Short communication

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

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## ARTICLE INFO

## 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.

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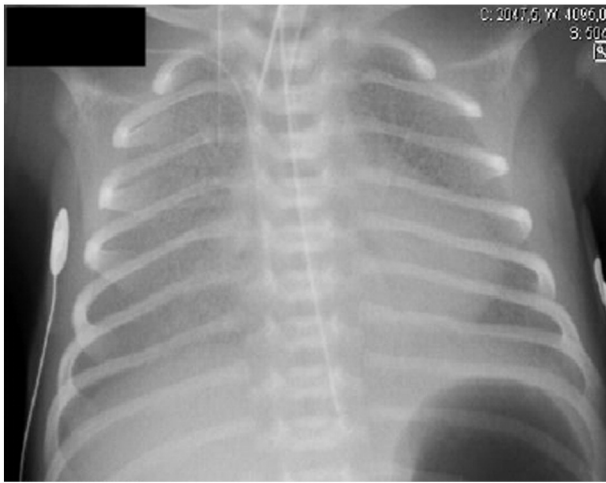
**Fig. 1.** Chest radiograph at day 4 of life.

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
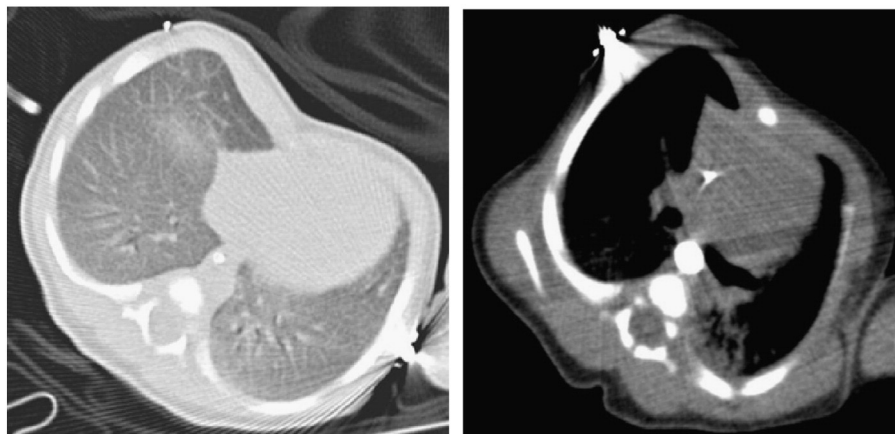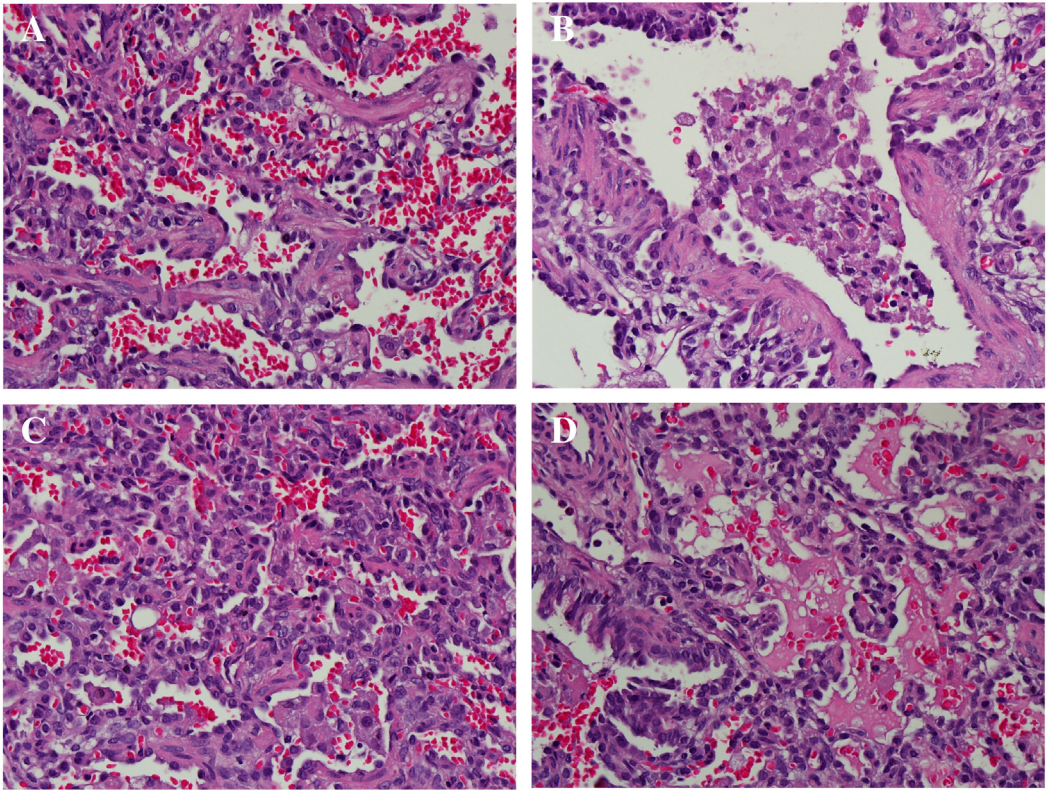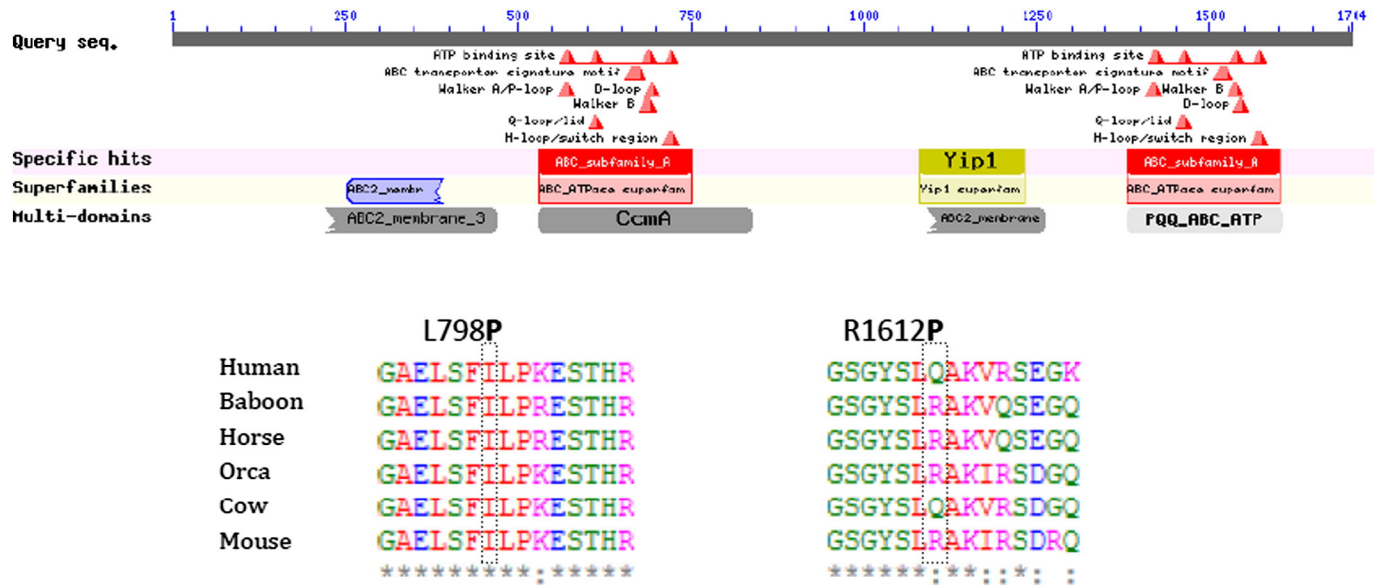


**Fig. 2.** CT scan at 13 days of life.

**Fig. 3.** Histopathology of ABCA3-deficient lung tissue demonstrating interstitial thickening and septal fibrosis (A); clustered alveolar macrophages (B); collapsed alveoli (C); focal areas of hemorrhage and hypereosinophilic degeneration material in the air space (D).

et al., 2007). The mutations result in a loss or reduced function of the ABCA3 protein, and are inherited in an autosomal recessive manner. It is assumed that homozygosity or compound heterozygosity for *ABCA*3 mutations causes fatal respiratory distress syndrome in newborns (Shulenin et al., 2004). The severe neonatal form of the disease is thought to result from a lack of functional surfactant. Disease caused by *ABCA*3 mutations varies in its presentation depending upon the degree of *ABCA*3 dysfunction. Fatal surfactant deficiency due to *ABCA*3 mutation may be classified into two categories, type I and type II, abnormal

intracellular trafficking and decreased ATP hydrolysis activity. These distinct pathophysiologies may be useful in assessing both the severity and effective therapy for surfactant deficiency (Matsumura et al., 2006). Approximately 150 different variants have been reported in the literature, most leading to severe neonatal lung disease (Whitsett et al., 2010). While most mutations in *ABCA*3 are unique, some have been identified among multiple symptomatic individuals including E292V(Bush, 2004; Garmany et al., 2008). The carrier rate of *ABCA*3 mutation in European-decent individuals was reported to be 3.6% and it is



**Fig. 4.** Degree of conservation of residues involved in missense mutations in the ABCA3 protein.

estimated that *ABCA*3 deficiency occurs approximately in 1:3100 among this population (Wambach et al., 2012). Our case report was unfortunate to have inherited two different mutations from her parents, leading most likely to abnormal surfactant function and clinical features of severe RDS. The histolopathologic findings in the lung biopsy revealed a non-specific interstitial pneumonia and features of chronic pneumonitis of infancy.

To our knowledge, both mutations have not been published before and are not found among variants reported in NHLBI GO Exome Sequencing Project (ESP) (Tennessen et al., 2012).

Experimental approaches to analyze protein expression, location and function are highly laborious and were not performed. The computational analysis is less informative than direct experimental evidence but do not involve additional labor, cost and can be applied to any gene. To infer the causality and functional significance of these mutations computational prediction algorithms were performed (PolyPhen-2) (Sunyaev, 2012). In this case report PolyPhen-2 predicts that L798P is probably damaging (high confidence supposed to affect protein function and structure) and R1612P is benign (most likely lacking any phenotypic effect). It appears that L798P is located in a nucleotide binding domain (NBD1), while R1612P in NBD2.

A variant is more likely to be a deleterious mutation if it affects an amino acid residue that is conserved across species. The authors therefore aligned the human, mouse, domestic cow, baboon, horse, and orca amino acid sequences of ABCA3. The L798P mutations occur in a residue in the ATP-binding domain that is highly conserved, and it almost certainly disrupts the function of the protein.

The clinical phenotype and genetic findings support the notion that these mutations were deleterious rather than neutral variants.

Analysis of the genes involved in surfactant dysfunction disorders is recommended because it is noninvasive and a positive molecular diagnosis may obviate the need for lung biopsy. Genetic analysis in this case was of significant benefit to the patient's family because it provided an explanation for the child's severe lung disease and was important for proper counseling regarding prognosis.

To date, information of the treatment of this disease in newborns and infants are still scarce. Treatment modalities are extrapolated from adult practice or experience in the treatment of other investigated lung diseases. Although there is no clear evidence about their beneficial effect, systemic corticosteroids and hydroxychloroquine are widely used for interstitial lung disease (Vece and Fan, 2011). In a few cases with *ABCA*3 mutations, these drugs have been shown to have beneficial effects (Hayes et al., 2012; Yokota et al., 2008).

Lung transplantation is the only definitive treatment option for patients with severe disease due to *ABCA*3 deficiency. Although lung transplantation is not readily available and highly problematic in very young infants this treatment carries similar survival rates to older children with lung transplantation (Elizur et al., 2009). The majority of infants with severe dysfunction of *ABCA*3 and without lung transplantation do not survive beyond 3 to 6 months of life (Hayes et al., 2012).

## Disclosure statement

The authors declare that they have no conflict of interest.

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