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REVIEW ARTICLES

Genetic Disorders of Neonatal Respiratory Function

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ABSTRACT

Genetic risk for respiratory distress in infancy has been recognized with increasing frequency in neonatal intensive care units. Reports of family clusters of affected infants and of ethnic- and gender-based respiratory phenotypes point to the contribution of inheritance. Similarly, different outcomes among gestationally matched infants with comparable exposures to oxygen, mechanical ventilation, or nutritional deficiency also suggest a genetic risk for respiratory distress. Examples of inherited deficiency of surfactant protein B in both humans and genetically engineered murine lineages illustrate the importance of identifying markers of genetic risk. In contrast to developmental, inflammatory, or nutritional causes of respiratory distress that may resolve as infants mature, genetic causes result in both acute and chronic (and potentially irreversible) respiratory failure. The availability of clinically useful genetic markers of risk for respiratory distress in infancy will permit development of rational strategies for treatment of genetic lung disorders of infancy and more accurate counseling of families whose infants are at genetic risk for development of respiratory distress at birth or during early childhood. We review examples of genetic variations known to be associated with or cause respiratory distress in infancy. **(*Pediatr Res* 50: 157–162, 2001)**

Respiratory distress syndrome in newborn infants is the most frequent respiratory cause of death and morbidity in children <1 y of age in the United States (1). It is also predictive of risk for chronic pulmonary diseases in childhood (2–5). Survivors of respiratory distress syndrome with asthma and bronchopulmonary dysplasia consume 20 times more annualized dollars than unaffected children ($19,104 *versus* $955) and 5.9% of all dollars spent on children from 0 to 18 y of age (6). Since the original description of surfactant deficiency, respiratory distress syndrome has most commonly been attributed to developmental immaturity of pulmonary surfactant production (7, 8). Pulmonary surfactant is a mixture of phospholipids and proteins synthesized, packaged, and secreted by type II pneumocytes that line the distal airways. This mixture forms a monolayer at the air-liquid interface that lowers surface tension at end expiration of the respiratory cycle and thereby prevents atelectasis and ventilation-perfusion mismatch. Availability of surfactant replacement therapy has been associated with a decline during the last 10 y in the mortality of respiratory distress syndrome among premature infants (1, 9–11).

Despite improvement in neonatal survival, long-term respiratory morbidity and mortality have persisted in a significant fraction (5–25%) of affected infants (12–15). Pulmonary morbidity has been attributed to oxygen toxicity, barotrauma, 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. Genetic risk for respiratory distress in infancy has also been suggested by reports of family clusters of affected infants, by studies of different ethnic groups and sex, by characterization of infants with inherited deficiency of surfactant protein B, and by targeted gene ablation in murine lineages (16–28). In contrast to nongenetic causes of respiratory distress that may resolve as infants mature, genetic causes result in both acute and chronic (and potentially irreversible) respiratory failure. In contrast to cystic fibrosis or α_1-antitrypsin deficiency that leads to chronic respiratory phenotypes in childhood owing to gradual destruction of normal lung parenchyma, genetic disorders

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that present in the neonatal period acutely disrupt alveolar function. The availability of clinically useful genetic markers of risk for respiratory distress in infancy would permit development of anticipatory and therapeutic strategies to reduce their significant medical and economic costs. Genetic variation in human surfactant protein genes A, B, and C, and in other extrapulmonary genes (granulocyte-macrophage colony-stimulating factor and its receptor) has provided the first examples of such genetic tools. Two mechanistically distinct groups of genetic markers have been identified: those statistically linked to risk of respiratory distress (*e.g.* surfactant protein A) and those that result in loss of surfactant function (*e.g.* surfactant protein B).

SURFACTANT PROTEIN A

The human surfactant protein A locus consists of two functional genes (SP-A1 and SP-A2) and a pseudogene (19, 29). Each of the two functional genes contains four coding exons and directs the synthesis of a distinct primary translation product (30). Of the >15 alleles of the SP-A2 gene identified to date, one ($1A^0$) has recently been observed in significantly higher frequency among unrelated, premature white infants >28 wk gestation with respiratory distress (31). This allele has also been associated with low levels of surfactant protein A transcript in human lung tissue of unrelated individuals (32). In a recent study of 88 infants with respiratory distress syndrome and 88 control subjects from the genetically homogeneous Finnish population, both protective and susceptibility effects were observed with different surfactant protein A alleles (33). In murine lineages with targeted ablation of the surfactant protein A gene, prematurely delivered pups lack tubular myelin but do not develop respiratory distress (34–36).

In addition to its role in surfactant function, surfactant protein A is a member of the collectin subgroup of mammalian C-type lectins that also includes surfactant protein D, mannose-binding protein, and conglutinin (37, 38). The role of surfactant protein A in host defense has been suggested by increased susceptibility to viral and bacterial infections observed in murine lineages with genetically ablated surfactant protein A production (39–41). Murine and human studies thus suggest that alleles associated with low concentrations of surfactant protein A may increase the genetic risk of respiratory distress and infection (19). However, to date, no human infants who lack surfactant protein A have been identified, and the human respiratory phenotype associated with the SP-A2 $1A^0$ allele has been demonstrated to be variable (19). The complexity of the genetic contribution of surfactant protein A to respiratory distress in infancy and to risk of infection makes surfactant protein A polymorphisms promising but not currently useful for estimation of individual risk of having an affected infant.

SURFACTANT PROTEIN B

In contrast to surfactant protein A, genetic disruption of surfactant protein B expression causes an unambiguous neonatal respiratory phenotype in both human infants and mice. The surfactant protein B gene has been sequenced and its regulatory regions characterized (Fig. 1) (42). The gene spans approximately 10 kilobases (kb) and has 11 exons. Exons 1 through 11 encode a 2-kb transcript that directs the synthesis of a 381-amino acid preproprotein that is subsequently glycosylated and proteolytically processed before incorporation into pulmonary surfactant (43, 44). The mature 8-kD protein is encoded in exons 6 and 7. Identified mutations and single nucleotide polymorphisms occur throughout the surfactant protein B gene, as indicated in Figure 1.

Surfactant protein B deficiency was the first reported genetic cause of lethal respiratory distress syndrome in infants (45). Affected infants in the initial kindred were homozygous for a mutation that involved a 1-bp deletion and 3-bp insertion at codon 121 in exon 4 of the surfactant protein B gene (121ins2). This mutation results in a frameshift and premature translation stop signal at codon 214 that accounts for the lack of protein by immunohistochemical staining and in tracheal effluent (46). Nuclear run-on assays performed with nuclei from lungs of affected infants suggest that the mutated gene is transcribed normally, but the transcript is unstable (47). The mutation creates a new *Sfu*I restriction site that facilitates its rapid detection. In addition, pro-surfactant protein C peptides with aberrant mobility on SDS-PAGE and with increased abundance by immunohistochemical staining and by Western blot analysis have been observed in affected infants and in mice with genetically engineered abrogation of surfactant protein B synthesis (45–48). Abnormal processing of pro-surfactant protein C most likely accounts for its altered mobility (47, 48). The mechanism for enhanced accumulation is unknown.

The clinical phenotype for infants homozygous for this mutation is consistent: full-term infants develop respiratory distress within the first 12–24 h of life and, without lung transplantation, expire within the first 1–6 mo of life (49, 50). Surfactant replacement therapy, corticosteroid treatment, and mechanical ventilatory support fail to reverse this outcome (51). The only available treatment for affected infants is lung transplantation (52). These family studies suggested that a single-gene, loss-of-function mutation results in irreversible respiratory distress syndrome in infancy. To make this genetic marker useful for individual risk assessment, animal, human, and population-based studies are necessary to correlate biochemical and clinical phenotype with genotype.

Genetic and biochemical studies of compound heterozygote infants, heterozygote adults, and mice heterozygous for targeted disruption of the surfactant protein B gene suggest that approximately 50% of normal surfactant protein B synthesis

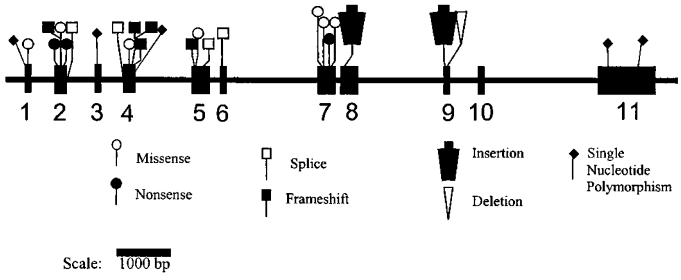

Figure 1. Molecular structure and genetic variation of the surfactant protein B gene.

may be sufficient for normal pulmonary function at birth (17, 18, 53–56). The minimum surfactant protein B production required for normal surfactant metabolism and lung function in humans and mice is unknown. An infant who produced only 8–10% of the normal amount of surfactant protein B because of different loss-of-function mutations on each chromosome experienced acute and chronic respiratory distress after birth and died of respiratory causes at 9.5 mo of age (53). Two other unrelated children homozygous for a mutation in exon 5 (479G→T) that creates an alternative donor splice site had reduced surfactant protein B and chronic respiratory distress: one required lung transplantation, and the other has chronic respiratory insufficiency (57). 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 (58). These observations suggest that acute and chronic respiratory distress syndrome may result from genetic variation in the human surfactant protein B gene.

To determine whether microsatellite markers would be useful in prediction of genetic risk of respiratory distress, population-based studies to evaluate genotype-phenotype correlation were necessary (Table 1). Among the 1260 individuals examined, polymorphic tandem-repeat sequences in intron 4 and intergenic microsatellite markers have been linked to risk of respiratory distress (31, 59–63). However, control populations of insufficient size have limited the ability to use these markers in clinical practice or parental counseling.

To determine the frequency of the 121ins2 mutation, we used clinical and molecular ascertainment in two large, population-based cohorts (64). We found one 121ins2 allele per 3300 individuals from a New York cohort by molecular ascertainment and one 121ins2 allele per 1000 individuals from a Missouri cohort by clinical ascertainment. The rare population frequency of the 121ins2 mutation, the consistent phenotype exhibited by infants with a homozygous genotype, and the absence of biologic redundancy for surfactant protein B function permit unambiguous counseling of parents of fetuses or infants homozygous for this mutation about disease progression, prognosis, and treatment options. In a cohort of infants with hereditary surfactant protein B deficiency, the 121ins2 mutation was the most frequently identified mutation and was found on approximately 60% of chromosomes (65). Other investigators and we have identified 27 mutations or single-nucleotide polymorphisms in the surfactant protein B gene (Fig. 1) (59–65). Other loss-of-function mutations appear to be family specific and result in respiratory distress, but sometimes with more gradually progressive respiratory failure. These findings permit prediction of acute or chronic respiratory distress in infants who carry any loss-of-function mutations on both alleles.

SURFACTANT PROTEIN C

Surfactant protein C is a hydrophobic protein that is synthesized from a precursor of either 191 or 197 amino acids (depending on differential splicing of the primary transcript) and proteolytically cleaved (66–68). Interestingly, pro-surfactant protein C does not contain an *N*-terminal signal peptide but does have hydrophobic domains (66). Mature surfactant protein C contains 35 amino acid residues and can be found in both airways and in lamellar bodies. A murine lineage with targeted ablation of the surfactant protein C gene has been reported to exhibit no respiratory distress at birth (69). In addition, human respiratory disease in the neonatal period caused by loss-of-function mutations in the surfactant protein 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 surfactant protein C gene, development of chronic interstitial lung disease in affected family members, and an autosomal dominant inheritance pattern (70). This report and the results of the surfactant protein C knockout lineage suggest that genetic variation in the surfactant protein C gene results in chronic respiratory disease rather than acute respiratory distress syndrome of infancy.

SURFACTANT PROTEIN D

Surfactant protein D is a member of the collectin family and is expressed in extrapulmonary tissues. Its functions include carbohydrate-domain recognition on the surface of pathogens (71, 72). Murine lineages with targeted ablation of the surfac-

Table 1. *Human surfactant protein B gene microsatellite markers*

Markers*	Ascertainment (number of patients)	Investigators (ref)
C-A tandem repeats in intron 4	Infants with RDS (82); infants without RDS (137)	Floros *et al.* 59
	20 unrelated individuals	Todd and Naylor 62
	Control white and black infants (94); RDS white and black (102)	Kala *et al.* 31
Intron 4 alleles	103 white controls; 34 black controls; 69 Nigerian controls; 40 black RDS	Veletza *et al.* 61
$(AAGG)_n$ marker alleles D2S388, D2S2232, and GATA41E01	CEPH families (32); control black and white infants (200); black and white RDS infants (365); Nigerian adults (200)	Kala *et al.* 60
C-A bp1013, T-C bp1580	15 individuals in affected family	Lin *et al.* 63
Total 671 controls; 589 with RDS		

Abbreviations used: ref, reference; CEPH, Centre d'Etude du Polymorphisme Humain; RDS, respiratory distress syndrome.
* Genomic numbering.

tant protein D gene have no respiratory abnormalities at the time of birth (73). 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 were observed during the first 8 wk of age (73, 74). No human infant or older individual with respiratory distress and mutation in the surfactant protein D gene has been identified. Although differences between genomic and cDNA sequences for surfactant protein D have been observed (75), human genetic variation in this gene has not been studied in detail. Two biallelic polymorphisms of the surfactant protein D gene have been reported (76). Because of the role of the amino terminal region in multimerization to dodecamers critical for many functions of surfactant protein D, amino acid substitution in this region may have greater impact on function. However, the contributions of sequence variants in the surfactant protein D gene to respiratory distress syndrome in infancy await further study.

EXTRAPULMONARY GENE PRODUCTS

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 (77). These observations demonstrated the important role of extrapulmonary gene products in surfactant production and function. 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 (78–80). Success in restoring surfactant homeostasis in mice with disrupted expression of granulocyte-macrophage colony-stimulating factor or its receptor by aerosolization of granulocyte-macrophage colony-stimulating factor or by bone marrow transplantation of wild-type bone marrow cells suggests novel strategies for treatment of respiratory distress syndrome in infancy (81, 82). However, to date, studies of infants and informative kindreds, genotype-phenotype studies, and population studies necessary to permit use of these genetic markers for counseling of individual families have not been performed.

SUMMARY

Antenatal or postnatal identification of infants homozygous for the 121ins2 mutation in the surfactant protein B gene permits unambiguous counseling concerning lethal prognosis of affected infants and treatment options. Identification of infants with loss-of-function mutations in this gene permits reliable prediction of development of acute or chronic respiratory distress. A family history of neonatal or infant death caused by unexplained respiratory distress should prompt consideration of genetic testing for these mutations. Additional population-based analyses of genotype-phenotype correlation, including both respiratory distress in the neonatal period and long-term respiratory morbidity, are required to quantify the contribution of mutations in surfactant protein genes and extrapulmonary genes to the frequency of respiratory distress syndrome and subsequent pulmonary disease. For example, although the frequency of the 121ins2 mutation in the general population is rare, the frequencies of mutations that cause reduced surfactant protein B production have not been assessed. Molecular amplification and high throughput analysis methods for identifying genetic variation in DNA samples from large populations make possible such frequency estimation without the need for sequencing DNA of each individual (83, 84). Population-based studies are critical to avoid exaggeration or underestimation of the contribution of specific mutations or polymorphisms to respiratory distress syndrome in infants owing to ethnic stratification, environmental selection, or genotype-phenotype heterogeneity (85–87). Ongoing state programs for detection of inherited diseases provide access to DNA samples that may be linked anonymously to birth and infant death certificate databases that contain clinical information or sufficient identifiers to evaluate respiratory phenotype (88, 89). Once identified, these mutations will permit development of more rational strategies for treatment of genetic lung disorders of infancy and more accurate counseling for families whose infants are at genetic risk for development of respiratory distress at birth or during early childhood.

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