Clinical, Radiologic, and Pathologic Features of ABCA3 Mutations in Children

Minh L Doan, a R Paul Guillerman, b Megan K Dishop, c Lawrence M Nogee, d Claire Langston, c George B Mallory, a Marianna M Sockrider, a Leland L Fan a

a Pediatric Pulmonary Section, Department of Pediatrics, Baylor College of Medicine, Texas Children's Hospital, Houston, Texas, U.S.A.
b Department of Radiology, Baylor College of Medicine, Texas Children's Hospital, Houston, Texas, U.S.A.
c Department of Pathology, Baylor College of Medicine, Texas Children's Hospital, Houston, Texas, U.S.A.
d Division of Neonatology, Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland, U.S.A.

Correspondence and requests for reprints:
Leland L. Fan, M.D.
Texas Children's Hospital
6621 Fannin, CC1040.00
Houston, TX 77030
U.S.A.
E-mail: llfan@texachildrenshospital.org
Phone: 832-822-3300
Fax: 832-825-3308

Keywords: surfactant deficiency, interstitial lung disease, ABCA3
Word count: 3,485

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive license (or non-exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article to be published in Thorax editions and any other BMJPGL products to exploit all subsidiary rights, as set out in our license http://thorax.bmjournals.com/ifora/licence.pdf”.
ABSTRACT

**Background:** Mutations in the ABCA3 gene can result in fatal surfactant deficiency in term newborns and chronic interstitial lung disease in older children. Previous studies on ABCA3 mutations focused primarily on the genetic abnormalities and reported limited clinical information about the resultant disease.

**Objective:** To systematically analyze the clinical presentation, pulmonary function, diagnostic imaging, pathologic features, and outcomes of children with ABCA3 mutations.

**Methods:** The records of nine children with ABCA3 mutations evaluated at Texas Children’s Hospital between 1992 and 2005 were reviewed and their current clinical status updated. Previous diagnostic imaging studies and lung biopsy specimens were reexamined. Results of DNA analyses were confirmed.

**Results:** Age at symptom onset ranged from birth to four years. Cough, crackles, failure to thrive, and clubbing were frequent findings. Mean lung function was low but tended to remain static. Computed tomography scans commonly revealed ground-glass opacification, septal thickening, parenchymal cysts, and pectus excavatum. Histopathologic patterns included pulmonary alveolar proteinosis, desquamative interstitial pneumonitis, and nonspecific interstitial pneumonitis and varied with age. Dense abnormalities of lamellar bodies, characteristic of ABCA3 mutations, were seen by electron microscopy in all adequate specimens. Outcomes varied with the age at which the severity of lung disease warranted open lung biopsy, and some patients have had prolonged survival without lung transplantation.

**Conclusions:** The presentation and course of interstitial lung disease due to ABCA3 mutations are variable, and open lung biopsy and genetic testing are warranted early in the evaluation of children with a consistent clinical picture.
INTRODUCTION

An important development in the field of childhood interstitial lung disease (chILD) has been the discovery of inborn errors of surfactant metabolism, including mutations in the surfactant protein B (SP-B), surfactant protein C (SP-C), and ABCA3 genes.1-3 Mutations in these genes cause chILD with varying histopathologic patterns, including desquamative interstitial pneumonitis (DIP), chronic pneumonitis of infancy (CPI), pulmonary alveolar proteinosis (PAP), and nonspecific interstitial pneumonitis (NSIP).1-10 ABCA3 is an ATP-binding cassette transporter of lipids that is found in the limiting membrane of lamellar bodies in alveolar type II cells.11 Initially reported as a cause of fatal lung disease in term neonates, with a clinical picture similar to that of SP-B deficiency,3 mutations in the ABCA3 gene are now known also to cause chronic interstitial lung disease (ILD) in older patients.8 Previous reports on ABCA3 mutations focused primarily on the genetic abnormalities and gave limited clinical information about the resultant disease beyond the neonatal period. We present an analysis of nine children with ABCA3 mutations evaluated at a single medical center in order to more fully describe the clinical presentations, pulmonary function, diagnostic imaging, pathologic features, and outcomes of children with this disorder.

METHODS

Nine children who were eventually diagnosed with mutations in the ABCA3 gene were evaluated at Texas Children’s Hospital (TCH), a tertiary chILD and pediatric lung transplantation referral center, between 1992 and 2005. Five of the patients were transferred to or primarily cared for at our center, while 4 were seen on an outpatient referral basis. The records of patients were reviewed to document the neonatal history, interval history prior to initial evaluation at TCH, presenting symptoms, family and environmental history, physical findings at initial evaluation, pulmonary function tests, and treatment regimen. Each patient’s current clinical status, including growth parameters, pulmonary function, and ILD score,12 was obtained from clinical records and communications with parents and referring physicians. Records of the initial evaluation were available and adequate for analysis in each case, and the clinical status was updated for all survivors. All available chest radiographs, computed tomography (CT) scans, and lung pathology specimens were systematically reexamined (without blinding) by a pediatric radiologist (R.P.G) and a pediatric lung pathologist (M.K.D), respectively, at TCH. DNA samples from 7 patients were initially analyzed for ABCA3 mutations by direct sequence analysis of the 30 coding exons as previously described under a research protocol at the Johns Hopkins University School of Medicine,8 and their mutations were subsequently confirmed in the CLIA certified Johns Hopkins DNA Diagnostic Laboratory, where the mutations of the remaining 2 patients were also identified. Two patients (1 and 6) have been described in previous publications.8,13 This study was approved by the Institutional Review Board of Baylor College of Medicine.

RESULTS
Clinical Presentation

All 9 patients in our cohort were born at term. Five had respiratory symptoms in the newborn period, including 3 patients who developed respiratory failure (2 were extubated after two days; 1 had progressive deterioration) and 2 patients who were treated for pneumonia with intravenous antibiotics and/or oxygen. In the 4 patients who were eventually discharged home, 3 continued to have either persistent or intermittent respiratory symptoms (described below), while 1 infant was apparently well until 8 months of age. In the other 4 children who had an unremarkable neonatal course, the age at symptom onset ranged from three months to four years. Known environmental exposures were present in 3 patients: 1 to birds and 2 to cigarette smoke. A family history of childhood interstitial lung disease was absent at the time of initial evaluation in all 9 cases.

**TABLE 1. CHARACTERISTICS OF 9 CHILDREN WITH ABCA3 MUTATIONS**

<table>
<thead>
<tr>
<th>Pt</th>
<th>Age of Onset, Manifestation</th>
<th>Clinical Features (age at evaluation)</th>
<th>CT Imaging (age at exam)</th>
<th>Mutational Analysis</th>
<th>Outcomes (current age)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Newborn, Resp failure</td>
<td>Ta, Cr, Wh, Cl, Hy (4 yrs)</td>
<td>GGO, ST, PE (2 wks)</td>
<td>Nt622C&gt;T (R208W)</td>
<td>Transplanted (died) (5 yrs)</td>
</tr>
<tr>
<td>2</td>
<td>Newborn, Resp failure</td>
<td>Ta, Hy (1 mo)</td>
<td>None</td>
<td>Nt289insA</td>
<td>Transplanted (died) (3 mos)</td>
</tr>
<tr>
<td>3</td>
<td>3 mos, Acute resp distress</td>
<td>Ta, FTT, Hy (2 yrs)</td>
<td>GGO, ST (3 mos)</td>
<td>Nt2646insC (P1253S)</td>
<td>Died (4 mos)</td>
</tr>
<tr>
<td>4</td>
<td>2 yrs, Acute resp distress</td>
<td>Ta, Cr, Cl, FTT, Hy (2 yrs)</td>
<td>GGO, ST, PE (2 yrs)</td>
<td>Nt4732G&gt;A (E1578K)</td>
<td>Alive, ILD score 4 (15 yrs)</td>
</tr>
<tr>
<td>5</td>
<td>1 yr, Recurrent hypoxemia</td>
<td>Ta, Cl, FTT, Hy (3 yrs)</td>
<td>GGO, ST, PE, cysts (2 yrs)</td>
<td>Nt590G&gt;T (R20L)</td>
<td>Alive, ILD score 4 (8 yrs)</td>
</tr>
<tr>
<td>6</td>
<td>Newborn, Pneumonia</td>
<td>Ta, Cr, Cl, FTT, Hy (10 yrs)</td>
<td>GGO, ST, PE (4 yrs)</td>
<td>Nt875A&gt;T (E292V)</td>
<td>Transplanted (alive) (12 yrs)</td>
</tr>
<tr>
<td>7</td>
<td>Newborn, Respiratory failure</td>
<td>Ta, Cl, FTT (6 yrs)</td>
<td>GGO, ST, PE, cysts (6 yrs)</td>
<td>Nt875A&gt;T (E292V)</td>
<td>Alive, ILD score 1 (18 yrs)</td>
</tr>
<tr>
<td>8</td>
<td>Newborn, Pneumonia</td>
<td>Ta, Cr, Cl, Hy (6 yrs)</td>
<td>GGO, PE (6 yrs)</td>
<td>Nt629G&gt;T (G210V)</td>
<td>Alive, ILD score 3 (11 yrs)</td>
</tr>
<tr>
<td>9</td>
<td>4 yrs, Recurrent hypoxemia</td>
<td>Ta, Cr, Hy (exertional) (7 yrs)</td>
<td>GGO, ST (7 yrs)</td>
<td>Nt128G&gt;A (R43H)</td>
<td>Alive, ILD score 2 (13 yrs)</td>
</tr>
</tbody>
</table>

*Definitions of abbreviations:* Ta = tachypnea; Cr = crackles; Wh = wheezing; Cl = clubbing; Hy = hypoxemia; FTT = failure to thrive; GGO = ground-glass opacification; ST = septal thickening; PE = pectus excavatum.

The 8 patients who did not have persistent respiratory failure from the newborn period all presented later with cough, tachypnea, dyspnea, and exercise intolerance (Table 1). Most had hypoxemia at rest, while one had hypoxemia only with exertion. On initial evaluation at our center, the majority of patients were < 5th percentile for weight and had crackles and retractions on examination. Only 1 child had wheezing. The 7 children who were older than two years at initial evaluation all had clubbing, and 5 of them also had pectus excavatum apparent by physical exam.
Pulmonary Function Tests

Six patients were able to perform spirometry starting at age 6-8 years (Figure 1A). The mean (±SD) of the initial forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1) percent predicted were 43.6% (±13.9%) and 41.2% (±12.0%), respectively, consistent with severe restrictive lung disease. Most of the patients were unable to perform the maneuver for measurement of the diffusion capacity for carbon monoxide (DLCO).

Diagnostic Imaging

Chest radiographs were available for review for all 9 patients at various ages. They consistently showed bilateral diffuse or patchy hazy granular or streaky opacities (Figure 2). One or more helical or high-resolution axial CT (HRCT) exams were available for review in 8 patients; the ninth was too critically ill to undergo CT imaging. On CT scan, all patients had ground glass opacification (GGO) that was either diffuse throughout the lungs or patchy but involving multiple or all lobes. The imaging appearance during infancy is very similar to neonatal respiratory distress syndrome. In some patients, the intensity of the GGO decreased with age, but this did not correlate with improvement in lung function or degree of hypoxemia. All patients imaged beyond infancy developed fine or coarse peripheral interstitial septal thickening. Five patients eventually developed small (few millimeters in diameter), air-filled, parenchymal lung cysts that tended to increase in number and size over time. Hilar and mediastinal lymphadenopathy (4 patients), consolidation and atelectasis that cleared over time (4 patients), pleural thickening (3 patients), and air trapping (3 patients) were also seen. Pectus excavatum (defined as Haller index > 2.7) developed in all patients surviving beyond infancy.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age at biopsy</th>
<th>Major histologic pattern</th>
<th>Pneumocyte hyperplasia</th>
<th>Reactive interstitium</th>
<th>Proenolysis</th>
<th>Cholesterol clefts</th>
<th>Alveolar macrophages</th>
<th>Lobular remodeling</th>
<th>Lympophcyte inflammation</th>
<th>Lympoid aggregates</th>
<th>Fibrosis</th>
<th>Iron</th>
<th>Lamellar dense bodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>3 wks</td>
<td>PAP</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1 mo</td>
<td>DIP</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3 mos</td>
<td>PAP</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>2 yrs</td>
<td>DIP</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2 yrs</td>
<td>NSIP/ ELP</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>+/-</td>
<td>+</td>
<td>++</td>
<td>ND</td>
</tr>
<tr>
<td>1B</td>
<td>4 yrs</td>
<td>NSIP/ ELP</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>6A</td>
<td>4 yrs</td>
<td>NSIP/ ELP</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>6 yrs</td>
<td>NSIP/ ELP</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>6 yrs</td>
<td>NSIP/ ELP</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>8 yrs</td>
<td>NSIP</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>6B</td>
<td>9 yrs</td>
<td>NSIP/ ELP</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

Definitions of abbreviations: PAP = pulmonary alveolar proteinosis; DIP = desquamative interstitial pneumonia, NSIP = non-specific interstitial pneumonia, ELP = endogenous lipoid pneumonia. ||| dense bodies. ND = no data (not done or inadequate specimen). ++ = rare/few foci/mild; +++ = occasional/scattered foci/moderate; ++++ = frequent/many foci/severe; +/- = early fibroplasia without organized fibrosis.
Pathology

Diagnostic lung biopsy slides were available for review in all 9 patients, including 3 with a second biopsy and 2 with subsequent lung pathology specimens after transplantation and at autopsy. The time interval between specimens was less than one month for 3 patients and similar histologic features were seen in the paired specimens in each case. Two subjects (Patients 1 and 6) had an extended interval between biopsies (4 years in both cases), and these biopsies are considered separately. Histologic features and major patterns of disease for these 11 biopsies are summarized in Table 2. The spectrum of overlapping histologic findings were grouped in three major categories: pulmonary alveolar proteinosis (PAP) pattern, desquamative interstitial pneumonitis (DIP) pattern, and non-specific interstitial pneumonitis (NSIP) pattern (Figure 3). Features present in all 11 cases included some degree of lobular remodeling, at least focally increased alveolar macrophages, and at least focal pneumocyte hyperplasia. All cases showed either alveolar proteinosis material or cholesterol clefts (endogenous lipoid pneumonia), with both features present in 5 biopsies. Alveolar proteinosis material and diffuse alveolar epithelial hyperplasia were most prominent in the infants, but inconspicuous in the older children at time of biopsy. Iron-laden macrophages were present in all patients except for the two youngest (ages 3 weeks and 1 month). Lobular remodeling was most prominent in the older children, although this finding was variable, with some biopsies showing a patchy distribution of architectural abnormalities.

The two patients with follow-up biopsies at prolonged intervals allowed for examination of the progression of histologic findings over time. When biopsied at 3 weeks of age, Patient 1 initially had PAP pattern with early lobular remodeling and a reactive widened interstitial (Figure 4, A and B). At follow-up biopsy 4 years later, the lung showed more extensive lobular remodeling (airspace enlargement and increased interstitial smooth muscle), mildly increased interstitial inflammatory cells, but less reactive interstitial widening (Figure 4, C and D). Alveolar proteinosis was present in both biopsies, with the additional finding of endogenous lipoid pneumonia at 4 years of age. Patient 6 underwent biopsies at 4 years (Figure 4, E) and 9 years of age (Figure 4, F), both showing NSIP pattern with endogenous lipid pneumonia and extensive lobular remodeling. Both specimens showed increased lymphoid infiltrates and cholesterol clefts, although these findings were more prominent in the second biopsy.

Tissue was available for electron microscopic study in 6 biopsies, with adequate preservation of ultrastructure in 5 cases. Characteristic small lamellar dense bodies were identified in all 5 patients, but were more frequent and well formed in the younger children (age range, 3 weeks to 2 years) and inconspicuous in the one biopsy from an older child (age 6 years) (Figure 5). The dense bodies varied in size and location, including both centrally positioned and eccentric round electron-dense forms. The lamellar structure associated with the dense bodies was delicate and whorled in some areas and more compact with increased electron density in other areas. In addition to these dense bodies, the type II alveolar epithelial cells also showed increased lysosomal material, forming dense black globular cytoplasmic aggregates focally containing components of lamellar membranes, suggesting intralysosomal degeneration of abortive lamellar bodies. Composite lamellar bodies with apparent fusion of two or three whorled lamellar bodies were also detected at least focally in each case. The abnormal dense bodies and degenerating lysosomal lamellar structures were also admixed with a few lamellar bodies of normal size and structure in each case, and these normal lamellar forms predominated in the oldest child (age 6 years).
Genetics
There were 8 Caucasians and 1 Hispanic in the cohort. DNA analysis for mutations in the ABCA3 gene revealed two mutations in all 9 patients, consistent with autosomal recessive inheritance. Five of these mutations (R20L, ΔF1203, E292V, T114M, Q1591P) have been previously identified in subjects with chILD, whereas the remainder are novel. The E292V mutation was the only one found in more than one patient. Eight patients had been followed for many years with a diagnosis of idiopathic chILD, while one (Patient 1) was prospectively diagnosed at two months of age.

Treatment and Outcome
All 9 patients were treated with systemic corticosteroids, 7 with pulses of intravenous methylprednisolone given on a monthly basis for varying durations. Hydroxychloroquine was added to the treatment regimen of 7 patients. Records were not sufficiently complete to determine the short-term response to therapy. Long-term outcomes within our cohort were variable. One infant died from unremitting respiratory failure at 4 months of age, one month after initial presentation (Table 1). Three patients underwent lung transplantation at ages 3 months, 5 years, and 12 years; the youngest was the child with persistent respiratory failure from the neonatal period. The two youngest transplant recipients have died (1 from primary graft dysfunction, 1 from bronchiolitis obliterans), while the oldest is still alive at less than one year post-transplant.

The 5 survivors who have not required transplantation currently range in age from 8 to 18 years. Three of them have had an improvement in the degree of their hypoxemia (as evaluated by ILD score), while 2 have not. None have evidence of secondary pulmonary arterial hypertension by electrocardiogram or echocardiogram. The lung function in 4 of these 5 patients has not changed significantly over time; the fifth has had a slow decline from a peak FEV1 of 80% to 40% predicted over 10 years, although he currently reports no symptoms and is very active physically. The mean (±SD) of the latest FVC and FEV1 percent predicted were 48.9% (±14.8%) and 48.1% (±18.5%), respectively, in the 5 survivors without transplant. In the 6 patients who are still alive, all 4 who had a weight < 5th percentile at the initial evaluation are still at < 3rd percentile for weight, while the 2 children who did not have failure to thrive have continued to have normal growth, with follow-up intervals ranging from 2-12 years (Figure 1B).

Within our cohort, no specific clinical feature correlated with outcome, as measured by the need for lung transplantation or death, although these analyses were difficult due to the small number of patients. There was a suggestion that younger age at the time of initial lung biopsy might indicate a lower likelihood of surviving without transplantation (Figure 6).

DISCUSSION
Our study is the first to present a systematic review of the clinical features, lung function, imaging changes, and histopathology of children with ABCA3 mutations. The results of our analysis expand upon the descriptions of previous studies and reveal several clinically significant findings not previously described.

Although ABCA3 mutations are inherited in an autosomal recessive fashion,3 none of our patients had a positive family history at the time of presentation, underscoring the need to consider this entity even in the absence of such history. The higher proportion of cases with a positive family history reported in previous studies may reflect selected patient populations.3 8 10 The age at symptom onset for nearly half of our patients was beyond the neonatal period and
extended to early childhood, reemphasizing the findings of the Bullard et al. that ABCA3 mutations can present as chronic ILD in young children.

This study provides the first description of the serial lung function, growth, and radiographic changes in children with ABCA3 mutations. The FVC percent predicted, while initially low, tended to remain stable over many years. Amongst the survivors without lung transplantation, a majority had a mild improvement in oxygenation. Most of the children who presented with chronic ILD beyond the neonatal period had failure to thrive, and all of these patients continued to grow poorly. The remainder, who underwent open lung biopsy at older ages (suggesting milder disease), did not have growth abnormalities at initial evaluation, and they continued to grow well. Serial CT imaging of the lungs consistently showed bilateral ground-glass opacification (GGO) that in some cases decreased in intensity with age, while peripheral interstitial thickening and small parenchymal lung cysts became more prominent findings over time. Interestingly, pectus excavatum was seen in all patients who were imaged beyond infancy; one child underwent surgical correction of his pectus before he was diagnosed with ABCA3 mutations. We are aware of only one previous report of pectus excavatum in association with chronic ILD in a patient with pulmonary alveolar microlithiasis. Changes in CT findings over time did not correlate with lung function, hypoxemia, or outcome, suggesting that once the diagnosis is established, frequent routine imaging studies are not warranted.

The systematic review of all open lung biopsy specimens in our series, including repeat biopsies at older ages in 2 children, demonstrated that the pathologic phenotypes of ABCA3 mutations are age-dependent. In the biopsies obtained from the young infants, PAP and DIP were the predominant patterns. In the 2 biopsies of toddlers, one showed DIP pattern, and the other had NSIP pattern. Finally, the older children, who underwent biopsy between the ages of 4 to 9 years, all had NSIP pattern, often with superimposed endogenous lipoid pneumonia. There were no cases that showed a complete constellation of features seen in chronic pneumonitis of infancy (CPI). As this pattern is most often recognized in older infants (6 to 18 months) with SP-C mutations, its absence in our series may reflect this gap in age distribution. While different histologic patterns have been reported in previous studies of ABCA3 mutations, an age-dependent variation in such patterns has not been described. Together with earlier reports, our findings emphasize that disease-causing mutations in the ABCA3 gene can lead to multiple pathologic diagnoses. It is important to note that several of our patients had been followed for many years (in one case into adulthood) with the pathologic diagnosis NSIP or DIP, raising the possibility that some adults with long-standing chronic ILD who have these pathologic patterns may actually have ABCA3 mutations.

The presence of characteristic small lamellar dense bodies in all 5 patients in our cohort who had adequately preserved samples for EM examination suggests that this finding may be a sensitive diagnostic feature of ABCA3 mutations. Absence of mature lamellar bodies and accumulation of these central or eccentrically placed dense bodies have previously been reported in patients with ABCA3 mutations. In 3 of our 5 patients, some normal lamellar bodies were also seen in type II alveolar cells, which previously had been described in only one patient. This finding further underscores the variability in the histology that can occur with ABCA3 mutations. More importantly, however, given that results of DNA analyses are not quickly available, emphasis must be placed on the need to preserve a portion of any open lung biopsy done for evaluation of chILD for electron microscopy analysis, as it may provide a rapid, preliminary diagnosis of ABCA3 mutations. Guidelines for the processing of pediatric lung biopsies have recently been published.
The significant heterogeneity and number of novel mutations in the ABCA3 gene found in our patients is similar to the report by Brasch et al.\textsuperscript{10} and suggest that like other genetic conditions (e.g. cystic fibrosis), many more mutations may yet be discovered. The E292V mutation found in 2 of our patients has been reported as the most common defect in the ABCA3 gene leading to chILD.\textsuperscript{20} Our study shows that the outcome in children with ABCA3 mutations is also quite variable. Almost half of our patients have survived into the second decade of life without need for lung transplantation. However, we realize that a selection bias may exist in our study due to the referral pattern.

The clinical and radiologic features described above for ABCA3 mutations can overlap with those seen with the other forms inborn errors of surfactant metabolism: the SP-B and SP-C mutations. Respiratory distress syndrome (RDS) in term newborns can result from either SP-B or ABCA3 mutations,\textsuperscript{1} while chronic ILD starting in infancy or early childhood can be the manifestation of either SP-C or ABCA3 mutations.\textsuperscript{2,4-5,7,21-24} Young children with ILD due to SP-C mutations can have similar findings on CT imaging as those with ABCA3 mutations.\textsuperscript{7,21-22} While histologic findings can also overlap,\textsuperscript{1,4-7,25} analysis of the lung ultrastructure using electron microscopy (EM) may be able to distinguish amongst these three entities. Patients with SP-B mutations have large, membrane-bound pleomorphic, cytoplasmic inclusions with irregularly disposed lamellae and multiple associated vesicular structures,\textsuperscript{17} while patients with SP-C mutations can have normal or less commonly somewhat disorganized appearing lamellar bodies.\textsuperscript{4,23-24}

Based upon the results of our analysis, we conclude that ABCA3 mutations should be considered in the differential diagnoses for newborns with unexplained respiratory distress syndrome and for older infants and young children who have chronic ILD of unclear etiology. Findings on HRCT, particularly widespread ground-glass opacification, septal thickening, and parenchymal lung cysts, can provide supportive evidence of a possible inborn error surfactant metabolism. DNA analysis for mutations in the ABCA3 gene is necessary for definitive diagnosis and should also be considered for the SP-B and SP-C genes because of similarities in the clinical, radiologic, and pathologic features of these entities. In patients with suspected ABCA3 mutations and chronic stable disease, confirmatory genetic testing will eliminate the need for lung biopsy. If genetic testing is equivocal or nondiagnostic, lung biopsy to look for one of the compatible histologic patterns and to evaluate for characteristic lamellar dense bodies by electron microscopy should be done. Lung biopsy should also be considered in patients with suspected ABCA3 mutations and rapidly progressive disease. These patients could be candidates for lung transplantation, and awaiting genetic testing results would delay a timely diagnosis. Outcomes in patients with ABCA3 mutations are variable, ranging from severe irreversible respiratory failure in early infancy to chronic static or progressive interstitial lung disease, with many patients surviving well into their second decade without lung transplantation.
ACKNOWLEDGMENTS
The authors thank Drs. Phil Black, Fran White, Stuart Sweet, Susan Wert, Gail Deutsch, Lisa Young, Aaron Hamvas, Robert Zwerdling, Jason Fullmer, and Tarak Patel for their care of these patients and their assistance in our research. We recognize Mr. Jim Barrish for his technical expertise in electron microscopy. We also express our appreciation to the patients and their families for their assistance and willingness to participate in the study.

COMPETING INTERESTS
No author has any conflicts of interest involved with this research.

FUNDING
LMN was supported by an NIH grant (HL-54703).
REFERENCES


FIGURE LEGENDS

FIGURE 1
(A) Changes in FVC percent predicted over time in 6 patients with ABCA3 mutations. (B) Changes in weight percentile in the same 6 patients plotted on a representation of the standard growth curve. Data points in the bottom bar simply represent < 5th percentile.

FIGURE 2
Major radiographic patterns seen with ABCA3 mutations. (A) AP CXR from a 3-month-old female shows diffuse hazy granular pulmonary opacification. (B) Axial HRCT image of the same infant reveals diffuse ground glass opacification of the lungs. (C) Axial HRCT image from a 6-year-old female exhibits mosaic lung attenuation with widespread ground glass opacification. (D) Imaging of the same child at 9 years of age depicts continued widespread ground glass attenuation in a similar distribution and development of small air-filled lung cysts and thickening of the peripheral pulmonary interstitium.

FIGURE 3
Major histologic patterns associated with ABCA3 mutations. (A, B) Pulmonary alveolar proteinosis (PAP) pattern in infancy is characterized by early lobular remodeling, interstitial widening, diffuse alveolar epithelial hyperplasia, and predominantly fine granular proteinosis material admixed with foamy macrophages. There are no significant inflammatory infiltrates. (A, B, 3-month-old female, 4x and 20x original magnification, respectively). (C, D) Desquamative interstitial pneumonitis (DIP) pattern in infancy is characterized by prominent clustered alveolar macrophages (C, 2-month-old female, 10x original magnification). Other findings may include interstitial widening, diffuse reactive alveolar epithelial hyperplasia, and focal globular proteinaceous material (D, 2-month-old female, 20x original magnification). Inflammatory infiltrates are inconspicuous. (E, F) Nonspecific interstitial pneumonitis (NSIP) pattern in older children is characterized by lobular remodeling, increased interstitial smooth muscle, and patchy mild interstitial lymphocyte infiltrates (E, 6-year-old female, 10x original magnification). These findings are often associated with cholesterol granulomas (endogenous lipid pneumonia), with or without lymphoid hyperplasia (F, 2-year-old male, 20x original magnification).

FIGURE 4
Progression of disease in follow-up biopsies. (A,B) Patient 1 initially had a pattern of variant PAP at 3 weeks of age (A), followed by a predominant pattern of NSIP at 4 years of age (B). In comparison to the first biopsy, the second shows increased airspace enlargement with more pronounced lobular remodeling and interstitial smooth muscle, decreased interstitial widening, similar granular and globular proteinosis material, and occasional foci of endogenous lipid pneumonia. (A, 4x original magnification. B, 2x original magnification.) (C, D) Two biopsies in Patient 6 both show significant lobular remodeling and increased interstitial smooth muscle, as well as clusters of foamy macrophages. The biopsy at 4 years of age shows an NSIP pattern with patchy mild interstitial lymphocyte infiltrates and only focal endogenous lipid pneumonia (C, 4x original magnification). The follow-up biopsy at 9 years of age also shows an NSIP pattern,
but with much more prominent lymphocytic infiltrates and frequent cholesterol clefts (D, 4x original magnification).

**FIGURE 5**
Electron microscopic features of ABCA3 mutations. Type II alveolar epithelial cells contain characteristic abnormal lamellar bodies with distinctive central and eccentric round dense bodies (A, 2-month-old female, 4,000x original magnification; B, 2-year-old male, 25,000x original magnification). A few degenerating forms suggesting lysosomal degradation are also noted in some cases. Many cases also contain a few normal lamellar bodies (B, upper right) admixed with the abnormal forms. Findings in a 6-year-old male include only rare characteristic dense bodies, with many lamellar bodies that are small with vague irregular densities (C, 12,000x original magnification), while others show normal size with appropriate delicate whorled structure (D, 10,000x original magnification). Occasional fusion of lamellar bodies is also noted (D).

**FIGURE 6**
Outcomes in the 9 patients with ABCA3 mutations as related to age at open lung biopsy.
Clinical, radiologic, and pathologic features of ABCA3 mutations in children

Minh L Doan, R Paul Guillerman, Megan K Dishop, Lawrence M Nogee, Claire Langston, George B Mallory, Marianna M Sockrider and Leland L Fan

Thorax published online November 16, 2007

Updated information and services can be found at: http://thorax.bmj.com/content/early/2007/11/16/thx.2007.083766

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

- Interstitial lung disease (559)
- Child health (843)
- Pulmonary alveolar proteinosis (12)
- Cardiotoracic surgery (676)
- Radiology (diagnostics) (812)
- Transplantation (184)

Notes

To request permissions go to: http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to: http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to: http://group.bmj.com/subscribe/