Three Infants with Pathogenic Variants in the *ABCA3* Gene: Presentation, Treatment, and Clinical Course

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ABCA3 deficiency is a rare cause of neonatal respiratory failure. Biallelic complete loss of function variants lead to neonatal demise without lung transplantation, but children with partial function variants have variable outcomes. The favorable clinical course of 3 such infants presenting with respiratory distress at birth is described. *(J Pediatr 2021;231:278-83).*

The importance of surfactant for neonatal lung function was elucidated in 1959 by Avery and Mead, who noted infants dying of hyaline membrane disease had dramatically higher lung surface tension than infants dying of nonpulmonary causes.[1] This finding built on Clements' discovery of pulmonary surfactant, which is produced by type II alveolar epithelial cells and composed of phospholipids and proteins that are assembled in lamellar bodies and released into the alveolar space to form a lipid layer at the air-liquid interface.[2,3] This lipid layer decreased the surface tension and is necessary to prevent alveolar collapse during expiration.[4] Multiple genes are responsible for the proper assembly and function of surfactant. One such gene, ATP-binding cassette transporter A3 (*ABCA3*), encodes for a multimembrane spanning protein of the same name found on lamellar bodies, crucial for phospholipid transport and creation of this lipid layer.[5] Enrichment of homozygous *ABCA3* variants in full-term infants with fatal neonatal respiratory failure were first identified in 2004.[6] Pathologic *ABCA3* variants are inherited in an autosomal recessive manner and lead to ABCA3 deficiency.[7] More than 200 disease associated *ABCA3* variants have been identified, including complete loss-of-function (null) and partial function (missense, splice site, insertion/deletions) with notable genotype and phenotype correlations.[8] Biallelic null variants universally present with respiratory failure at birth and result in death without lung transplantation in infancy, whereas the presentation of compound heterozygous partial function variants is variable, ranging from lethal neonatal respiratory failure to adult onset interstitial lung disease.[8,9]

We report favorable clinical outcomes in 3 infants with neonatal respiratory distress and compound heterozygous variants in *ABCA3* treated with a 3-drug therapeutic regimen of monthly methylprednisolone pulses and daily azithromycin and hydroxychloroquine. In addition, each patient was treated with noninvasive ventilation and therapies chosen to mitigate extrapulmonary complications.

ABCA3	ATP-binding cassette transporter A3
LFNC	Low-flow nasal cannula
CT	Computed tomography

Methods

The details of the clinical history, laboratory findings, chest imaging, and genetic testing were obtained from the electronic medical record. This case series was written under Stanford University's Institutional Review Board and Privacy Board approved protocols, and parental written permissions were obtained.

Clinical Presentations

Patient 1 was a 740-g male infant born at 25 weeks of gestation with immediate respiratory distress. He was intubated, mechanically ventilated, and given exogenous surfactant. Examination was notable for diminished air entry and bibasilar crackles. At 36 weeks postmenstrual age, he remained on high-frequency oscillatory ventilation without tolerance of ventilator weans and with increasing oxygen needs. Based on these ventilatory needs, genetic testing for heritable causes of neonatal lung disease was done at 38 weeks postmenstrual age revealing 2 missense variants in *ABCA3*: c.875 A>T (p.E292V), the most commonly reported deleterious variant, and c.3241 C>T (p.R1081W), a variant positioned close to other disease-causing variants.[7,8,10-18] Computed tomography (CT) angiography of the chest obtained at approximately 40 weeks postmenstrual age demonstrated areas of "crazy paving" with interlobular thickening on a background of diffuse ground glass opacities, cystic changes, and air trapping (**Figure 1**, 1a). At 3 months of age, the infant was started on daily azithromycin (5 mg/kg/d) and hydroxychloroquine

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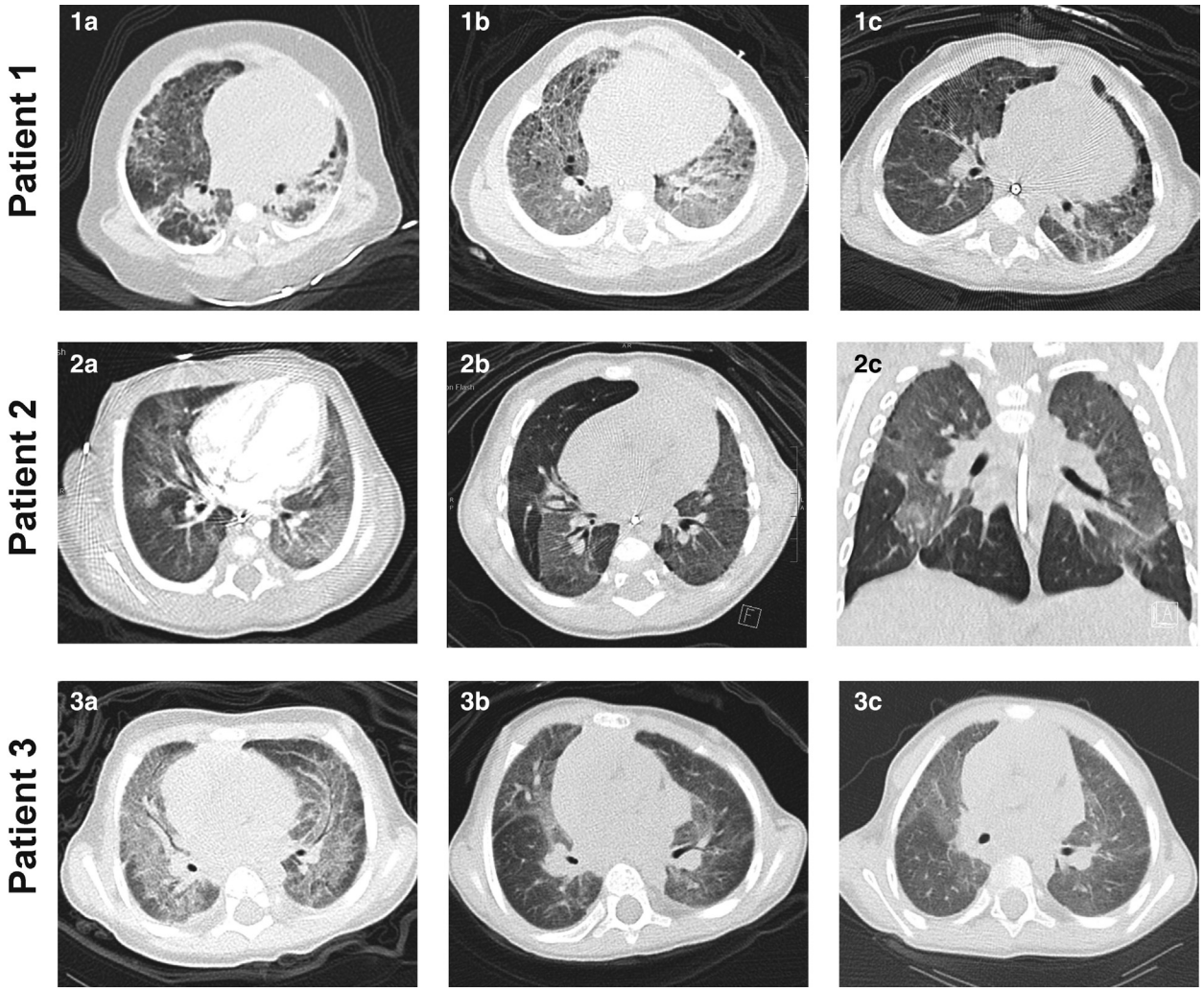

Figure 1. CT imaging demonstrates variable improvement vs progression of lung disease after at least 6 monthly steroid pulses. Serial imaging of patient 1 show diffuse, confluent ground glass opacities, patchy interlobular septal thickening, areas of lobular overinflation and scattered small cysts at 3 months of age (1a), which progressed 2 weeks after initial steroid course at 6 months of age (1b) but improved after 6 monthly steroid pulses at 11 months of age (1c). Serial imaging of patient 2 show diffuse ground glass opacities with left-sided predominance and a relative sparing of the inferior right middle and lower lobes at 2 weeks of age (2a) with worsening ground glass opacities in posterior and superior lung fields with development of subpleural cystic changes in bilateral upper lobes and scattered hyperinflated pulmonary lobules after 6 monthly steroid pulses at 7 months of age (2b). Serial imaging of patient 3 show extensive ground glass opacities more prominently in upper lobes with interlobular septal thickening and mild hyperinflation at 4 months of age (3a), which improved after 6 monthly steroid pulses at 11 months of age (3b) and continue to improve 4 months after last steroid pulse at 18 months of age (3c).

(5 mg/kg/d). He underwent gastrostomy tube placement with Nissen fundoplication for gastroesophageal reflux before extubation at 4.5 months of age. Monthly intravenous methylprednisolone pulse therapy (10 mg/kg/d for 3 days) was started, given persistent tachypnea and evidence of worsening respiratory acidosis, which improved the respiratory rate within the first 48 hours after initial steroid pulse. Two weeks later, an interval CT chest scan demonstrated worsening cystic changes and diffuse ground glass opacities (**Figure 1**, 1b). The infant was transitioned to nocturnal bilevel noninvasive ventilation and daytime low-flow nasal cannula (LFNC). After 6 monthly methylprednisolone pulses, a repeat chest CT scan (**Figure 1**, 1c) revealed a decrease in ground glass opacities, and steroid therapy was discontinued. He was discharged home at 13 months of age and is tolerating weans of respiratory support. Developmental assessment at 2 years of age revealed severe motor, language, and cognitive delays (**Table I**). Both weight and length started well below the third percentile and improved to the third percentile at 27 months of age.

Patient 2 was a 2645-g male infant was born at 38 weeks of gestation. The infant was initially vigorous at birth, but developed respiratory distress within the first few hours of life and

Table I. Patient characteristics

Characteristics	Patient 1	Patient 2	Patient 3
Sex	Male	Male	Male
Race/ethnicity	Caucasian	Hispanic	Chinese
Birth history			
Gestational age, weeks	25	38	41
Birth weight, g	740	2645	4035
APGAR scores	2, 8	9, 9	7, 9
Delivery room resuscitation	Mechanical ventilation	None	None
Family history	None	Older sister with sudden death at 8 mos; older brother with asthma*	None
Age at presentation	Birth	Hours of life	Hours of life
Age at diagnosis, months	3	<1	5
Pulmonary presentation	Severe RDS, PIE	Persistent tachypnea, grunting, hypoxemia	Hypoxemia, tension pneumothorax, pneumomediastinum, PPHN
Extrapulmonary symptoms	GERD, poor weight gain, gastric dysmotility, developmental delay, single pulmonary vein narrowing, pectus carinatum	GERD, poor weight gain, oral aversion, severe neutropenia, developmental delay, asymmetric chest wall with right prominence	GERD, poor weight gain, axial hypotonia, formula intolerance, asymmetric chest wall with right prominence
ABCA3 deficiency treatment regimen	Monthly methylprednisolone† (6), azithromycin,‡ hydroxychloroquine‡	Monthly methylprednisolone† (12), azithromycin,‡ hydroxychloroquine‡	Monthly methylprednisolone† (8), azithromycin,‡ hydroxychloroquine‡
Additional therapies	Palivizumab, diuretics, PPI, erythromycin, amoxicillin-clavulanate	Palivizumab, PPI, filgrastim	Palivizumab, diuretics, iNO
Outcome at 1 year	Alive	Alive	Alive
Maximal respiratory support	HFOV	CPAP	HFOV
Current age, months	27 (24 corrected)	22	19
Current respiratory support	Nocturnal bilevel, daytime LFNC	HFNC	Nocturnal LFNC
Developmental assessment§: chronological age (developmental age)	24 months: motor (5-6 months); language (8-9 months); cognition (6-9 months)	14.8 months: motor (9 months); language (10 months); cognition (6 months)	13.3 months: motor (12 months); language (10.8 months); cognition (17 months)

CPAP, continuous positive airway pressure; *GERD*, gastroesophageal reflux; *HFNC*, high-flow nasal cannula; *HFOV*, high-frequency oscillatory ventilation; *iNO*, inhaled nitric oxide; *PIE*, pulmonary interstitial emphysema; *PPHN*, persistent pulmonary hypertension of the newborn; *PPI*, proton pump inhibitor; *RDS*, respiratory distress syndrome.
*Sibling genetic testing not performed.
†Methylprednisolone dosed at 10 mg/kg for 3 days.
‡Azithromycin and hydroxychloroquine dosed 5 mg/kg/d.
§Assessments were based on Bayley Scales of Infant Development III Gross Motor Domain, Clinical Linguistic & Auditory Scale, and the Cognitive Adaptive Test.

was supported with continuous positive airway pressure and 60% oxygen. After surfactant administration, supplemental oxygen was weaned to 30%. Although his lungs were clear to auscultation, he was notably tachypneic and grunting. His chest CT angiography demonstrated extensive ground glass opacities bilaterally (**Figure 1**, 2a). Genetic testing for heritable causes of neonatal respiratory distress revealed 2 variants in *ABCA3*: c.2274T>G (p.Y758*), a likely pathogenic nonsense variant and c.2745G>C (p.K915N), a missense variant in a codon that is described with other disease-causing variants. The family later disclosed that, several years prior, a full sibling sister died unexpectedly at 8 months of age of an unknown etiology (autopsy and genetic testing were not completed). The patient was started on monthly intravenous methylprednisolone pulse therapy (10 mg/kg/d for 3 days) at 1.5 months of age with improvement in work of breathing and was weaned from continuous positive airway pressure to LFNC support. He was discharged home at 2 months of age on 0.25 L/min. He had 8 admissions for increased work of breathing and hypoxemia over the following 6-month period. At 4 months of age, the infant developed profound neutropenia temporally associated with a respiratory syncytial virus infection. An evaluation for congenital and autoimmune causes of neutropenia was unrevealing, and the neutropenia ultimately resolved. A gastrostomy tube with Nissen fundoplication was performed at 8 months of age owing to failure to thrive, feeding intolerance, and severe gastroesophageal reflux. A repeat CT chest at 8 months of age demonstrated progression of disease and subpleural cysts with new areas of hyperinflated lobules (**Figure 1**, 2b). He was transitioned from LFNC to high-flow nasal cannula owing to persistent tachypnea and subsequently has not required further hospitalizations. He was started on hydroxychloroquine (5 mg/kg/d) and azithromycin (5 mg/kg/d) at 10 and 11 months of age, respectively. Monthly pulse methylprednisolone therapy was discontinued at 12 months of age, when the clinical benefit was unclear. A developmental assessment at 14 months of age revealed significant language, cognition, and motor delays (**Table I**), but he continues to gain milestones with ongoing outpatient developmental therapies. Linear gains are consistent around the third to fifth percentile. Weight gain, although improving remains, an ongoing issue, and he tracks below the third percentile at 22 months of age.

Patient 3 was a 4035-g male infant born at 41 weeks of gestation. Meconium-stained fluid was noted at delivery, but the infant was initially vigorous. He became dusky and hypoxemic at a few hours of life and placed on oxygen. A

chest radiograph revealed a right-sided tension pneumothorax and pneumomediastinum, which was evacuated by chest tube placement. An echocardiogram revealed right atrial enlargement, septal flattening, and bidirectional flow through a patent ductus arteriosus, consistent with persistent pulmonary hypertension of the newborn. He was intubated and started on inhaled nitric oxide and diuretics, which were later weaned. A follow-up echocardiogram at 2 weeks of age was normal. Extubation to continuous positive airway pressure took place on day of life 8 with progressive weaning to high-flow nasal cannula and LFNC support by 1.5 months of age. His lungs were clear to auscultation, but he was notably tachypneic. The patient underwent gastrostomy with Nissen fundoplication at 2 months of age before discharge home on 0.5 L/min LFNC support.

At 3 months of age, he was admitted for worsening tachypnea and hypoxemia. A CT of the chest revealed extensive ground glass opacities bilaterally, with smooth interlobular septal thickening and mild hyperexpansion (**Figure 1**, 3a). Owing to the progression of respiratory symptoms, genetic testing for causes of neonatal respiratory distress revealed 2 likely pathogenic variants in the *ABCA3* gene: c.1285+1G>A, a splice site variant, and c.599A>G (p.D200G), a missense variant. At 5 months of age, therapy with monthly intravenous methylprednisolone pulse therapy (10 mg/kg/d for 3 days) was initiated along with azithromycin (5 mg/kg/d) and hydroxychloroquine (5 mg/kg/d). During the first 6 months of therapy, he was able to wean off daytime supplemental oxygen with improvement in his tachypnea. A follow-up CT scan of the chest demonstrated improvement in diffuse ground glass opacities more prominent in the apices (**Figure 1**, 3b). After 6 doses of monthly methylprednisolone pulse therapy, he was transitioned to every other month therapy for 2 pulses before discontinuation of steroids, and a follow-up CT scan of the chest 4 months later revealed ongoing improvements in diffuse ground glass opacities (**Figure 1**, 3c). Oral feeds were reinitiated by 1 year of age with intensive feeding therapies, and gastrostomy tube was removed at 18 months of age. Weight gain is improving on a high-calorie diet, and his weight is tracking at the third percentile with linear growth consistently around the 50th percentile. Developmental assessment at 13 months of age revealed only a mild delay in language (**Table I**).

Genetic testing was performed by a College of American Pathologists accredited and Clinical Laboratory Improvement Amendments certified clinical diagnostic laboratories. Variant pathogenicity was reported by each clinical laboratory using variant classification standards and guidelines published by the American College of Medical Genetics and Genomics.[19] **Table II** (available at www.jpeds.com) provides a summary of variant details including in silico prediction models. **Figure 2** (available at www.jpeds.com) provides a visual representation of the variant locations within the ABCA3 protein. For patient 1, whole exome sequencing was performed by the Stanford Clinical Genomics Program. Parental samples were included in the exome analysis and confirmed that the *ABCA3* variants were in trans. For patient 2, a gene panel sequence analysis was performed using the Johns Hopkins DNA Diagnostic Laboratory Diffuse Lung Disease Gene Panel. Targeted parental testing by Sanger sequencing was performed on a research basis to confirm the ABCA3 variants were in trans. For patient 3, a gene panel sequence analysis was performed using the Blueprint Genetics Interstitial Lung Disease Panel (version 3). Targeted parental testing by Sanger sequencing confirmed that the ABCA3 variants were in trans. Of the 6 variants identified, 4 are novel and have not been previously reported in association with disease. All variants have minor allele frequencies less than 0.5% in gnomAD.

Lung biopsy was not pursued for any of the children based on alignment of phenotype to genetic results. In patient 3, bronchoscopy with bronchoalveolar lavage was completed to evaluate for the presence and structure of lamellar bodies under electron microscopy. The histopathology of bronchoalveolar lavage fluid showed numerous foamy macrophages with evidence of mixed inflammation. Ultrastructure evaluation by electron microscopy showed both normal lamellar bodies and abnormal lamellar bodies with an irregular whorl-like pattern with and without a dense core (**Figure 3**; available at www.jpeds.com).

Discussion

We describe 3 children with compound heterozygous variants in *ABCA3* with neonatal symptom onset and diagnosis via genetic testing revealing known and novel variants. This series underscores the usefulness of primary genetic testing for neonatal onset ABCA3 deficiency over the more invasive approach of lung biopsy. We also report success with the use of noninvasive respiratory support and a standardized approach to medical therapy that includes azithromycin, hydroxychloroquine and monthly pulse steroid therapy. We speculate that these therapies enhance the efficacy of endogenous surfactant function and decrease pulmonary inflammation in children with compound heterozygous variants in *ABCA3*.

Lung biopsy, the gold standard for the diagnosis of childhood diffuse lung disease, was not needed for diagnosis in this series.[20] Although lung biopsy is still recommended by expert opinion, the classic histologic findings of abnormal lamellar bodies with small, densely packed, fried egg appearance are nonspecific and may be absent in patients with ABCA3 deficiency.[10,20-24] Given the morbidity associated with these procedures, our series suggests that genetic testing in concert with advanced imaging can be used as the primary mode of diagnosis. In patients for whom genetic testing is unrevealing, further diagnostic procedures may be warranted.

Prior case series of ABCA3 deficiency have linked respiratory distress at birth with higher mortality regardless of genotype, although we found a favorable outcome even in infants presenting with respiratory distress at birth.[8,17] We speculate that the early initiation of pulse steroid therapy (within the

first 6 months of life) was beneficial in halting the rapid progression of pulmonary disease. We chose monthly pulse intravenous methylprednisolone to mitigate the side effects of chronic daily glucocorticoid therapy. Our use of steroids was informed by prior case reports demonstrating improved clinical symptoms and outcomes in patients with surfactant deficiencies treated with steroids, as well as consensus guidelines for childhood diffuse lung disease.[10,25-27] Steroids likely decrease the marked alveolar inflammation that characterizes surfactant deficiencies and upregulate ABCA3 transcription by glucocorticoid responsive elements in the promoter region of ABCA3.[28,29] The clinical response to steroids can occur quickly with a delayed radiographic response, as seen in patients 1 and 2.

The mechanism of azithromycin and hydroxychloroquine relative to surfactant deficiencies remains poorly understood.[30] Azithromycin may promote lung parenchymal repair by promoting autophagy of intracellular protein aggregates and play a role in surfactant homeostasis by altering phospholipid gene expression.[30,31] Hydroxychloroquine is commonly used in rheumatologic disorders for its immunomodulatory effects, and also mitigates inflammation by increasing the pH of intracellular vacuoles to disrupt antigen binding, disrupting calcium-dependent intracellular signaling, decreasing macrophage mediated cytokine production, and inhibiting toll like receptor signaling.[32-35] Given the variable response to these therapies in patients with ABCA3 deficiency, we suspect that the impact of glucocorticoids, azithromycin, and hydroxychloroquine on ABCA3 protein function is variant dependent.[17] Future studies using advanced gene editing technology in animal, organoid, and cell-based model systems could yield mechanistic insight into the degree of functional ABCA3 protein and response to therapy. Preliminary studies have shown this to be feasible for a small subset of variants.[36-38]

Extrapulmonary complications were noted in our patients. All 3 patients demonstrated poor weight gain before gastrostomy tube and Nissen fundoplication, with subsequent improvement in their growth trajectory. We observed developmental delay of varying severity in each patient which is likely attributable to the sequelae of chronic illness as opposed to a specific neurologic consequence of *ABCA3*.[26]

The limitations of this series include the small sample of patients followed at a single tertiary outpatient center and the relatively circumscribed duration of follow-up. However, the single-center experience permitted us to monitor the clinical response in greater detail. Two of the patients had a confounding clinical picture at presentation with severe prematurity in patient 1 and possible meconium aspiration syndrome in patient 3. Debate remains over when to perform genetic testing in infants with other causes for respiratory distress. In patients 1 and 3, we note that concerns about persistent respiratory symptoms over many months led to genetic testing. We also acknowledge that, although the clinical status of the 3 infants improved after the initiation of 3-drug therapy, it is unknown whether that was attributable to the medical regimen or the natural history of the lung disease. Given the highly variable clinical effects of partial function variants, with approximately one-third of children surviving past 1 year of life, functional studies of the specific variants might be particularly instructive.[8,17]

In conclusion, our series of patients with ABCA3 deficiency with presumed residual ABCA3 function highlights the importance of a establishing a genetic diagnosis for management and prognosis and adoption of therapeutic strategies that limit extrapulmonary complications. A standardized treatment regimen with early initiation of pulse steroid therapy, azithromycin, and hydroxychloroquine may be of benefit. Finally, the clinical course in infants with partial function *ABCA3* variants is highly variable, and supportive care and medical therapy is indicated. even in the presence of neonatal respiratory distress. Further studies should focus on individualized, precision based therapeutic regimens for patients with ABCA3 deficiency. ■

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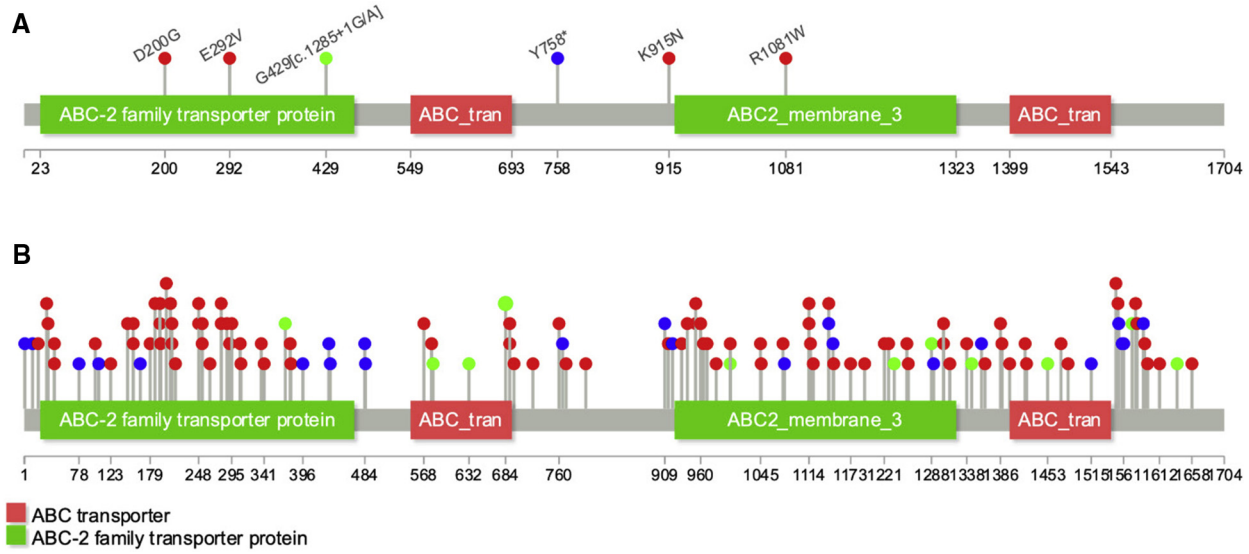

Figure 2. Mutation plots showing disease causing variants and their amino acid positions plotted along a representation of the ABCA3 protein. **A,** Plot shows variants described in this report. **B,** Plot shows single nucleotide coding variants cataloged in Human Gene Mutation Database as disease causing. *Blue dots* indicate truncating variants, *red dots* indicate missense variants, and *green dots* indicate splice site variants mapped to the nearest amino acid position. The splice site variant c.1285+1G>A is noted at amino acid position G429. *Green and red bars* represent protein domains conserved across multiple proteins within the ABC transporter family of proteins identified in the pfam database (http://pfam.xfam.org/). The *red bars* represent the ATP-binding domain.

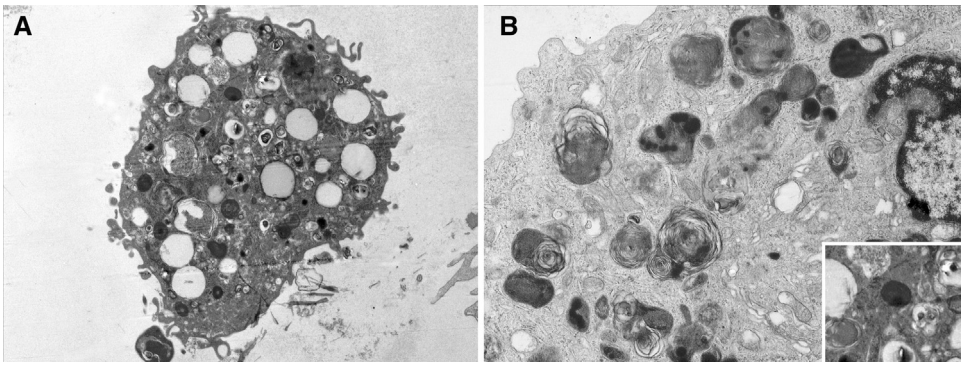

Figure 3. Electron microscopy of a macrophage in bronchoalveolar fluid containing atypical lamellar bodies in patient 3. **A,** Macrophage with a heterogeneous mixture of atypical lamellar bodies and other cytoplasmic contents. **B,** Heterogeneous lamellar bodies with regular and irregular concentric lamellae, and others with one or more electron dense bodies. *Inset,* A lamellar body with regular concentric lamellae and a large electron dense body characteristic of ABCA3 deficiency.

Table II. ABCA3 variant characteristics

Patients	*ABCA3* variant	Classification	Variant type	Reference SNP	MAF	Mutation taster	Predictsnp2	CADD	Prior publications	
									Authors	Year
1	c.875A>T (p.E292V)	Pathogenic	Missense	rs149989682	0.002337	Disease causing	Deleterious	34	Bullard et al[7]	2005
									Wambach et al[8]	2014
									Doan et al[10]	2008
									Copertino et al[11]	2012
									Wambach et al[12]	2012
									Turcu et al[13]	2013
									Soares et al[14]	2013
									Epaud et al[15]	2014
									Coghlan et al[16]	2014
									Kröner et al[17]	2017
									Akil and Fischer[18]	2018
1	c.3241C>T (p.R1081W)	VUS	Missense	rs369277188	0.000053	Disease causing	Deleterious	32	Wambach et al[8]	2014
2	c.2274T>G (p.Y758*)	Likely pathogenic	Nonsense	N/A	N/A	Disease causing	Deleterious	37	None	
2	c.2745G>C (p.K915N)	VUS	Missense	rs1459105468	0.000005	Disease causing	Neutral	24.1	None	
3	c.1285+1G>A	Likely pathogenic	Splice site	rs1366444219	0.000004	Disease causing	Deleterious	29	None	
3	c.599A>G (p.D200G)	Likely pathogenic	Missense	rs767050480	0.000004	Disease causing	Deleterious	25	None	

CADD, Combined Annotation Dependent Depletion tool (http://cadd.gs.washington.edu); *MAF*, minor allele frequency in the Genome Aggregation Database (GnomAD); *VUS*, variant of unknown significance.

CADD scores of >23.71 are predicted to be pathogenic and scores of <17.21 are predicted to be benign based on GAVIN (https://molgenis20.gcc.rug.nl/).

Classification is based on American College of Medical Genetics and Genomics guidelines followed by individual clinical laboratories: patient 1 through the Stanford Clinical Genomics Program, patient 2 through the Johns Hopkins DNA Diagnostic Laboratory, and patient 3 through Blueprint Genetics.