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Short Communication

Novel ABCA3 mutations as a cause of respiratory distress in a term newborn

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ABSTRACT

We report here the case of a term female newborn that developed severe respiratory distress soon after birth. She was found to be a compound heterozygote for both novel mutations in the ABCA3 gene.
ABCA3 deficiency should be considered in mature babies who develop severe respiratory distress syndrome.

1. Introduction

Respiratory distress syndrome (RDS) is due to deficiency of surfactant and commonly occurs in preterm babies (Avery, 2000). In term babies this condition can also occur and may be due to abnormalities of surfactant production.

In addition to surfactant protein-B deficiency (*SFTPB*), protein-C deficiency (*SFTPC*) and the gene encoding thyroid transcription factor-1 (*NKX2-1*) mutations, inherited severe neonatal respiratory distress has recently been attributed to mutations in the ATP-binding cassette A3 transporter (*ABCA3*) gene (Brasch et al., 2006; Bullard et al., 2005; Cutz et al., 2000; Hamvas et al., 2013; Shulenin et al., 2004). ABCA3 protein is thought to transport lipids into the lamellar bodies (LB), where the components of mature pulmonary surfactant are assembled before being secreted into the alveolar airspaces (Ban et al., 2007; Nagata et al., 2004).

The clinical spectrum and severity of lung disease caused by *ABCA3* deficiency is variable, ranging from fulminant neonatal respiratory failure resulting in death during the first days or months of life or later-onset interstitial lung disease (Bullard et al., 2005; Doan et al., 2008; Shulenin et al., 2004; Young et al., 2008).

Genetic testing for mutations in *ABCA3* may obviate the need for lung biopsy in some patients. Approximately 150 mutations in *ABCA3* have been reported to date (Gower et al., 2010; Park et al., 2010; Shulenin et al., 2004; Whitsett et al., 2010).

In this report, we present a newborn that developed immediate respiratory distress and was discovered to be a compound heterozygote for two novel *ABCA3* gene mutations.

2. Patients and methods

2.1. Patients

A 2.41 kg term female infant was born at 37 weeks gestation, via a spontaneous vaginal delivery, to healthy and non-consanguineous parents. There was no family history of birth defects, mental retardation, infant deaths or metabolic disorders.

Her mother was a primigravida. Pregnancy, labor and delivery were uncomplicated. No meconium was noted at birth and there was no history of perinatal asphyxia, birth trauma or perinatal infection risk. Within four hours from birth, she developed severe respiratory distress.

White cell count and C-reactive protein concentration had fallen to within the normal range. Initial chest radiograph showed diffuse bilateral interstitial infiltrates, consistent with RDS (Fig. 1). An echocardiogram revealed no structural or functional heart abnormalities and excluded pulmonary hypertension.

The newborn was treated with an empiric course of antibiotics and started nasal continuous positive airway pressure. On day 4 of life invasive ventilation with high fraction of inspired oxygen and mean airway pressures to maintain adequate oxygenation was started. Natural porcine surfactant (Curosurf®) was administered on day 13, 15, 18 and

Abbreviations: RDS, respiratory distress syndrome; LB, lamellar bodies; *SFTPB*, surfactant protein-B; *SFTPC*, surfactant protein-C; *NKX2-1*, thyroid transcription factor-1; *ABCA3*, ATP-binding cassette A3 transporter; CT, computed tomography; BAL, bronchoalveolar lavage; DIP, desquamative interstitial pneumonitis; NBD, nucleotide binding domain.

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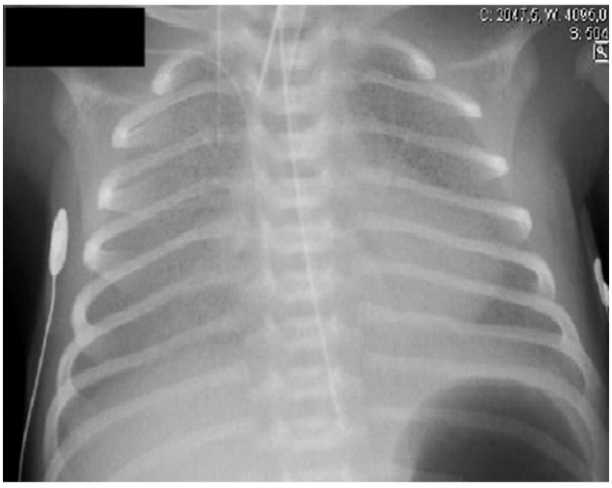

Fig. 1. Chest radiograph at day 4 of life.

20 (100 mg/kg/dose). There were brief periods of decreased ventilator requirements and improvement in blood gasses following each dose of surfactant, but the effects wore off 18 to 24 h after each treatment.

On day 13 of life, a chest radiograph revealed a white-out of both lung fields. A computed tomography (CT) scan revealed areas of condensation predominantly in left lung (Fig. 2). The esophagram revealed no abnormalities. Cultures of blood, urine and tracheal aspirate were negative for bacteria (including ureaplasma) and fungus. Cultures for viral respiratory pathogens were negative.

A bronchoalveolar lavage (BAL) was performed and the cytology, cultures and polymerase chain reaction of *Pneumocystis jirovecii* in bronchoalveolar lavage fluid were normal.

A repeated echocardiogram showed no signs of pulmonary hypertension. An open lung biopsy was performed, but the results did not reveal an etiology. Microscopic examination of the lung sample showed interstitial thickening and septal fibrosis, clustered alveolar macrophages, uneven aeration, hypereosinophilic degeneration cellular material in the air space and focal areas of hemorrhage were also noted (Fig. 3). Electron microscopy was not performed. Based on lung histology, desquamative interstitial pneumonitis (DIP) was diagnosed but pulmonary alveolar proteinosis could not be firmly excluded.

The patient`s condition continued to deteriorate and corticosteroids pulse therapy (metilprednisolone: 30 mg/kg/dose) was continued in 3-day courses on a monthly basis and oral hydroxychloroquine (10 mg/kg/day) have been used but no clinical improvement was observed.

2.2. Cytogenetic analysis

Peripheral blood was sent for genetic analysis of the gene enconding, the surfactant-related proteins SP-B, SP-C and *ABCA*3 (Institute of Laboratory Medicine and Human Genetics, Singen, Germany). No mutations were detected in the genes for SP-B and SP-C.

Genomic DNA was extracted from EDTA whole blood with the DNA Midi Kit® (Qiagen, Hilden/Germany) according to the manufacturer's protocol. Thus, a second purification step with the DNA Mini Kit® (Qiagen) had to be performed to minimize the amount of phospolipids that interfere with the DNA sequencing procedure. Each of the 30 coding exons of the *ABCA*3 gene was amplified by PCR using exon specific primers located in the flanking intronic regions and sequenced by direct sequencing using an ABI Prism 3100 Genetic Analyser® (Applied Biosystems, Foster City, CA).

*ABCA*3 gene analysis revealed two undescribed mutations. She is a compound heterozygote carrier of a leucine$_{798}$ (CTT) → proline (CCT)/p.Leu798Prol/L798P exchange and of an arginine$_{1612}$ (CGG) → proline (CCG)/p.Arg1612Pro/R1612P substitution encoded by exons 18 and 31 of the ABCA3 gene. One of these mutations was detected in analysis of the mother's DNA and the other mutation in the father's.

2.3. Phylogenetic analysis

To analyze the evolutionary (phylogenetic) relation between ABCA3 and related proteins from different organisms, the authors have used the deduced amino acid sequence of ABCA3 (GenBank accession number NP_001080) to search the sequence data base using BLAST program (http://www.ncbi.nlm.nih.gov/blast/). Similar proteins were also identified in other species.

The protein relations from different organisms (human (*Homo sapiens*), baboon (*Papio anubis*), horse (*Equus caballus*), orca (*Orcinus orca*), domestic cow (*Bos taurus*), and mouse (*Mus musculus*)) were evaluated using amino acid alignment (Clustal X program) (Thompson et al., 1997) (Fig. 4).

3. Results

Over the ensuing weeks, the infant's work of breathing increased significantly and she required increasingly higher levels of supplemental oxygenation. On 101 days of life, after discussions with parents and family, intensive care was withdrawn and she died peacefully.

4. Discussion

Mutations in *ABCA*3 appear to be the most common cause of genetic surfactant dysfunction in humans (Shulenin et al., 2004; Somaschini

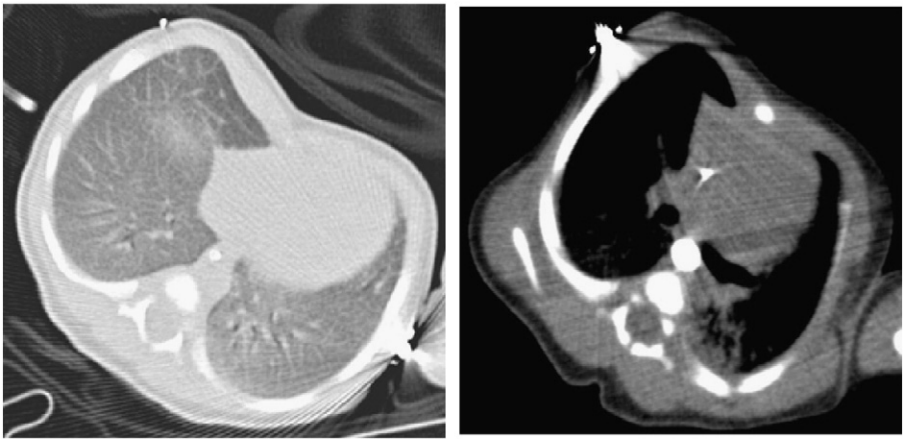

Fig. 2. CT scan at 13 days of life.