HEREDITARY SURFACTANT PROTEIN B DEFICIENCY RESULTING FROM A NOVEL MUTATION

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Abstract

Hereditary surfactant protein B (SP-B) deficiency is an autosomal recessive disease in which affected infants are unable to produce normally functional surfactant, resulting in neonatal respiratory failure and death within the first year. The most common cause of SP-B deficiency is a frameshift mutation in exon 4 (121ins2) of the SP-B gene. We report a newborn infant who had onset of respiratory distress during the first days, unresponsive to corticosteroids, surfactant, prostacyclin, high frequency oscillatory ventilation, inhaled nitric oxide and died after 27 days. Immunostaining of lung tissue obtained at biopsy demonstrated absent staining for SP-B, and robust extracellualr staining for proSP-C, findings characteristic for SP-B deficiency. DNA analysis revealed the 121ins2 mutation on one of her SP-B alleles and a novel mutation, 122 delC, on her other SP-B allele. The proximity of the novel mutation in exon 4 allele found in this infant to the 121ins2 supports the notion that this region may represent a "hot spot" for SP-B gene mutations, and confirms the heterogeneity of mechanisms which leads to SP-B deficiency.

Hereditary SP-B deficiency is a rare, newly diagnosable and probably underrecognised disease, which should be suspected in term newborn infants with unexplained respiratory failure.

Key words:

Respiratory distress syndrome

Congenital pulmonary alveolar proteinosis

Surfactant protein B deficiency

INTRODUCTION

Hereditary surfactant protein B deficiency is a rare autosomal recessive disease of newborn infants causing unremitting respiratory failure and death within the first year of life. The disease was first recognized in 1993 in a family in which 3 siblings died from respiratory disease associated with an accumulation of protein rich material filling distal airspaces, a condition known as congenital pulmonary alveolar proteinosis (CPAP) (1). A genetic basis for CPAP had been suspected because of its occurrence in siblings within several affected families (2,3). A frameshift mutation consisting of a net 2 base pair insertion in exon 4 of the SP-B gene and the introduction of a premature signal for termination of translation of the SP-B transcript was identified in infants from the initial kindred as well as unrelated infants who had died from CPAP, although not all infants with the histologic and biochemical features of CPAP have SP-B deficiency as the basis for their disease (4,5). All infants homozygous for the 121ins2 mutation have had a similar phenotype with the onset of respiratory failure in the first days of life which is unresponsive to maximal respiratory support including administration of corticosteroids, surfactant and ECMO (6-8). The mechanism for the finding of a common mutation in unrelated infants is unknown. Recently a frameshift mutation in codon 122 was identified in a kindred of middle eastern descent, suggesting that this region of the SP-B gene may therefore be a "hot spot" for mutations in this gene (9). We now report an Italian infant with a typical clincial course for surfactant protein B deficiency, who was a compound heterozygote for the 121ins2 mutation and a novel frameshift mutation located in codon 122 on her other SP-B allele.

METHODS

Case Report:

A 40-week-gestation female infant with birth weight of 3050 grams was delivered by cesarean section to a 25 years women after an uncomplicated pregnancy. Apgar scores were 9 and 9 at 1 and 5 minutes, respectively. She was the second child of a nonconsanguineous marriage. The family history was unremarkable and the first child, aged 3 years, was normal. At 12 hours of life she had a cyanotic spell and tachypnea, requiring supplemental oxygen administration. After 3 days the cyanotic spells persisted and the infant was transferred to our center. After intubation and mechanical ventilation, the oxygenation and the general condition improved rapidly; however, an increase of FiO2 up to 100% was required to maintain adequate oxygenation. Chest radiography showed a diffuse granular pattern (Figure 1). Echocardiography showed an anatomically normal heart with moderate pulmonary hypertension. Treatments with corticosteroids (dexamethasone, 0.3 mg/kg/day), prostacyclin (from 5 up to 50 ng/kg/min), inhaled nitric oxide (NO) up to 20 parts per million (ppm) did not significantly improve oxygenation. Dopamine and dobutamine were administered to maintain normal blood pressure. An evaluation for infection was negative for pathogens. During the next days the diffuse granular markings increased and air bronchograms were evident on the chest radiograph. Porcine surfactant (Curosurf, 100 mg/kg) administration resulted in a transient improvement of her oxygenation. High frequency oscillatory ventilation (HFOV) did not improve her respiratory status. Chest CT demonstrated hypoventilation of both lungs, without evident malformations. 4 additional doses of surfactant did not improve her respiratory status. Despite mechanical ventilation with 100% oxygen, peak inspiratory pressure of 40 cm/H2O, respiratory rate of 70/min and end expiratory pressure of 4 cm/H2O, she had severe hypoxemia and respiratory acidosis. An open lung biopsy was performed after 25 days; two days later the infant died. Histopathologic examination of the lung tissue obtained at

biopsy showed an accumulation of granular eosinophilic material that filled distal airspaces which stained positively with periodic acid-Schiff reagent (Figure 2), with macrophages trapped within the proteinaceous debris.

Immunohistochemical staining:

Formalin fixed lung tissue was stained for the surfactant proteins using antisera and methods as previously described (10).

DNA Analysis:

DNA was prepared from blood leukocytes and analyzed for the 121ins2 mutation by PCR amplification and restriction analysis, and analyzed for novel SP-B gene mutations by heteroduplex analysis, direct DNA sequencing, and subcloning of PCR products amplified from genomic DNA as previously described (10, 11). Automated sequencing was performed in the DNA Analysis Facility of the Genetics Core at the Johns Hopkins University School of Medicine.

RESULTS:

Immunohistochemical staining of the lung tissue demonstrated the absence of staining for both proSP-B and mature SP-B, even with the aid of antigen retrieval techniques to enhance sensitivity of the immunostaining. In contrast, both epithelial cells and the extracellular proteinaceous material stained positively using antisera directed to both SP-A, and the precursor protein of SP-C.

Analysis of genomic DNA amplified by PCR demonstrated the presence of the 121ins2 mutation on one allele. Direct sequence analysis of

exon 4 indicated a frameshift mutation on the other allele, which was confirmed to be a single base deletion in a sequence of 5 cytosines spanning codons 120 to 122 of the SP-B cDNA (Figure 3).

DISCUSSION

Pulmonary surfactant is the mixture of lipids and specific proteins needed to reduce alveolar surface tension and prevent end-expiratory collapse. Surfactant deficiency commonly results from pulmonary immaturity leading to the respiratory distress syndrome observed in prematurely born infants, and from lung injury contributing to acute respiratory distress syndrome in older children and adults. Hereditary SP-B deficiency is a rare cause of surfactant deficiency due to genetic mechanisms, wherein affected newborn infants are unable to produce normally functional surfactant due to the inability to produce SP-B. SP-B is essential for lung function, enhancing the spreading and stability of surfactant phospholipids that serve to reduce surface tension at the alveolar air-liquid interface. The lack of SP-B also results in secondary abnormalities in surfactant metabolism including the accumulation of aberrantly processed SP-C peptides, and accumulation of protein rich material in the distal airspaces producing the pathology of alveolar proteinosis (12, 13).

The most frequent molecular defect accounting for the deficiency of SP-B is a substitution of three bases (GAA) for the single nucleotide C in exon 4 of the SP-B gene, resulting in a net 2 base pair insertion into codon 121 of the SP-B cDNA causing a frameshift and introduction of a premature codon for the termination of translation thereby accounting for the lack of SP-B, and is referred to as 121 ins2. Other mutations may be associated with SP-B deficiency, and different genotypes may be associated with different clinical phenotypes. Partial and transient deficiencies of SP-B deficiency have been reported, associated with missense mutations in SP-B genes (11, 14).

The infant in this report had clinical findings fairly typical of SP-B deficiency, with early and unremitting respiratory failure, and the typical histopathologic findings that have been associated with this disorder. The initial degree of respiratory disease was not as severe however as has been observed in other children homozygous for null mutations (6, 7, 12). This case thus illustrates

the difficulties and importance of making a precise etiologic diagnosis in a crtically ill child. Additionally, while the 121 ins2 mutation was found on one allele, a novel mutation that would have the same effects of the 121ins2 mutation was identified on her other allele, thus accounting for her inability to make SP-B. This mutation consisted of a deletion of a single cytosine a string of 5 such nucleotides. By convention, we have termed this mutation 122delC, listing the most 3' nucleotide as the one deleted (15), although it is impossible to determine exactly which base was deleted. The location and nature of this second mutation provide further support for the notion that this region of the SP-B gene may be one that is particularly susceptible to mutation.

The patient in this report had severe respiratory disease from birth that was unresponsive to all medical therapy, including exogenous surfactant and administration of pulmonary vasodilators. As in other reported cases, the disease was fatal early in infancy, with the child dying at less than one month of age. The diagnosis of hereditary SP-B deficiency was suggested by the findings of open lung biopsy, and confirmed post-mortem by immunostaining of the biopsy tissue and by molecular methods. The diagnosis of SP-B deficiency may be made non-invasively however. Analysis of surfactant proteins in bronchoalveolar lavage or tracheal aspirate fluid and analysis of DNA prepared from a blood sample for the mutations of SP-B gene known to cause SP-B deficiency may be sufficient to establish the diagnosis. If pulmonary tissue is available then immunohistochemical analysis for the surfactant proteins using antisera directed against SP-A, SP-B, ProSP-B and ProSP-C can confirm the diagnosis.

SP-B deficiency should be suspected in term infants who develop respiratory distress syndrome in the first hours or days, whose lung radiographs are similar in appearance to that of the premature infants with surfactant deficiency, and who fail to improve with optimal medical management. A history of death of a previous child due to unexplained respiratory failure should also indicate prompt evaluation for SP-B deficiency. If a diagnosis of SP-B

deficiency is confirmed, until gene therapy is available, lung transplantation seems to be the only currently effective treatment (16). If such an option is not available or refused by parents, compassionate management could be introduced, avoiding prolonged intensive care and costly, invasive and probably useless treatments.

The incidence of hereditary SP-B deficiency is unknown. It is a rare disease that is probably underecognised because of the phenotypic variability and lack of awareness of such a newly diagnosable condition (14). Genetic and immunostaining analysis for SP-B deficiency are not widely available, and this limitation makes the diagnosis of the disease more difficult. Prompt diagnosis is important not only to establish appropriate management and evaluation of treatment options, but is essential for accurate genetic counselling (6, 12). Prenatal diagnosis of this condition has recently been reported (17). This is the first report of an SP-B deficient infant from Italy. The majority of cases of hereditary SP-B deficiency reported to date have been from the United States, and reported cases from Europe include only 2 kindreds each from the United Kingdom, Germany, and the Netherlands (7, 9, 17, 18). It is important that both data on the prevalence of this condition in Europe be obtained, and that European centers capable of making such a diagnosis be identified (17).

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Figure 1

Chest radiography swowing diffuse granular markings and air bronchogram

Figure 2

Lung biopsy specimen shows intraalveolar accumulation of proteinaceous material reactive to periodic acid-Shiff reagent. (hematoxilin-eosin stain, original magnification x 25)

Figure 3

SP-B mRNA sequences.

The normal SP-B mRNA sequence (top) contains 5 cytosines spanning codons 120 to 122 (underlined). In the 121ins2 mutation (middle), present on one allele on the case patient, there is a substitution of the nucleotides GAA for a single cytosine in codon 121, resulting in a frameshit and introduction of a premature signal for the termination of translation after codon 214. On the second allele in the case patient (bottom), there are only 4 cytosines instead of 5 (underlined), as shown in the chromatograph from the automated sequencing reaction, which also results in a frameshift.