ORIGINAL ARTICLE

Genotype–Phenotype Correlations for Infants and Children with ABCA3 Deficiency

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Abstract

Rationale: Recessive mutations in the ATP-binding cassette transporter A3 (*ABCA3*) cause lethal neonatal respiratory failure and childhood interstitial lung disease. Most *ABCA3* mutations are private.

Objectives: To determine genotype–phenotype correlations for recessive *ABCA3* mutations.

Methods: We reviewed all published and unpublished *ABCA3* sequence and phenotype data from our prospective genetic studies of symptomatic infants and children at Washington and Johns Hopkins Universities. Mutations were classified based on their predicted disruption of protein function: frameshift and nonsense mutations were classified as "null," whereas missense, predicted splice site mutations, and insertion/deletions were classified as "other." We compared age of presentation and outcomes for the three genotypes: null/null, null/other, and other/other.

Measurements and Main Results: We identified 185 infants and children with homozygous or compound heterozygous *ABCA3* mutations and lung disease. All of the null/null infants presented with respiratory failure at birth compared with 75% of infants with null/other or other/other genotypes ($P = 0.00011$). By 1 year of age, all of the null/null infants had died or undergone lung transplantation compared with 62% of the null/other and other/other children ($P < 0.0001$).

Conclusions: Genotype–phenotype correlations exist for homozygous or compound heterozygous mutations in *ABCA3*. Frameshift or nonsense *ABCA3* mutations are predictive of neonatal presentation and poor outcome, whereas missense, splice site, and insertion/deletions are less reliably associated with age of presentation and prognosis. Counseling and clinical decision making should acknowledge these correlations.

Keywords: surfactant; childhood interstitial lung disease; neonatal respiratory distress

ATP-binding cassette transporter A3 (ABCA3) is a member of a large family of proteins that hydrolyze ATP to move substrates across biologic membranes (1). The 80-kb gene encoding ABCA3 (*ABCA3*, NM_001089.2, Gene ID 21) is located on chromosome 16 and encodes a 1,704 amino acid protein that contains two membrane-spanning domains and two nucleotide-binding domains. ABCA3 is expressed in alveolar type II cells and localized to the membrane of lamellar bodies, which are lysosomally derived, intracellular, storage organelles where the final assembly and processing of the pulmonary surfactant occurs (2, 3). ABCA3 is important for lamellar body biogenesis (4), and the transport of

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At a Glance Commentary

Scientific Knowledge on the Subject: Recessive mutations in *ABCA3* cause lethal neonatal respiratory failure and childhood interstitial lung disease. Most *ABCA3* mutations are private. Pulmonary phenotype varies in terms of age of presentation, disease severity, and progression.

What This Study Adds to the Field: Recessive frameshift or nonsense *ABCA3* mutations are predictive of neonatal presentation and poor outcome, whereas missense, splice site, and insertion/deletions are less reliably associated with age of presentation and prognosis.

phospholipids (phosphatidylcholine and phosphatidylglycerol) into the lamellar body for assembly of the pulmonary surfactant (5–7).

Recessive, loss-of-function mutations in *ABCA3* were first identified among racially and ethnically diverse full-term neonates who died from severe neonatal respiratory distress syndrome (RDS) (8). Those ABCA3-deficient neonates developed severe respiratory failure shortly after birth, which required significant ventilatory support and often extracorporeal membrane oxygenation (8). Some infants responded transiently to surfactant replacement therapy, but failed to improve after the first week of life (9). Lamellar bodies from ABCA3-deficient patients were small with densely packed phospholipid membranes and eccentrically placed, dense inclusion bodies (8, 10, 11), although cases with more normal-appearing lamellar bodies have also been reported (12, 13). Surfactant deficiency in infants with lethal *ABCA3* mutations was suggested by reduced phosphatidylcholine content and failure to lower surface tension in bronchoalveolar lavage or tracheal aspirates (14). Mutations in *ABCA3* were subsequently identified among older children with interstitial lung disease (13, 15).

Most of the more than 150 distinct *ABCA3* mutations among infants and children with lung disease are unique to individuals and families. Mutations associated with ABCA3 deficiency are distributed throughout the gene and include nonsense, frameshift, missense, splice site, and insertions/deletions. Lung disease resulting from *ABCA3* mutations is expressed in an autosomal-recessive manner, requiring mutations on both alleles. However, among individuals with two *ABCA3* mutations, the pulmonary phenotype varies in terms of age of presentation, disease severity, and progression. The basis for this variability is not known, but may be related to the amount of residual protein function (no functional protein vs. decreased protein function), type of mutation (trafficking vs. ATP hydrolysis/impaired lipid transport) (16), activation of intracellular stress pathways (17), or other genetic (18) or environmental modifiers. To determine whether genotype–phenotype correlations exist for *ABCA3* mutations, we analyzed genotypes and clinical outcomes of cases (both unpublished and previously published) of ABCA3-deficient children identified through prospective studies. Some of the results of these studies have been previously reported in the form of an abstract (19).

Methods

Subject Selection

The subjects in this study were identified among symptomatic infants and children suspected of having genetic surfactant dysfunction and referred for candidate gene sequencing in research laboratories at Washington University School of Medicine (A.H., F.S.C.) and Johns Hopkins University School of Medicine (L.M.N.). We only included those subjects who met a strict case definition including severe neonatal respiratory failure or childhood interstitial lung disease, and homozygous or compound heterozygous *ABCA3* mutations. We did not include subjects in whom only a single *ABCA3* mutation was identified in this primary analysis, even if their phenotype was very consistent with surfactant dysfunction, because we could not fully characterize their genotype. Parents provided informed consent for participation in the study. These studies were reviewed and approved by the human research protection offices at both institutions.

Mutational Analysis

DNA was isolated from blood (n = 180), frozen lung tissue (n = 4), and saliva (n = 1) using commercially available kits as previously described (15, 20). Sequence analysis of the coding exons and flanking introns was performed for all subjects as previously described (15, 20).

Classification of Mutations

We classified frameshift and nonsense mutations as "null" mutations because they are predicted to result in truncated or nonfunctional proteins. We classified missense, splice site, and in-frame insertion/deletions as "other" mutations because their effect on protein function is more difficult to predict.

We used the Exome Variant Server (EVS; NHLBI Exome Sequencing Project, Seattle, WA; [http://evs.gs.washington.edu/EVS/] release ESP6500 [accessed 2014 April]), a database of 6,500 adult individuals of European and African descent from up to 18 different US populations, who participated in longitudinal cardiovascular- and pulmonary-related research, to determine population-based frequencies of *ABCA3* mutations.

Statistics

We used chi-square and Fisher's exact test to compare the clinical characteristics of children with *ABCA3* mutations, including age of presentation and outcome at 1 year of age (alive, lung transplantation, or death).

Results

We resequenced all coding exons of *ABCA3* for 632 subjects referred for candidate gene analysis for whom surfactant protein B deficiency and *SFTPC* mutations were excluded as the cause of their lung disease. We identified 185 infants and children with lung disease and homozygous or compound heterozygous *ABCA3* mutations (Table 1). The subjects represent diverse racial and ethnic backgrounds, and there was no sex predilection. Most subjects of Middle Eastern descent had a history of consanguinity. Of all subjects with recessive *ABCA3* mutations (all genotypes), 149 (81%) presented with respiratory symptoms at birth, 25 (14%) presented during infancy (first year of

age), and 9 (5%) presented during childhood (presentation age unknown for two subjects) (Table 1). By 1 year of age, 108 (62%) subjects died, 17 (10%) underwent lung transplantation, and 50 (28%) were alive (outcome unknown for 10 subjects) (Table 1).

We identified 47 (25%) subjects with two frameshift or nonsense mutations (null/null genotype); 38 (21%) subjects with one complete loss of function mutation and one missense, splice site, or insertion/deletion (null/other genotype); and 100 (54%) subjects with two missense, splice site, or insertion/deletions (other/other genotype) (Table 1; *see* Tables E1–E3 in online supplement). There were no differences in sex or race among the three genotype groups. There were no differences in the age of presentation or outcome at 1 year of age for children with null/other and other/other mutations (*see* Table E4). Therefore, these two genotype groups were combined for statistical purposes and compared with the null/null subjects. All subjects with null/null genotypes presented with respiratory symptoms at birth as compared with 75% of subjects with null/other and other/other genotypes ($P = 0.00011$) (Table 1, Figure 1). Among subjects with null/other and other/other genotypes, 18% presented with respiratory symptoms later during infancy and 7% presented during childhood (Table 1, Figure 1).

By 1 year of age, all of the subjects with null/null genotypes for whom outcome information was available had died or undergone lung transplantation as compared with 62% of the null/other and other/other subjects ($P < 0.0001$) (Table 1, Figure 2). Of the subjects who presented with respiratory symptoms at birth with any of the three genotypes, only 16% were alive at 1 year of age without lung transplantation (22 of 139; 10 outcomes unknown). Conversely, of the 25 infants who presented beyond the newborn period, but at less than 1 year of age, 18 (72%) were alive without transplantation at 1 year of age.

Clinical and genetic data for individual subjects are included in Tables E1 and E2 in the online supplement. We found 75 (41%) subjects were homozygous and 110 (59%) were compound heterozygous for *ABCA3* mutations. Although most mutations (80%) were private and observed in only a single family, 37 mutations were identified in more than one unrelated individual. Five mutations were identified in more than five unrelated individuals: (*1*) p.E292V, (*2*) p.Y1515X, (*3*) IVS25–98 C > T, (*4*) F1203del, and (*5*) c.3997_3998delAG. p.E292V is the most common *ABCA3* mutation associated with childhood interstitial lung disease (13, 15, 21) and was identified among 16 subjects (Subjects 53, 129–143; *see* Table E2). One infant was homozygous for p.E292V, presented with neonatal respiratory failure, and died shortly after birth (Subject 129; *see* Table E2). The remaining 15 individuals were compound heterozygous for p.E292V and had variable outcomes (*see* Table E2). All of the 19 Middle Eastern infants with the p.Y1515X/p.Y1515X genotype for whom complete clinical information was known presented with severe neonatal RDS and died before 3 months of age (Subjects 22–40; *see* Table E1; two infant outcomes unknown). Four Hispanic neonates were homozygous for the splice site mutation IVS25–98 C > T (22),

Table 1. Characteristics of Subjects with *ABCA3* Mutations

	Null/Null (*n = 47*)	Null/Other and Other/Other* (*n = 138*)	*P* Value
Sex			
Female	23	63	0.70
Male	24	75	
Race/ethnicity			
White	41	116	0.32
Hispanic	5	22	
Middle Eastern	20	13	
African descent	1	9	
Asian	5	8	
Other	0	5	
Age of presentation			
Birth	47	102	0.00011
Infant (≤1 yr)	0	25	
Childhood (>1 yr)	0	9	
Unknown	0	2	
Outcome at 1 yr			
Died (≤1 yr)	43	65	<0.0001
Transplanted (≤1 yr)	2	15	
Alive (>1 yr)	0	50	
Unknown	2	8	

*There were no differences in the sex distribution, race or ethnicity, age of presentation, or outcome at 1 year for children with null/other and other/other genotypes; therefore, these two groups were combined for statistical purposes and compared with the null/null infants (*see* Table E4).

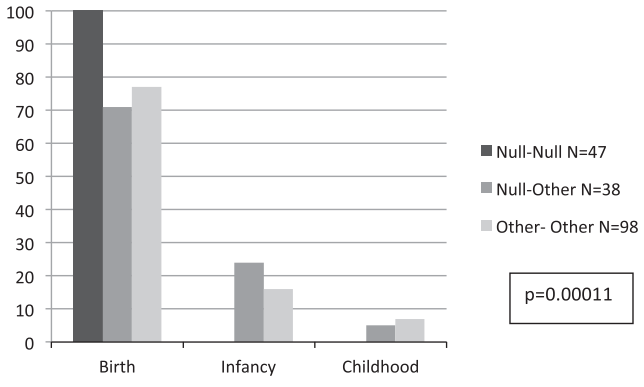

Figure 1. Age of presentation for subjects with ABCA3 mutations. Data are presented as percentage of infants.

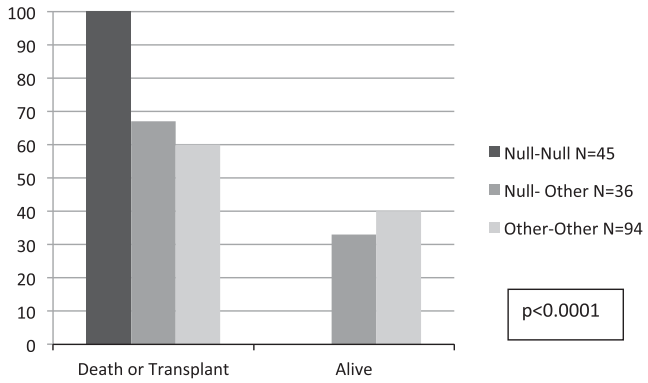

Figure 2. Outcome at 1 year of age by ABCA3 genotype. Data are presented as percentage of infants.

presented with severe neonatal RDS, and died during infancy (Subjects 169–172; *see* Table E2; one infant outcome unknown). Five additional infants were compound heterozygotes for the IVS25–98 C > T mutation and had variable outcomes (Subjects 173–177; *see* Table E2). Eight subjects were compound heterozygous for the F1203del mutation and had variable outcomes ranging from death during infancy, transplantation, and survival into adulthood (Subjects 67, 77, 92–97; *see* Table E2). The c.3997_3998delAG mutation was found in subjects from widely distributed geographic and ethnic backgrounds, including two siblings of Mexican descent (subjects 14 and 15; *see* Table E1) and a white child from Australia (Subject 60; *see* Table E2). There were 45 (24%) subjects with an affected sibling with the same genotype including one set of twins. Although siblings exhibited minor discordance in their early clinical courses, they were generally concordant for outcome at 1 year of age (death or transplant vs. alive). Two or more *ABCA3* variants were identified to be in *cis* among 21 subjects based on parental testing (Table 2). Fifteen subjects had a phenotype consistent with surfactant dysfunction and only a single mutation in *ABCA3* identified; although these patients were not included in the primary analysis, their mutations are presented in the online supplement (*see* Table E5).

Discussion

This study includes the largest collective experience of subjects with ABCA3 deficiency reported to date and demonstrates a consistent genotype–phenotype correlation for patients homozygous or compound heterozygous for frameshift and/or nonsense (null) mutations. All subjects with the null/null genotype presented with respiratory distress at birth and died or underwent lung transplantation before 1 year of age, thereby emphasizing the critical role of ABCA3 in neonatal surfactant metabolism and lung function. Given the uniformly poor prognosis for infants with null/null genotypes, expeditious discussions with families about lung transplantation or compassionate care should occur to begin the transplant evaluation if desired.

The outcome of subjects with null/other and other/other genotypes is more challenging to predict because the mechanisms by which ABCA3 function might be disrupted are likely to be far more diverse. Of the subjects with null/other or other/other genotypes who presented with respiratory distress at birth, 23% were alive at 1 year without transplantation (22 of 94 infants, eight outcomes unknown). This observation suggests that, in the absence of the null/null genotype, definitive assessment of prognosis is difficult even in infants with severe neonatal- onset disease. Also highlighting that the prognosis is more variable for these genotypes is the finding that most infants who presented after the newborn period were alive at 1 year (72%). Some have survived into adolescence (23) and even adulthood (24). Although all siblings in our study were concordant for outcome at 1 year of age (death or transplant vs. alive) (*see* Tables E1 and E2), discordant outcomes between siblings have been reported (25, 26). Thus, treatment options should be discussed on an individual patient basis and should be informed with genotype data. Further study of factors (genetic, environmental, and treatment) that contribute to more prolonged survival should be conducted.

This study also has significant implications for ABCA3 genetic testing. Because 21 (11%) subjects had two *ABCA3* mutations in *cis*, confirmation that mutations are on opposite alleles is critical before making irreversible clinical decisions and for providing counseling for future reproductive decisions. Determination of mutation orientation is most easily accomplished by demonstrating that each parent carries one of the mutations. Because 25% (34 of 136; two age of presentations unknown) of subjects with null/other and other/other genotypes presented beyond the newborn period, clinical sequencing for ABCA3 deficiency should be strongly considered for patients with less severe, but unexplained lung disease, as has been recently recommended (2013 American Thoracic Society statement) (27). Although this study did not include subjects presenting with interstitial lung disease beyond childhood, a recent report of an adult patient with ABCA3 deficiency and the increased availability of clinical *ABCA3* sequencing suggests that additional cases in adults will be identified (24).

Most *ABCA3* mutations identified in this study are private and not listed in the NHLBI EVS or other public databases, further amplifying the lack of precedent for predicting prognosis. Although 16 individuals are homozygous and three individuals are heterozygous for the F1203del mutation in the EVS, the lack of phenotype information and the variance of this mutation from Hardy-Weinberg equilibrium in the EVS population make the significance of these findings uncertain. Because all eight subjects with the F1203del mutation in our study were heterozygous for a second disease-causing mutation, our data suggest that F1203del is a disease-causing mutation. The c.3997_3998delAG mutation was found in subjects from geographically and ethnically diverse backgrounds, and c.3997delA has been reported by other research and clinical laboratories (28–30). Although haplotypes have not been examined, a recurrent mutation at this site may be the

Table 2. Alleles with *ABCA3* Variants in *Cis*

Allele	Number of Subjects with Allele
R43C-P1653L	1
D115E-**D253H**	1 (2 alleles, 1 subject homozygous)
V129M-V1495M	1
W179C-P770L	3 (3 subjects heterozygous)
E195K-R1271Q	1
R280C-**Q1589X**	2 (3 alleles, 1 subject homozygous, 1 subject heterozygous)
R288K-**S693L**	2 (2 subjects heterozygous)
c.1474_1475insT-D953N	4 (3 siblings homozygous, 1 subject heterozygous)
P766S-**L960F**	4 (4 subjects heterozygous)
H778R-**L1252P**	1
A54T-R1482W-IVS25-98 C > T	1 subject (segregation of variants not determined because of lack of parental DNA for analysis)

Bold indicates predominantly suspected pathogenic variants.

mechanism underlying these observations. Seven unrelated individuals from diverse ethnic and geographic origins had a mutation in codon 43 (p.R43C, p.R43H, and p.R43L, Subjects 92 and 93 [siblings], 101, 104, 105, 118, 173, 174) and these mutations have been reported in other ABCA3-deficient patients from diverse geographic locations (12, 28, 29), suggesting that this codon may be particularly susceptible to mutation.

In vitro mechanistic studies of a limited number of ABCA3 mutations (*see* Table E3) have demonstrated that ABCA3 function may be disrupted either through intracellular misrouting of ABCA3 protein or through defective ATP hydrolysis and impaired phospholipid transport into the lamellar body (16). These studies have demonstrated that not only is it difficult to predict the effect on protein function based on a mutation's location, but also that the type of mutation does not explain the variable presentations or outcomes. Thus, in the absence of lung tissue to examine gene expression and high-throughput surrogate cell systems to study the effects of these mutations in depth, it is difficult to functionally refine the categorization of "other" mutations. Understanding the mechanisms by which these mutations result in variable severity of disease and identifying compounds that can alter the mutant ABCA3 protein expression or function are paramount.

There are several important limitations of our study. Subjects were included because they were suspected of having neonatal respiratory failure or childhood interstitial lung disease caused by surfactant dysfunction and usually had severe disease. Because of this ascertainment bias, subjects with less severe disease or other phenotypes would have been less likely to be enrolled, which could include individuals with null/null genotypes. Conversely, some of the missense mutations identified in our subjects may represent benign variants and not disease-causing mutations, and some of the subjects with milder disease may not have been functionally ABCA3 deficient, but rather had a different mechanism for their lung disease. Heterozygous *ABCA3* missense variants were identified in 1.5–3.7% of African and European descent individuals from control and population-based subjects in a recent study (31). We may also have not included all subjects with ABCA3 deficiency in our study populations because of our strict case definition. Subjects with clinical history and histology findings consistent with ABCA3 deficiency, but who had only a single or no mutations detected by sequencing, were not included in the primary analysis because we could not be certain of the presence of a second disease-causing mutation (*see* Table E5). Deep intronic or promoter region mutations affecting ABCA3 expression or large deletions, as have recently been reported (22, 32, 33), would not have been detected by our sequencing approach, and thus subjects with such mutations may have been missed. Analysis of parental DNA was used to confirm that identified *ABCA3* mutations were in *trans*; however, DNA was not available from the parents of every subject and thus we may have included subjects with two variants in *cis* and thus only one identified *ABCA3* mutated allele. Finally, long-term subject follow-up information is limited, so the current status of many of the individuals who survived is unknown, although several individuals are known to be living into their teenage years and beyond. We also do not have specific information about therapeutic interventions and how such empiric treatments (or the lack thereof) might have modified disease outcomes. Understanding the factors associated with prolonged survival could contribute to the development of more refined and individualized therapeutic approaches.

In conclusion, infants homozygous or compound heterozygous for frameshift or nonsense *ABCA3* mutations presented with neonatal respiratory failure and died within the first year of life without lung transplantation. The presentation and outcome for infants and children with null/other and other/other genotypes were more variable and less predictable, and therefore infants with such genotypes require more complex decision-making. Because these infants with a relatively less severe pulmonary phenotype had at least one mutation that could result in some residual ABCA3 function, strategies aimed at augmenting functional ABCA3 might improve their outcome. Continued studies of the underlying genetic mechanisms, current options for therapeutic interventions, and identification of novel compounds to restore mutant protein function are needed. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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