lavage fluid can be analyzed for the presence of SP-B after birth. Diagnosis of the index case is very important for the detection of the mutation in the prenatal diagnosis of subsequent pregnancies[46].

Mild SP-B Deficiency

Surfactant protein B gene mutations may also result in milder phenotypes. These mutations resulting in reduced synthesis of SP-B appear to be family-specific and result in respiratory distress, but sometimes with more gradually progressive or chronic respiratory failure.

Approximately 50% of normal SP-B synthesis may be sufficient for normal pulmonary function at birth[47-50]. The minimum SP-B production required for normal surfactant metabolism and lung function in humans is unknown. But 8-10% of the normal amount is not compatible with life[47].

In mice heterozygous for targeted disruption of surfactant protein B production, reduced synthesis led to air trapping and chronic lung damage when exposed to hyperoxia[51].

A mutation in exon 5 (479G→T) causes reduced surfactant protein B and chronic respiratory distress. Two unrelated children

homozygous for this mutation have been reported; one of them required lung transplantation. These two children had less severe symptoms than have been previously observed in SP-B deficiency. Immunostaining patterns for pulmonary surfactant proteins were consistent with SP-B deficiency in both children. DNA sequence analysis indicated that both children were homozygous for a mutation in exon 5 which resulted in a premature termination codon in exon 7. Western blot analysis detected reduced amounts of mature SP-B as well as an aberrant SP-B proprotein that corresponded to the size expected from translation of the abnormal transcript[52].

Partial SP-B Deficiency

Partial deficiency of SP-B in a patient who is a compound heterozygote with a new mutation has been reported. Respiratory distress developed in an infant delivered at term, and he required extracorporeal bypass support for two weeks. Until his unexpected death at 9.5 months, he was ventilator- and oxygen-dependent and required continual dexamethasone therapy. Tracheobronchial lavage samples contained SP-A but not SP-B, and DNA restriction analysis indicated that the patient and his mother were heterozygous for the previously described 121ins2 mutation of SP-B. Postmortem lung tissue contained normal levels of SP-A and its mRNA, a low level of SP-B, and near normal content of SP-B mRNA. SP-C was abundant on staining, and some 6kd precursor was present in tissue. On DNA sequencing, a point mutation was found in exon 7 of the patient's SP-B gene allele resulting in a cysteine for arginine substitution, and the father was a carrier for the same mutation[49].

Transient SP-B Deficiency

A 38-day-old male infant with persistent pulmonary hypertension and respiratory failure since birth was found to have a complete absence of SP-B along with an aberrant form of SP-C in his tracheal aspirate fluid, findings consistent with the diagnosis of hereditary SP-B deficiency. Surprisingly, SP-B and SP-B messenger ribonucleic acid were present in lung biopsy tissue. However, DNA sequence analysis demonstrated a point mutation in exon 5 of one of the SP-B gene alleles. The infant's mother was found to be a carrier of this mutation. The infant's other SP-B allele did not differ from the published DNA sequence for the SP-B gene. It has been speculated that this patient had a transient deficiency of SP-B, in contrast to that of previously described infants with irreversible respiratory failure caused by hereditary SP-B deficiency. The new mutation found in one of this patient's SP-B genes was in part responsible for the transient deficiency of SP-B[47].

Abnormal SP-B mRNA

Surfactant protein B mRNA abnormalities may also cause SP-B deficiency without DNA mutations. Analysis of the SP-B mRNA showed that the region between exons 7 and 8 was abnormal in tracheal aspirates of two full-term male siblings who were the children of first-cousin healthy Turkish parents. The patients died due to congenital alveolar proteinosis. The abnormal SP-B mRNA in these patients led to an altered SP-B precursor protein. It is possible that the altered SP-B precursor protein was processed into defective SP-B, i.e., altered SP-B function[53].

SP-B Polymorphism

The SP-B gene is known to be polymorphic. Of the two SP-B polymorphisms genotyped, the Ile131Thr variation and the length variation of intron 4 have previously been suggested to associate with RDS[27,28].

The last codon of SP-B gene exon 4 showed a single nucleotide polymorphism T/C, encoding amino acid variation Ile131Thr that is substitution of isoleucine for threonine in the SP-B peptide at amino acid 131. This polymorphism affects a putative N-linked glycosylation site of proSP-B which plays a role in the genetic susceptibility to RDS after very premature birth. Alternatively, the SP-B Ile131Thr variation may be linked to an as yet unidentified variable genetic element that, together with the SP-A allelic variants, has a co-operative biological role in the risk of RDS.

Surfactant protein B intron 4 is composed of different short sequence motifs separated by 3-14 $(CA)_n$ repeats. Allelic variation results from deletion or insertion of one or several motif-$(CA)_n$ sequence stretches. These length variations in intron 4 of the SP-B gene (SP-B Δi4) may be a determinant of the RDS association, similar to the Thr/Thr genotype.

Although these two SP-B polymorphisms have previously been suggested to associate with RDS, more recent data suggest that neither the SP-B Ile131Thr nor the SP-BΔi4 polymorphism associates directly with RDS[27]. Instead, association between SP-A alleles and RDS is dependent on the SP-B Ile131Thr genotype. Among the infants born before 32 weeks of gestation and having the SP-B genotype Thr/Thr, the SP-A1 allele $6A^2$ is over-represented in the RDS group compared with controls. In the same comparison, the SP-A1 allele $6A^3$ is underrepresented in RDS. Therefore SP-B Ile131Thr polymorphism is a determinant for certain SP-A alleles as factors causing genetic susceptibility to RDS ($6A_2$, $1A^0$) or protection against it ($6A^3$, $1A^2$)[27]. SP-B Ile131Thr polymorphism also contributes towards the discordance rather than the concordance of RDS in mono- and dizygotic twin pairs[54].

A familial SP-B deficiency in a multigenerational consanguineous pedigree with 14 infant deaths following respiratory distress at birth has been reported. Immunostaining of the lungs from three such infants revealed decreased or absent SP-B. Nine SP-B polymorphisms were found in this pedigree, but none of them could explain the observed SP-B deficiency. Although the nature of the genetic basis of SP-B deficiency in this family is currently unknown, the existence of aberrant SP-B mRNA may, at least in part, be responsible for the SP-B deficiency in this pedigree[55].

Surfactant Protein C

Surfactant protein C (SP-C) is the smallest of the surfactant proteins with 35 amino acids and its gene is located on the short arm of chromosome 8. SP-C is synthesized from a precursor of either 191 or 197 amino acids. Mature SP-C can be found in both airways and in lamellar bodies. Although functions like SP-B, it does not play an essential role in the structure of tubular myelin. SP-C plays a role in the stabilization of surfactant at low lung volumes and may also have a role in the intracellular processing of the surfactant complex. SP-B is important in the intracellular processing and production of SP-C[56].

A SP-C knockout murine lineage has been reported to cause no respiratory distress at birth. Although SP-C-deficient mice are viable and survive to adulthood without obvious pulmonary abnormalities, their lungs have reduced viscoelasticity[57].

Human respiratory disease in the neonatal period caused by loss-of-function mutations in the SP-C gene has not been identified. However, a recent report describes a family with a splice site mutation at the first base of intron 4 of the SP-C gene, development of chronic interstitial lung disease in affected family members, and an autosomal dominant inheritance pattern[58,59].

Another large familial kindred, including adults with desquamative and non-specific interstitial pneumonitis (a common form of familial pulmonary fibrosis) and children with cellular non-specific interstitial pneumonitis, was characterized as a dominant mutation in carboxy-terminal proSP-C[60]. Similar dominant mutations were identified in 11 out of 34 infants with chronic lung disease of unknown etiology[59].

Genetic analysis showed a mutation in one allele of the SP-C gene. The heterozygous substitution of A to G was located in the first base of intron 4, abolishing the normal donor splice site and resulting in the skipping of exon 4 and the deletion of 37 amino acid residues in the SP-C precursor protein. The abnormal accumulation of SP-C proprotein results in chronic respiratory disease rather than acute RDS in newborn infants[60].

Surfactant Protein D

The vast majority of surfactant protein D (SP-D) is found in the lung, but it is expressed in extrapulmonary tissues such as gastric mucosa. SP-D is a member of the collectin family like SP-A, therefore SP-D opsonizes pathogens and enhances their phagocytosis by alveolar macrophages and neutrophils. Unlike SP-A, it does not contribute to lowering surface tension.

The SP-D gene is located on chromosome 10q22-q23 close to the SP-A genes. The exons of SP-D contain at least three single-nucleotide polymorphisms affecting the amino acid sequence[61].

Murine lineages with targeted ablation of the SP-D-deficient mice) have no respiratory abnormalities at the time of birth. But intraalveolar clearance of surfactant is significantly reduced, which causes progressive extracellular accumulation of surfactant

lipids and of surfactant proteins A and B, activation of alveolar macrophages and peribronchiolar-perivascular inflammation, and development of emphysema. The animals were also found to be susceptible to specific infections[62,63].

No human infant or child with respiratory distress and mutation in the surfactant protein D gene has been identified.

Deficiency of Lamellar Bodies in Alveolar Type II Cells

Abnormalities of lamellar bodies without any surfactant protein deficiency may also cause severe respiratory distress in newborn infants. This abnormality was presented in a case of a full-term female infant who died due to severe respiratory distress shortly after birth. Infectious and other known causes of respiratory disease in this clinical setting were excluded. Histochemical investigations indicated normal alveolar type II cell marker expression including all surfactant protein, and mutations in the coding sequences of the SP-B gene were excluded as a cause of disease. Ultrastructural studies revealed that alveolar type II cells lacked cytoplasmic lamellar bodies, while other organelles appeared normal. This case appears to be a novel congenital defect affecting the pulmonary surfactant system. The cellular abnormality may involve the assembly of cytoplasmic lamellar bodies in alveolar type II cells, the principal storage site of pulmonary surfactant[64].

Others

Functional entities other than the surfactant system influence respiratory adaptation. These include the proteins that catalyze ion transport across the alveolar epithelium, factors affecting the growth of the respiratory airways, mechanisms controlling pulmonary vascular flow, and those primarily involved in the inflammatory response[65-67].

Targeted disruption of the genes that encode either granulocyte-macrophage colony-stimulating factor or its receptor (GM-CSF/IL-3/IL-5 receptor common β-chain) in mice resulted in pulmonary alveolar proteinosis[68]. Subsequently, patients with acquired alveolar proteinosis have been identified who either fail to express the GM-CSF/IL-3/IL-5 receptor common β-chain or have neutralizing antibody of IgG isotype against granulocyte-macrophage colony-stimulating factor in bronchoalveolar lavage[69-71]. These observations demonstrated the important role of extrapulmonary gene products in surfactant production and function.

The surfactant proteins serve as targets of hormones and growth factors that regulate their differentiation. A hormone or a growth factor either decreases or increases the expression of surfactant proteins, the direction of the effect depending on the length of gestation[72]. In addition, the degree of responsiveness to hormones and other factors may be restricted to a specific SP-A genotype[73].

Conclusion

The etiology of most diseases is the result of the interplay between multiple factors and/or genes. What differs among various diseases is the extent of the genetic contribution, and whether the genetic contribution is made by single or multiple genetic factors. The degree to which genetics contribute and the number of genetic factors involved in the etiology of a given disease determine the individual contributing genetic factors.

A locus is considered polymorphic if the less frequent allele has a population frequency of at least 1% and heterozygosity frequency of at least 2%. Below these frequencies, nucleotide variations are allelic variants or, if very rare, they are described as mutations. A number of mutations have been identified in association with hereditary surfactant deficiencies. If an allele or a genotype is found to occur with higher frequency in a disease group, that particular allele or genotype is considered to be associated with the disease phenotype. A significant association may indicate that the allele is a susceptibility/modifier factor for the disease, or is linked to a locus that is a susceptibility/modifier locus for the given disease, or the association may be an artifact due to population admixture. Matching the groups under study (at least for the known confounding parameters) is of primary importance because several confounding factors, such as gestational age, sex, and/or race, may contribute to the pathogenesis of RDS.

REFERENCES

1. Cole FS, Hamvas A, Nogee LM. Genetic disorders of neonatal respiratory function. Pediatr Res 2001; 50: 157-162.
2. Hallman M, Haataja R. Genetic influences and neonatal lung disease. Semin Neonatol 2003; 8: 19-27.

3. Hamvas A, Cole FS, Nogee LM. Inherited disorders of surfactant protein metabolism. Biol Neonate 2002; 82: 276-277.
4. Khoury MJ, Marks JS, McCarthy BJ, Zaro SM. Factors affecting the sex differential in neonatal mortality: the role of respiratory distress syndrome. Am J Obstet Gynecol 1985; 151: 777-782.
5. Hulsey TC, Alexander GR, Robillard PY, Annibale DJ, Keenan A. Hyaline membrane disease: the role of ethnicity and maternal risk characteristics. Am J Obstet Gynecol 1993; 168: 572-576.
6. Martilla R, Haataja R, Ramet M, et al. Evaluation of the genetic susceptibility to RDS in monozygotic and dizygotic twins. Pediatr Res 2001; 49: 387A.
7. Marttila R, Haataja R, Ramet M, Lofgren J, Hallman M. Surfactant protein B polymorphism and respiratory distress syndrome in premature twins. Hum Genet 2003; 112: 18-23.
8. Hamvas A, Wise PH, Yang RK, et al. The influence of the wider use of surfactant therapy on neonatal mortality among blacks and whites. N Engl J Med 1996; 334: 1635-1640.
9. Khoury MJ, Calle EE, Joesoef RM. Recurrence of low birth weight in siblings. J Clin Epidemiol 1989; 42: 1171-1178.
10. Raven SN, Misenheimer HR. Respiratory distress syndrome and the high risk mother. Am J Dis Child 1965; 109: 489-494.
11. Lankenau HM. A genetic and statistical study of the respiratory distress syndrome. Eur J Pediatr 1976; 123: 167-177.
12. Nagourney BA, Kramer MS, Klebanoff MA, Usher RH. Recurrent respiratory distress syndrome in successive preterm pregnancies. J Pediatr 1996; 129: 591-596.
13. McColley SA. Bronchopulmonary dysplasia: impact of surfactant replacement therapy. Pediatr Clin North Am 1998; 45: 573-586.
14. Parker RA, Lindstrom DP, Cotton RB. Evidence from twin study implies possible genetic susceptibility to bronchopulmonary dysplasia. Semin Perinatol 1996; 20: 206-209.
15. Mallory GB Jr. Surfactant proteins: role in lung physiology and disease in early life. Paediatr Resp Rev 2001; 2: 151-158.
16. Korfhagen TR, Bruno MD, Ross GF, et al. Altered surfactant function and structure in SP-A gene targeted mice. Proc Natl Acad Sci USA 1996; 93: 9594-9599.
17. Ikegami M, Korfhagen TR, Whitsett JA, et al. Characteristics of surfactant from SP-A-deficient mice. Am J Physiol 1998; 275: L247-L254.
18. Ikegami Mm, Korfhagen TR, Bruno MD, Whitsett JA, Jobe AH. Surfactant metabolism in surfactant protein A-deficient mice. Am J Physiol 1997; 272: L479-L485.
19. Levine AM, Kurak KE, Bruno MD, Stark JM, Whitsett JA, Korfhagen TR. Surfactant protein-A deficient mice are susceptible to Pseudomonas aeruginosa infection. Am J Respir Cell Mol Biol 1998; 19: 700-708.
20. Levine AM, Gwozdz J, Stark J, Bruno M, Whitsett J, Korfhagen T. Surfactant protein-A enhances respiratory syncytial virus clearance in vivo. J Clin Invest 1999; 103: 1015-1021.
21. Meyer KC, Zimmerman J. Inflammation and surfactant. Paediatr Resp Rev 2002; 3: 308-314.
22. Wright JR. Pulmonary surfactant: a front line of lung host defense. J Clin Invest 2003; 111: 1453-1455.
23. McCormack FX, Whitsett JA. The pulmonary collections, SP-A and SP-D, orchestrate innate immunity in the lung. J Clin Invest 2002; 109: 707-712.
24. Pantelidis P, Veeraraghavan S, du Bois RM. Surfactant gene polymorphisms and interstitial lung diseases. Respir Res 2002; 3: 14-21.
25. Floros J, Hoover RR. Genetics of the hydrophilic surfactant proteins A and D. Biochim Biophys Acta 1998; 1408: 312-322.
26. Hamvas A. Surfactant protein B deficiency: insights into inherited disorders of lung cell metabolism. Curr Probl Pediatr 1997; 27: 325-345.
27. Haataja R, Ramet M, Martilla R, Hallman M. Surfactant proteins A and B as interactive genetic determinants of neonatal respiratory distress syndrome. Hum Mol Genet 2000; 9: 2751-2760.
28. Kala P, Ten Have T, Nielsen H, Dunn M, Floros J. Association of pulmonary surfactant protein A (SP-A) gene and respiratory distress syndrome. Interaction with SP-B. Pediatr Res 1998; 43: 169-177.
29. Ramet M, Haataja R, Martilla R, Floros J, Hallman M. Association between the surfactant protein A (SP-A) gene locus and respiratory-distress syndrome in the Finnish population. Am J Hum Genet 2000; 66: 1569-1579.
30. Haataja R, Martilla R, Uimari P, Logren J, Ramet M. Respiratory distress syndrome: evaluation of genetic susceptibility and protection by transmission disequilibrium test. Hum Genet 2001; 109: 351-355.
31. Karinch AM, deMello DE, Foloros J. Effect of genotype on the levels of surfactant protein A mRNA and on the SP-A2 splice variants in adults humans. Biochem J 1997; 321: 39-47.
32. Floros J, Kala P. Surfactant proteins: molecular genetics of neonatal plumonary diseases. Annu Rev Physiol 1998; 60: 365-384.
33. Hawgood S, Derrick M, Poulain F. Structure and properties of surfactant protein B. Biochim Biophys Acta 1998; 1408: 150-160.
34. Clark JC, Wert SE, Bachurski CJ, et al. Targeted disruption of the surfactant protein B gene disrupts surfactant homeostasis, causing respiratory failure in newbron mice. Proc Natl Acad Sci USA 1995; 92: 7794-7798.
35. Whitsett JA, Weaver TE. Hydrophobic surfactant proteins in lung function and disease. N Engl J Med 2002; 347: 2141-2148.
36. Nogee LM, Wert SE, Proffit SA, Hull WM, Whitsett JA. Allelic heterogeneity in hereditary surfactant protein B (SP-B) deficiency. Am J Respir Crit Care Med 2000; 161: 973-981.
37. Hamvas A, Cole FS, deMello DE, et al. Surfactant protein B deficiency: antenatal diagnosis and prospective treatment with surfactant replacement. J Pediatr 1994; 125: 356-361.
38. Nogee LM, de Mello DE, Dehner LP, Colten HR. Brief report: deficiency of pulmonary surfactant protein B in congenital alveolar proteinosis. N Engl J Med 1993; 328: 406-410.

39. Cole FS, Hamvas A, Rubinstein P, et al. Population-based estimates of surfactant protein B deficiency. Pediatrics 2000; 105: 538-541.
40. Nogee LM, Wertt SE, Proffit SA, Hull WM, Whitsett JA. Allelic heterogeneity in hereditary surfactant protein B (SP-B) deficiency. Am J Respir Crit Care Med 2000; 161: 973-981.
41. Nogee LM. Surfactant protein-B deficiency. Chest 1997; 111 (Suppl): 129S-135S.
42. Nogee LM, Garnier G, Dietz HC, et al. A mutation in the surfactant protein B gene responsible for fatal neonatal respiratory disease in multiple kindreds. J Clin Invest 1994; 93: 1860-1863.
43. Hamvas A, Nogee LM, Mallory GB Jr, et al. Lung transplantation for treatment of infants with surfactant protein B deficiency. J Pediatr 1997; 130: 231-239.
44. Beers MF, Hamvas A, Moxley MA, et al. Pulmonary surfactant metabolism in infants lacking surfactant protein B. Am J Respir Cell Mol Biol 2000; 22: 380-391.
45. Vorbroker DK, Profitt SA, Nogee LM, Whitsett JA. Aberrant processing of surfactant protein C in hereditary SP-B deficiency. Am J Physiol 1995; 268: L647-L656.
46. Stuhrmann M, Bohnhorst B, Peters U, Bohle RM, Poets CF, Schmidtke J. Prenatal diagnosis of congenital alveolar proteinosis (surfactant protein B deficiency). Prenat Diagn 1998; 18: 953-955.
47. Klein JM, Thompson MW, Snyder JM, et al. Transient surfactant protein B deficiency in a term infant with severe respiratory failure. J Pediatr 1998; 132: 244-248.
48. Yusen RD, Cohen AH, Hamvas A. Normal lung function in subjects heterozygous for surfactant protein-B deficiency. Am J Respir Crit Care Med 1999; 159: 411-414.
49. Ballard PL, Nogee LM, Beers MF, et al. Partial deficiency of surfactant protein B in an infant with chronic lung disease. Pediatrics 1995; 96: 1046-1052.
50. Akinbi HT, Breslin JS, Ikegami M, et al. Rescue of SP-B knockout mice with a truncated SP-B proprotein: function of the C-terminal propeptide. J Biol Chem 1997; 272: 9640-9647.
51. Tokieda K, Ikegami M, Wert SE, Baatz JE, Zou Y, Whitsett JA. Surfactant protein B corrects oxygen-induced pulmonary dysfunction in heterozygous surfactant protein B-deficient mice. Pediatr Res 1999; 46: 708-714.
52. Dunbar AE III, Wert SE, Ikegami M, et al. Prolonged survival in hereditary surfactant protein B (SP-B) deficiency associated with a novel splicing mutation. Pediatr Res 2000; 48: 275-282.
53. Mildenberger E, de Mello DE, Lin Z, Kössel H, Hoehn T, Versmold HT. Focal congenital alveolar proteinosis associated with abnormal surfactant protein B messenger RNA. Chest 2001; 119: 645-647.
54. Marttila R, Haataja R, Ramet M, Löfgren J, Hallman M. Surfactant protein B polymorphism and respiratory distress syndrome in premature twins. Hum Genet 2003; 112: 18-23.
55. Lin Z, deMello DE, Batanian JR, et al. Aberrant SP-B mRNA in lung tissue of patients with congenital alveolar proteinosis (CAP). Clin Genet 2000; 57: 359-369. [Erratum in: Clin Genet 2000; 58: 156].
56. Johansson J. Structure and properties of surfactant protein C. Biochim Biophys Acta 1998; 1408: 161-172.
57. Glasser WS, Burhans MS, Korfhagen TR, et al. Generation of an SP-C deficient mouse by targeted gene inactivation (Abstract). Am J Respir Crit Care Med 2000; 161: A43.
58. Nogee LM, Dunbar AE, Wertü SE, Askin F, Hamvas A, Whitsett JA. A mutation in the surfactant protein C (SP-C) gene associated with familial interstitial lung disease. N Engl J Med 2001; 344: 573-579.
59. Nogee LM, Dunbar AE III, Wert S, Askin F, Hamvas A, Whitsett JA. Mutations in the surfactant protein C gene associated with interstitial lung disease. Chest 2002; 121 (Suppl): 20S-21S.
60. Thomas AQ,Lane K, Philips J 3rd, et al. Heterozygosity for a surfactant protein C gene mutation associated with usual interstitial pneumonitis and cellular nonspecific interstitial pneumonitis in one kindred. Am J Respir Crit Care Med 2002; 165: 1322-1328.
61. Lahti M, Logren J, Marttila R, et al. Surfactant protein D gene polymorphism associated with severe respiratory syncytial virus infection. Pediatr Res 2002; 51: 696-699.
62. Wert SE, Yoshida M, LeVine AM, et al. Increased metalloproteinase activity, oxidant production, and emphysema in surfactant protein D gene-inactivated mice. Proc Natl Acad Sci USA 2000; 97: 5972-5977.
63. Botas C, Poulain R, Akiyama J, et al. Altered surfactant homeostasis and alveolar type II cell morphology inmice lacking surfactant protein D. Proc Natl Acad Sci USA 1998; 95: 11869-11874.
64. Cutz E, Wert SE, Nogee LM, Moore AM. Deficiency of lamellar bodies in alveolar type II cells associated with fatal respiratory disease in a full-term infant. Am J Respir Crit Care Med 2000; 161: 608-614.
65. Pitkanen O. Lung epithelial ion transport in neonatal lung disease. Biol Neonate 2001; 80 (Suppl): 14-17.
66. Compernolle V, Brusselmans K, Acker T, et al. Loss of HIF-2alpha and inhibition of VEGF prevents fatal respiratory distress in premature mice. Nat Med 2002; 8: 702-710.
67. Waterer GW, Quasney MW, Cantor RM, Wunderink RG. Septic shock and respiratory failure in community-acquired pneumonia have different TNF polymorphism associations. Am J Respir Crit Care Med 2001; 163: 1599-1604.
68. Dranoff G, Crawford AD, Sadelain M, et al. Involvement of granulocyte-macrophage colony-stimulating factor in pulmonary homeostasis. Science 1994; 264: 713-716.
69. Dirksen U, Nishinakamura R, Groneck P, et al. Human pulmonary alveolar proteinosis associated with a defect in GM-CSF/IL-3/IL-5 receptor common beta chain expression. J Clin Invest 1997; 100: 2211-2217.
70. Kitamura T, Tanaka N, Watanabe J, et al. Idiopathic pulmonary alveolar proteinosis as an autoimmune disease with neutralizing antibody against granulocyte/macrophage colony-stimulating factor. J Exp Med 1999; 190: 875-880.
71. Thomassen MJ, Yi T, Raychaudhuri B, Malur A, Kavuru MS. Pulmonary alveolar proteinosis is a disease of decreased availability of GM-CSF rather than an intrinsic cellular defect. Cell Immunol 2000; 95: 85-92.
72. Vayrynenn O, Glumoff V, Hallman M. Regulation of surfactant proteins by LPS and proinflammatory cytokines in fetal and newborn lung. Am J Physiol Lung Cell Mol Physiol 2002; 282: L803-L810.
73. Hoover RR, Floros J. SP-A 3'UTR is involved in the glucocorticoid inhibition of human SP-A gene expression. Am J Physiol 1999; 276: L917-L924.