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ABCA3, A Key Player In Neonatal Respiratory Transition And Genetic Disorders Of The Surfactant System

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ABCA3 in congenital surfactant disorders

Abbreviations:

AEC2: alveolar epithelial type 2 cell; LB: lamellar body; SP-B: surfactant protein B;
SP-C: surfactant protein C; ABCA3: adenosine triphosphate binding cassette,
subfamily A, member 3; TTF-1: thyroid transcription factor 1; FOXA2: forkhead box
A2; C/EBP- α : CCAAT/enhancer-binding protein alpha; GATA6: GATA-binding
protein 6; NFATc3: nuclear factor of activated T-cells, cytoplasmic, calcineurin-
dependent 3; SREBP1a/c: sterol regulatory element-binding transcription factor
1a/c; ER: endoplasmic reticulum; PAP: pulmonary alveolar proteinosis; DIP:
desquamative interstitial pneumonitis; NSIP: non-specific interstitial pneumonitis;
proSP-B: surfactant protein B apoprotein; ProSP-C: surfactant protein C apoprotein;
RDS: respiratory distress syndrome; NKX2.1: NK2 homeobox 1; NBD: nucleotide-
binding domain; ECD: extracellular domain; ICL1: intracellular loop 1; EMT:
epithelial-mesenchymal transition; FEV1: forced expiratory volume in 1st second;
OR: odd ratio; COPD: chronic obstructive pulmonary disease; SIFT: sorting
intolerant from tolerant; PolyPhen2: polymorphism phenotyping version 2; ESP:
National Heart Lung and Blood Institute grand opportunity exome sequencing
project; 1000G: 1000 genomes.

Abstract

Genetic disorders of the surfactant system are rare diseases with a broad range of clinical manifestations, from fatal respiratory distress syndrome in neonates to chronic interstitial lung diseases in children and adults. *ABCA3* is a lung-specific phospholipid transporter critical for intracellular surfactant synthesis and storage in lamellar bodies. Its expression is developmentally regulated, peaking prior to birth under the influence of steroids and transcription factors. Bi-allelic mutations of the *ABCA3* gene represent the most frequent cause of congenital surfactant deficiency, indicating its critical role in lung function. Mutations affect surfactant lipid and protein processing, and lamellar bodies morphology, leading to partial or total surfactant deficiency. About 200 mutations have been reported, most of which are unique to individuals and families, which makes diagnosis and prognosis challenging. Various types of mutations, affecting different domains of the protein, account in part for phenotype diversity. Disease-causing mutations have been reported in most coding and some non-coding regions of the gene, but tend to cluster in the first extracellular loop and the second nucleotide-binding domain, leading to defective glycosylation and trafficking defects, and interfering with ATP binding and hydrolysis respectively. Mono-allelic damaging and benign variants are often subclinical but may act as disease modifiers in lung diseases such as respiratory distress syndrome of prematurity or associate with mutations in other surfactant-related genes. Diagnosis is complex but essential, and should combine pathology and ultrastructure studies on lung biopsy with broad-spectrum genetic testing of surfactant-related genes, made possible by recent technology advances in next-generation sequencing.

Introduction

Surfactant is a complex mixture of highly specific phospholipids and proteins synthesized by alveolar epithelial type II cells (AEC2), which plays a vital role in modulating alveolar surface tension and maintaining stable air space for gas exchanges in the lung. Surfactant is assembled and stored in lamellar bodies (LB), containing concentric layers of phospholipids associated with two small hydrophobic structural proteins, surfactant protein B (SP-B) and C (SP-C), with an ultrastructural aspect designated pseudomyelin. Surfactant is secreted by exocytosis into the alveolar lumen, where it spreads at the air-liquid interface of the alveolar fluid and forms a dynamic phospholipid film, expanding in monolayers and contracting into complex tridimensional subphase structures over repeated respiratory cycles, a process highly dependent on SP-B and SP-C [1]. Surfactant production is developmentally regulated by several nuclear factors and hormones, and numerous proteins are implicated in its synthesis, trafficking and homeostasis [2].

Adenosine triphosphate-binding cassette subclass A member 3 (ABCA3) is a transmembrane lipid carrier that belongs to the ABC transporter family, a large group of proteins highly conserved through species and involved in multiple physiological processes, with over 48 encoding genes in human. ABC subclass A members are mainly involved in sterols and lipids transport, and rare variants in their encoding genes are associated with several diseases in human, such as Tangier's disease (ABCA1), Stargardt's disease and recessive cone-rod dystrophy and retinitis pigmentosa (ABCA4), and harlequin ichthyosis (ABCA12), highlighting their critical role in various physiologic processes [3]. ABCA3 is a 150 kDa, 1704 amino acid protein encoded by an 80 kb gene composed of 33 exons -30 of which are transcribed- located at the 16p13.3 genomic locus. ABCA3 is mostly expressed in the lung, although it can be detected in other tissues including trachea, liver, stomach, kidney, adrenal gland, pancreas, platelets and brain [4]. Bi-allelic *ABCA3* mutations were found in 2004 to be the most frequent cause of congenital surfactant deficiency in neonates with fatal respiratory distress syndrome (RDS) [5]. These evidences support a primary role in respiration for ABCA3, which represents the focus of this review. However, the recently discovered association of mono-allelic *ABCA3* mutations with congenital cataract-microcornea syndrome [6] and the multidrug resistance induced by de-regulated ABCA3 expression in leukemia cells [7] suggest a much broader role in human physiology and disease.

Expression, regulation and function of the *ABCA3* gene

In human, ABCA3 is expressed at the highest level in the alveolar epithelium prior to birth, indicating that its gene is developmentally regulated. The ABCA3 protein can be readily detected by 26-27 weeks of gestation in normal fetuses, but as early as 23-24 weeks when lung inflammation is present, its expression increasing with lung infection and bronchopulmonary dysplasia [4, 8] . Several

factors and hormones regulate ABCA3 expression. Glucocorticoids such as dexamethasone up-regulate *ABCA3* gene expression at the transcriptional level through a glucocorticoid-responsive element located in the promoter region [8]. Thyroid transcription factor 1 (TTF-1), a nuclear factor encoded by the *NKX2.1* gene, regulating over 23 lung genes and playing a switch role in epithelial maturation and surfactant synthesis, induces the expression of ABCA3 as well as SP-A, SP-B and SP-C [4, 9]. STAT3, an IL-6 signal transducer, also induces ABCA3 expression and possibly mediates its response to inflammation [10]. Other factors binding to the distal promoter region and enhancing *ABCA3* transcription include FOXA2, C/EBP- α , GATA-6 and NFATc3 [11]. *ABCA3* transcription is also inducible by SREBP1a and -c proteins through a specific 5' regulatory region containing several sterol-responsive elements, linking lipid homeostasis with LB formation [11].

ABCA3 is predominantly associated with the LB membrane in AEC2 [10, 12]. The protein folds in the endoplasmic reticulum (ER) and is glycosylated in the Golgi, a process essential for its stability [13], then is trafficked towards multivesicular bodies, which represent lysosome-derived precursors of LBs, and undergoes N-terminus cleavage [14]. ABCA3 has the property of mediating phospholipid uptake and generating LBs in the presence of lipids in pulmonary cell lines, through a process involving ATP binding and hydrolysis [15, 16]. Four mice models of *ABCA3* targeted disruption were reported, which concurred in showing near-total absence of surfactant in alveolar spaces, deep reduction of phosphatidylcholine and phosphatidylglycerol -the two major surfactant phospholipids-, loss of pseudomyelin-like LBs, and early neonatal death in null animals, thus establishing a critical role for ABCA3 in lipid homeostasis and surfactant homeostasis [17-20]. Subsequently, a mouse model of conditional *ABCA3* deletion in AEC, resulting in partial survival and emphysema in adulthood, demonstrated its role in surfactant homeostasis modulation beyond the neonatal period [21].

ABCA3 deficiency in human

ABCA3 deficiency was initially identified in full-term infants who died from unexplained respiratory distress syndrome is caused by bi-allelic loss-of-function mutations of the *ABCA3* gene [5], and is the most frequent of the congenital surfactant deficiencies [22]. Subsequently, *ABCA3* mutations were also identified in infants with persistent or recurrent tachypnea [23, 24] or pulmonary arterial hypertension, older children and young adults with idiopathic interstitial lung disease [25-27], and even in adults with idiopathic pulmonary fibrosis and emphysema [28], indicating a broader and probably underrecognized spectrum of manifestations and severity. Radiologic features are nonspecific and vary with age, including diffuse ground-glass opacities, interlobular septa thickening, honeycombing and fibrosis, parenchymal cysts and emphysema [26, 29]. Histopathology is also variable, showing combinations of AEC2 hyperplasia, intra-alveolar eosinophilic material (PAP-like) and alveolar macrophages and desquamated epithelial cells (DIP-like), and inter-alveolar septa thickening, fibrosis

and inflammation (NSIP-like) [30, 31]. Conversely, transmission electron microscopy typically reveals decreased number/size or complete lack of mature LBs, and the presence of pleomorphic, electron-dense organelles with tightly packed membrane structures and denser aggregates, occasionally referred to as fried-egg morphology [30-32], and corresponding to abnormal LBs as a result of defective lipid transport (Figure 1). These anomalies are highly specific and distinct from those observed in other surfactant-related genetic disorders such as SP-B, SP-C and TTF-1 deficiencies [31-33]. Hence transmission electron microscopy analysis of lung biopsy may significantly contribute to directing towards or confirming genetic diagnosis, and should be performed whenever possible.

These ultrastructural anomalies result in a profound disruption of surfactant composition and function, with a decreased amount of phosphatidylcholine and other phospholipids and much higher alveolar surface tension values compared to controls and to SP-B deficiency subjects [34]. Surfactant proteins processing is altered as a consequence of *ABCA3* mutations. Mature SP-B peptide is overall diminished, and found aggregated in the dense core of *ABCA3*-deficient LBs. Uncleaved SP-B apoprotein (proSP-B) accumulates in large amount in LBs and leaks in the alveolar space. Some proSP-C -but no mature SP-C peptide- is detectable in LBs [30]. These data show that altered SP-B and SP-C processing and routing also occur in *ABCA3* deficiency and, likely, contribute to surfactant homeostasis disruption and respiratory symptoms.

Molecular genetics and phenotype correlations

Among the over 200 rare variants reported to date, most are unique to individuals and families and carried in compound heterozygosis, which makes it challenging to interpret individual genotypes. Homozygous mutations are presumably due to consanguinity [5], although uniparental disomy of chromosome 16 has been reported in some cases [35]. The basis for the large phenotypic variability is not known but may be related to several factors, including the net residual protein function, the type and severity of the mutation, the activation of intracellular stress pathways, the subject's genetic background, other unaccounted genetic variants, or environmental modifiers such as infections. Several types of coding and non-coding variants have been described: nonsense or missense single nucleotide variations, in-frame or frameshift insertions or deletions, intron splice-site mutations, large deletions and others. In the largest series published to date [36], the compound of two null alleles (frameshift, nonsense) resulted uniformly in neonatal onset and early lethal/transplant outcome, whereas missense, splicing variants and in-frame deletions/insertions were less correlated with poor outcome, hence potentially less deleterious, allowing some residual functionality of the protein. In this series, a significant subset of the subjects (11%) carried two or more mutations on the same allele (in *cis*), highlighting the importance of confirming, by parental inheritance determination or other methods, that compound mutations identified in a given individual should be on opposite alleles (in *trans*) in order to

result in ABCA3 deficiency, as this is a recessive disease. [36]. In another large series of children with chronic interstitial lung disease, whereas the most frequent variants were mutations of *SFTPC* (the SP-C-encoding gene), 10 infants were compound heterozygous carriers of the same p.Glu292Val *ABCA3* mutation, which appears to be more frequent and associated with a less severe phenotype [25].

The finding of a single, monoallelic mutation in a patient with a congenital surfactant deficiency phenotype raises the question of genetic mechanism, since ABCA3 deficiency is a recessive disorder and heterozygous family members are typically asymptomatic [36]. One possible explanation is the presence of an undiscovered *ABCA3* variant in the opposite allele. Complete loss of ABCA3 expression in the lung was documented in some affected subjects in whom no mutations could be identified, suggesting that yet-unexplored non-coding regions may be implicated in lung disease [37]. Large deletions spanning entire exons or the whole gene, undetectable by classic exon-based Sanger sequencing, have also been described in infants with RDS [38]. A single intronic mutation generating a new splice site (IVS25-98T) was found to be relatively frequent even though undetectable with the classic exon-restricted sequencing method, and was the co-etiology of respiratory symptoms in seven infants in whom initial genetic studies had revealed only one heterozygous mutation [39]. Alternatively, we and others have observed mono-allelic *ABCA3* mutations associated with a mutation in other genes affecting surfactant homeostasis on the opposite allele following a transheterozygosity model, such as *NKX2.1* (the TTF-1-encoding gene) [40] or *SFTPC* [33, 41]. Overall, complex molecular genetics mechanisms are involved, calling for broader gene coverage and genomic approaches for diagnostic purposes.

Molecular and cellular mechanisms of disease

The ABCA3 protein is constituted of two 6-unit transmembrane complexes, two nucleotide-binding domains (NBD1 and 2) oriented toward the cytosol and six so-called extracellular domains (ECD1-6), actually oriented towards LB's inner compartment [3]. Many deleterious mutations found in human tend to cluster on ECD1 and NBD2, highlighting the functional relevance of these domains (Figure 2). Mutagenesis experiments simulating severe genotypes showed that proteins with ECD1 and 4 and C-terminus mutations remained located in the ER and exhibited impaired glycosylation, while NBD1 and 2 mutant proteins led to decreased ATP binding and/or hydrolysis [42]. NBD2 mutants fail to accumulate phosphatidylcholine into organelles, which supports the critical role of ATP binding and hydrolysis for active lipid transport [19]. Conversely, a mutation located in transmembrane domain 11 led to proper protein trafficking to the lysosome membrane, but defective expression in LBs, suggesting that functional transmembrane complexes are a requirement for LB biogenesis and maturation [43]. The p.Glu292Val mutation, located on the intracellular loop 1 (ICL1) that mediates ATP-driven conformational change of the 1st transmembrane complex,

results in moderately impaired lipid transfer into LBs, and is indeed associated with a less severe, chronic interstitial lung disease phenotype [44].

Certain ECD1 mutants were shown to determine an ER stress response by activating chaperone proteins of the HSP70 complex, and to trigger apoptosis, which may represent an additional lung injury mechanism in the complex pathogenesis of ABCA3 deficiency [45]. Another potential mechanism, typically observed in pediatric or adult interstitial lung disease and lung fibrosis, is epithelial-mesenchymal transition (EMT). In a lung epithelial cell line mutagenesis experiment reproducing two common human *ABCA3* mutations, disruption of certain epithelial markers such as E-cadherin, and induction of mesenchymal markers such as matrix metalloproteinase 2 resulted in EMT, with the cell line gradually assuming a fibroblast-like aspect and behavior [46], a phenomenon that likely accounts for the intense interstitial cell proliferation and fibrosis observed in most *ABCA3* mutation carriers. Respiratory syncytial virus, typically implicated in ILD exacerbations in subjects with congenital surfactant deficiencies [33], potentiated the effect of *ABCA3* mutations on EMT. These observations corroborate the intense interstitial proliferation and fibrosis observed in certain patients with ABCA3 deficiency.

Taken together, these results indicate multiple pathophysiological processes involved in ABCA3 deficiency, and may contribute to better understanding genotype-phenotype correlations and identifying mutation-specific therapeutic targets in the future.

Heterozygous carriers and common variants

The impact of mono-allelic *ABCA3* rare and common variants on surfactant homeostasis and their connection to human disease are currently poorly defined. Heterozygous ABCA3 missense variants are present in 1.5–3.7% of African and European descent individuals [47]. The p.Glu292Val allele, which is carried by 1.3% of the Danish general population, resulted in a 5% FEV1 decrease and a 1.9 OR for COPD in a 10,000 subjects cohort from Copenhagen, but the findings were not replicated in the general population [48]. The impact of p.Glu292Val was also studied in a German cohort of >3000 very-low-birth-weight infants, showing no measurable difference on pulmonary outcome [49]. In a Finnish cohort of 267 infants <32 weeks gestational age, a common synonymous *ABCA3* variant (p.Phe353Phe) was shown to correlate with RDS and bronchopulmonary dysplasia incidence [50]. However, in a large US-based cohort (over 500 term/late preterm infants) that underwent much broader screening of *ABCA3* and other surfactant-related genes through next generation sequencing, single mono-allelic missense *ABCA3* rare variants resulted associated with a 10% increased risk for transient RDS incidence and severity [47], whereas common synonymous variants did not increase neonatal RDS risk [51].

In a cohort of infants with severe diffuse lung disease, we have observed a distinct ultrastructural phenotype in mono-allelic *ABCA3* mutation carriers, consisting of smaller LB diameter compared to controls, but larger LB number compared to bi-allelic mutants (figure 1), although the exon-targeted sequencing approach represents a technical limitation compared to other studies [31]. Our findings match the heterozygous phenotype observed in mice with *ABCA3* haploinsufficiency (*ABCA3*^{+/-}) in one of the published knock-out mouse models [19].

Overall these data suggest that non-critical *ABCA3* loss of function may interfere with surfactant homeostasis and contribute to multifactorial lung disease; with a threshold for pathogenicity depending of co-morbidities, genetic and environmental factors.

Diagnosis and therapeutic approaches

The uniqueness of *ABCA3* variants and complexity of their molecular genetic mechanisms, as well as the potential overlap with other surfactant-related genetic diseases strongly support the use of broad-spectrum, high-resolution, high-throughput approaches, such as next generation sequencing with custom panels targeted to surfactant-related genes or whole-exome sequencing [36]. However the high yield of variants revealed by these technologies increases the need for validation techniques. Mutation type and position, in-silico validation tools (impact predictors of amino acid substitution such as SIFT or PolyPhen2, human variation database such as ESP or 1000G, species conservation databases and others), inheritance patterns (such as targeted mutation sequencing or “trio” exome sequencing comparing parental and proband genotypes) should be complemented by strict phenotypical characterization. Albeit invasive, lung biopsy with electron microscopy may provide evidences of surfactant homeostasis disruption and show typical ultrastructural alterations pointing to specific genetic defects [31], thus allowing care decisions and meaningful family counseling.

Although lung transplantation remains the only option for end-stage lung disease and carries ethical challenges given its poor long-term prognosis, some less severe forms of ILD may benefit from pharmacological treatment. Inflammatory-modulating agents such as pulse methylprednisolone, hydroxychloroquine and azithromycin are occasionally used individually or in combination [29], but their use is not supported by proper prospective randomized clinical trials. A deeper understanding of pathogenic cellular mechanisms could lead to the development of mutation-specific drugs, such as proteasome inhibitors in mutations affecting glycosylation site [13], or chemical chaperones in those affecting intracellular trafficking [43].

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References

- 1 Parra, E. and Perez-Gil, J. (2015) Composition, structure and mechanical properties define performance of pulmonary surfactant membranes and films. *Chem Phys Lipids*. **185**, 153-175
- 2 Andreeva, A. V., Kutuzov, M. A. and Voyno-Yasenetskaya, T. A. (2007) Regulation of surfactant secretion in alveolar type II cells. *Am J Physiol Lung Cell Mol Physiol*. **293**, L259-271
- 3 Kaminski, W. E., Piehler, A. and Wenzel, J. J. (2006) ABC A-subfamily transporters: structure, function and disease. *Biochim Biophys Acta*. **1762**, 510-524
- 4 Stahlman, M. T., Besnard, V., Wert, S. E., Weaver, T. E., Dingle, S., Xu, Y., von Zychlin, K., Olson, S. J. and Whitsett, J. A. (2007) Expression of ABCA3 in developing lung and other tissues. *J Histochem Cytochem*. **55**, 71-83
- 5 Shulenin, S., Noguee, L. M., Annilo, T., Wert, S. E., Whitsett, J. A. and Dean, M. (2004) ABCA3 gene mutations in newborns with fatal surfactant deficiency. *N Engl J Med*. **350**, 1296-1303
- 6 Chen, P., Dai, Y., Wu, X., Wang, Y., Sun, S., Xiao, J., Zhang, Q., Guan, L., Zhao, X., Hao, X., Wu, R. and Xie, L. (2014) Mutations in the ABCA3 gene are associated with cataract-microcornea syndrome. *Investigative ophthalmology & visual science*. **55**, 8031-8043
- 7 Chapuy, B., Koch, R., Radunski, U., Corsham, S., Cheong, N., Inagaki, N., Ban, N., Wenzel, D., Reinhardt, D., Zapf, A., Schweyer, S., Kosari, F., Klapper, W., Truemper, L. and Wulf, G. G. (2008) Intracellular ABC transporter A3 confers multidrug resistance in leukemia cells by lysosomal drug sequestration. *Leukemia*. **22**, 1576-1586
- 8 Yoshida, I., Ban, N. and Inagaki, N. (2004) Expression of ABCA3, a causative gene for fatal surfactant deficiency, is up-regulated by glucocorticoids in lung alveolar type II cells. *Biochem Biophys Res Commun*. **323**, 547-555
- 9 Kolla, V., Gonzales, L. W., Gonzales, J., Wang, P., Angampalli, S., Feinstein, S. I. and Ballard, P. L. (2007) Thyroid transcription factor in differentiating type II cells: regulation, isoforms, and target genes. *Am J Respir Cell Mol Biol*. **36**, 213-225
- 10 Matsuzaki, Y., Besnard, V., Clark, J. C., Xu, Y., Wert, S. E., Ikegami, M. and Whitsett, J. A. (2008) STAT3 regulates ABCA3 expression and influences lamellar body formation in alveolar type II cells. *Am J Respir Cell Mol Biol*. **38**, 551-558
- 11 Besnard, V., Xu, Y. and Whitsett, J. A. (2007) Sterol response element binding protein and thyroid transcription factor-1 (Nkx2.1) regulate Abca3 gene expression. *Am J Physiol Lung Cell Mol Physiol*. **293**, L1395-1405

- 12 Mulugeta, S., Gray, J. M., Notarfrancesco, K. L., Gonzales, L. W., Koval, M., Feinstein, S. I., Ballard, P. L., Fisher, A. B. and Shuman, H. (2002) Identification of LBM180, a lamellar body limiting membrane protein of alveolar type II cells, as the ABC transporter protein ABCA3. *J Biol Chem.* **277**, 22147-22155
- 13 Beers, M. F., Zhao, M., Tomer, Y., Russo, S. J., Zhang, P., Gonzales, L. W., Guttentag, S. H. and Mulugeta, S. (2013) Disruption of N-linked glycosylation promotes proteasomal degradation of the human ATP-binding cassette transporter ABCA3. *Am J Physiol Lung Cell Mol Physiol.* **305**, L970-980
- 14 Engelbrecht, S., Kaltenborn, E., Griese, M. and Kern, S. (2010) The surfactant lipid transporter ABCA3 is N-terminally cleaved inside LAMP3-positive vesicles. *FEBS Lett.* **584**, 4306-4312
- 15 Nagata, K., Yamamoto, A., Ban, N., Tanaka, A. R., Matsuo, M., Kioka, N., Inagaki, N. and Ueda, K. (2004) Human ABCA3, a product of a responsible gene for *abca3* for fatal surfactant deficiency in newborns, exhibits unique ATP hydrolysis activity and generates intracellular multilamellar vesicles. *Biochem Biophys Res Commun.* **324**, 262-268
- 16 Matsumura, Y., Sakai, H., Sasaki, M., Ban, N. and Inagaki, N. (2007) ABCA3-mediated choline-phospholipids uptake into intracellular vesicles in A549 cells. *FEBS Lett.* **581**, 3139-3144
- 17 Fitzgerald, M. L., Xavier, R., Haley, K. J., Welti, R., Goss, J. L., Brown, C. E., Zhuang, D. Z., Bell, S. A., Lu, N., McKee, M., Seed, B. and Freeman, M. W. (2007) ABCA3 inactivation in mice causes respiratory failure, loss of pulmonary surfactant, and depletion of lung phosphatidylglycerol. *J Lipid Res.* **48**, 621-632
- 18 Hammel, M., Michel, G., Hoefler, C., Klatfen, M., Muller-Hocker, J., de Angelis, M. H. and Holzinger, A. (2007) Targeted inactivation of the murine *Abca3* gene leads to respiratory failure in newborns with defective lamellar bodies. *Biochem Biophys Res Commun.* **359**, 947-951
- 19 Cheong, N., Zhang, H., Madesh, M., Zhao, M., Yu, K., Dodia, C., Fisher, A. B., Savani, R. C. and Shuman, H. (2007) ABCA3 is critical for lamellar body biogenesis in vivo. *J Biol Chem.* **282**, 23811-23817
- 20 Ban, N., Matsumura, Y., Sakai, H., Takanezawa, Y., Sasaki, M., Arai, H. and Inagaki, N. (2007) ABCA3 as a lipid transporter in pulmonary surfactant biogenesis. *J Biol Chem.* **282**, 9628-9634
- 21 Besnard, V., Matsuzaki, Y., Clark, J., Xu, Y., Wert, S. E., Ikegami, M., Stahlman, M. T., Weaver, T. E., Hunt, A. N., Postle, A. D. and Whitsett, J. A. (2010) Conditional deletion of *Abca3* in alveolar type II cells alters surfactant homeostasis in newborn and adult mice. *Am J Physiol Lung Cell Mol Physiol.* **298**, L646-659
- 22 Somaschini, M., Noguee, L. M., Sassi, I., Danhaive, O., Presi, S., Boldrini, R., Montrasio, C., Ferrari, M., Wert, S. E. and Carrera, P. (2007) Unexplained neonatal respiratory distress due to congenital surfactant deficiency. *J Pediatr.* **150**, 649-653, 653 e641
- 23 Kinane, T. B., Shailam, R. and Mark, E. J. (2011) Case records of the Massachusetts General Hospital. Case 37-2011. A 9-month-old boy with recurrent tachypnea and respiratory distress. *N Engl J Med.* **365**, 2221-2228

- 24 Prestridge, A., Wooldridge, J., Deutsch, G., Young, L. R., Wert, S. E., Whitsett, J. A. and Noguee, L. (2006) Persistent tachypnea and hypoxia in a 3-month-old term infant. *J Pediatr.* **149**, 702-706
- 25 Bullard, J. E., Wert, S. E., Whitsett, J. A., Dean, M. and Noguee, L. M. (2005) ABCA3 mutations associated with pediatric interstitial lung disease. *Am J Respir Crit Care Med.* **172**, 1026-1031
- 26 Doan, M. L., Guillerman, R. P., Dishop, M. K., Noguee, L. M., Langston, C., Mallory, G. B., Sockrider, M. M. and Fan, L. L. (2008) Clinical, radiological and pathological features of ABCA3 mutations in children. *Thorax.* **63**, 366-373
- 27 Kitazawa, H., Moriya, K., Niizuma, H., Kawano, K., Saito-Nanjo, Y., Uchiyama, T., Rikiishi, T., Sasahara, Y., Sakamoto, O., Setoguchi, Y. and Kure, S. (2013) Interstitial lung disease in two brothers with novel compound heterozygous ABCA3 mutations. *Eur J Pediatr.* **172**, 953-957
- 28 Epaud, R., Delestrain, C., Louha, M., Simon, S., Fanen, P. and Tazi, A. (2014) Combined pulmonary fibrosis and emphysema syndrome associated with ABCA3 mutations. *Eur Respir J.* **43**, 638-641
- 29 Flamein, F., Riffault, L., Muselet-Charlier, C., Pernelle, J., Feldmann, D., Jonard, L., Durand-Schneider, A. M., Coulomb, A., Maurice, M., Noguee, L. M., Inagaki, N., Amselem, S., Dubus, J. C., Rigourd, V., Bremont, F., Marguet, C., Brouard, J., de Blic, J., Clement, A., Epaud, R. and Guillot, L. (2012) Molecular and cellular characteristics of ABCA3 mutations associated with diffuse parenchymal lung diseases in children. *Hum Mol Genet.* **21**, 765-775
- 30 Brasch, F., Schimanski, S., Muhlfeld, C., Barlage, S., Langmann, T., Aslanidis, C., Boettcher, A., Dada, A., Schroten, H., Mildenerger, E., Prueter, E., Ballmann, M., Ochs, M., Johnen, G., Griese, M. and Schmitz, G. (2006) Alteration of the pulmonary surfactant system in full-term infants with hereditary ABCA3 deficiency. *Am J Respir Crit Care Med.* **174**, 571-580
- 31 Citti, A., Peca, D., Petrini, S., Cutrera, R., Biban, P., Haass, C., Boldrini, R. and Danhaive, O. (2013) Ultrastructural characterization of genetic diffuse lung diseases in infants and children: a cohort study and review. *Ultrastructural pathology.* **37**, 356-365
- 32 Edwards, V., Cutz, E., Viero, S., Moore, A. M. and Noguee, L. (2005) Ultrastructure of lamellar bodies in congenital surfactant deficiency. *Ultrastructural pathology.* **29**, 503-509
- 33 Peca, D., Boldrini, R., Johannson, J., Shieh, J. T., Citti, A., Petrini, S., Salerno, T., Cazzato, S., Testa, R., Messina, F., Onofri, A., Cenacchi, G., Westermarck, P., Ullman, N., Cogo, P., Cutrera, R. and Danhaive, O. (2015) Clinical and ultrastructural spectrum of diffuse lung disease associated with surfactant protein C mutations. *Eur J Hum Genet*
- 34 Garmany, T. H., Moxley, M. A., White, F. V., Dean, M., Hull, W. M., Whitsett, J. A., Noguee, L. M. and Hamvas, A. (2006) Surfactant composition and function in patients with ABCA3 mutations. *Pediatr Res.* **59**, 801-805
- 35 Hamvas, A., Noguee, L. M., Wegner, D. J., Depass, K., Christodoulou, J., Bennetts, B., McQuade, L. R., Gray, P. H., Deterding, R. R., Carroll, T. R., Kammesheidt, A., Kasch, L. M., Kulkarni, S. and Cole, F. S. (2009) Inherited surfactant deficiency caused by

- uniparental disomy of rare mutations in the surfactant protein-B and ATP binding cassette, subfamily a, member 3 genes. *J Pediatr.* **155**, 854-859 e851
- 36 Wambach, J. A., Casey, A. M., Fishman, M. P., Wegner, D. J., Wert, S. E., Cole, F. S., Hamvas, A. and Nogee, L. M. (2014) Genotype-phenotype correlations for infants and children with ABCA3 deficiency. *Am J Respir Crit Care Med.* **189**, 1538-1543
- 37 Gower, W. A., Wert, S. E., Ginsberg, J. S., Golan, A., Whitsett, J. A. and Nogee, L. M. (2010) Fatal familial lung disease caused by ABCA3 deficiency without identified ABCA3 mutations. *J Pediatr.* **157**, 62-68
- 38 Henderson, L. B., Melton, K., Wert, S., Couriel, J., Bush, A., Ashworth, M. and Nogee, L. M. (2013) Large ABCA3 and SFTPC deletions resulting in lung disease. *Annals of the American Thoracic Society.* **10**, 602-607
- 39 Agrawal, A., Hamvas, A., Cole, F. S., Wambach, J. A., Wegner, D., Coghill, C., Harrison, K. and Nogee, L. M. (2012) An intronic ABCA3 mutation that is responsible for respiratory disease. *Pediatr Res.* **71**, 633-637
- 40 Peca, D., Petrini, S., Tzialla, C., Boldrini, R., Morini, F., Stronati, M., Carnielli, V. P., Cogo, P. E. and Danhaive, O. (2011) Altered surfactant homeostasis and recurrent respiratory failure secondary to TTF-1 nuclear targeting defect. *Respir Res.* **12**, 115
- 41 Bullard, J. E. and Nogee, L. M. (2007) Heterozygosity for ABCA3 mutations modifies the severity of lung disease associated with a surfactant protein C gene (SFTPC) mutation. *Pediatr Res.* **62**, 176-179
- 42 Matsumura, Y., Ban, N., Ueda, K. and Inagaki, N. (2006) Characterization and classification of ATP-binding cassette transporter ABCA3 mutants in fatal surfactant deficiency. *J Biol Chem.* **281**, 34503-34514
- 43 Cheong, N., Madesh, M., Gonzales, L. W., Zhao, M., Yu, K., Ballard, P. L. and Shuman, H. (2006) Functional and trafficking defects in ATP binding cassette A3 mutants associated with respiratory distress syndrome. *J Biol Chem.* **281**, 9791-9800
- 44 Matsumura, Y., Ban, N. and Inagaki, N. (2008) Aberrant catalytic cycle and impaired lipid transport into intracellular vesicles in ABCA3 mutants associated with nonfatal pediatric interstitial lung disease. *Am J Physiol Lung Cell Mol Physiol.* **295**, L698-707
- 45 Weichert, N., Kaltenborn, E., Hector, A., Woischnik, M., Schams, A., Holzinger, A., Kern, S. and Griese, M. (2011) Some ABCA3 mutations elevate ER stress and initiate apoptosis of lung epithelial cells. *Respir Res.* **12**, 4
- 46 Kaltenborn, E., Kern, S., Frixel, S., Fragnet, L., Conzelmann, K. K., Zarbock, R. and Griese, M. (2012) Respiratory syncytial virus potentiates ABCA3 mutation-induced loss of lung epithelial cell differentiation. *Hum Mol Genet.* **21**, 2793-2806
- 47 Wambach, J. A., Wegner, D. J., Depass, K., Heins, H., Druley, T. E., Mitra, R. D., An, P., Zhang, Q., Nogee, L. M., Cole, F. S. and Hamvas, A. (2012) Single ABCA3 Mutations Increase Risk for Neonatal Respiratory Distress Syndrome. *Pediatrics.* **130**, e1575-1582
- 48 Baekvad-Hansen, M., Nordestgaard, B. G. and Dahl, M. (2012) Heterozygosity for E292V in ABCA3, lung function and COPD in 64,000 individuals. *Respir Res.* **13**, 67
- 49 Hartel, C., Felderhoff-Muser, U., Gebauer, C., Hoehn, T., Kribs, A., Laux, R., Moller, J., Segerer, H., Teig, N., von der Wense, A., Wieg, C., Stichtenoth, G., Herting, E.,

- Gopel, W. and German Neonatal, N. (2012) ATP-binding cassette member A3 (E292V) gene mutation and pulmonary morbidity in very-low-birth-weight infants. *Acta Paediatr.* **101**, 380-383
- 50 Karjalainen, M. K., Haataja, R. and Hallman, M. (2008) Haplotype analysis of ABCA3: association with respiratory distress in very premature infants. *Ann Med.* **40**, 56-65
- 51 Wambach, J. A., Wegner, D. J., Heins, H. B., Druley, T. E., Mitra, R. D., Hamvas, A. and Cole, F. S. (2014) Synonymous ABCA3 variants do not increase risk for neonatal respiratory distress syndrome. *J Pediatr.* **164**, 1316-1321 e1313

Figure legends

Figure 1. Pathology and ultrastructural alterations with bi- and mono-allelic *ABCA3* mutations

A. Lung tissue in optical microscopy in a 1-month old infant with homozygous *ABCA3* frameshift mutation (complete *ABCA3* deficiency), hematoxylin-eosin, 20x, showing diffuse interstitial thickening, AEC2 hyperplasia and intra-alveolar eosinophilic material. Insert: 1-month old control. B. Masson trichrome, 40x, showing interstitial fibrosis (blue). C. Immunohistochemistry labeling with anti-CD45 monoclonal antibody, 40x, showing numerous alveolar macrophages and interstitial lymphocyte infiltrate. D/G. AEC2 transmission electron microscopy in control, with abundant LBs displaying a normal pseudomyelin aspect of intracellular surfactant. E/H. AEC2 in a 4-month old infant with interstitial lung disease carrying a mono-allelic *ABCA3* missense mutation, showing numerous smaller LBs at the apical pole. F/I. AEC2 in a 1-month old infant with homozygous frameshift mutation, with no detectable LBs. J. Confocal optical microscopy with anti-*ABCA3* antibody (red) and DAPI (blue) staining, 1-month old control, showing normal *ABCA3* granular expression at AEC2 apical pole. K. 1-month old infant with homozygous frameshift mutation, showing no detectable *ABCA3* expression. L. 1-month old infant with 2 compound heterozygous mutations affecting ECD1, showing increase *ABCA3* signal in lung tissue. M. Same subject as L, showing diffuse *ABCA3* cytoplasmic accumulation. J-M images published with permission of N. Inagaki and N. Ban.

Figure 2. Distribution of aminoacid substitutions due to *ABCA3* mutations

The *ABCA3* protein structure includes two transmembrane complexes composed of 6 domains each, coupled with two nucleotide-binding domains (NBD1 and 2) each containing two Walker domains A and B facing the cytosol, and one major and two minor extracellular domains for each transmembrane complex (ECD1-3 and ECD 4-6) facing the lamellar body (LB) inner compartment. Over 160 published aminoacid variations (red) (from Wambach J.A. et al. [36]) are distributed throughout the molecule but tend to cluster on ECD1 and NBD2, highlighting the functional importance of these two domains. In the context of an ongoing pediatric interstitial lung disease project [31, 33] we identified 15 different missense mutations in 14 infants, mostly localized on ECD1 (blue), NBD2 (orange) but also TMC2 (pink) and NBD1 (green). Based on a figure from M.F. Beers and S. Mulugeta [13], reproduced with permission.



