

ORIGINAL ARTICLE

The classification of ATP-binding cassette subfamily A member 3 mutations using the cystic fibrosis transmembrane conductance regulator classification system

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ABSTRACT

Importance: The ATP-binding cassette subfamily A member 3 (ABCA3) protein plays a vital role in surfactant homeostasis. Mutations in the *ABCA3* gene lead to the development of interstitial lung disease. In the most severe manifestation, mutations can lead to a fatal respiratory distress syndrome in neonates. ABCA3 belongs to the same ATP-binding cassette transporter superfamily as the cystic fibrosis transmembrane conductance regulator (CFTR), the gene that causes cystic fibrosis.

Objective: To classify ABCA3 mutations in a manner similar to CFTR mutations in order to take advantage of recent advances in therapeutics.

Methods: Sequence homology between the CFTR protein and the ABCA3 protein was established. The region of CFTR that is a target for the new potentiator class of drugs was of particular interest. We performed a literature search to obtain all published mutations that were thought to be disease causing. We classified these mutations using the established CFTR classification system. When possible, we drew on previous experimental classification of ABCA3 mutations.

Results: Although the proteins share the same overall structure, only a 19% identity was established between CFTR and ABCA3. The CFTR therapeutic target region has a 22% homology with the corresponding ABCA3 region. Totally 233 unique protein mutations were identified. All protein mutations were classified and mapped to a schematic diagram of the ABCA3 protein.

Interpretation: This new classification system for ABCA3, based on CFTR classification, will likely aid further research of clinical outcomes and identification of mutation-tailored therapeutics, with the aim for improving clinical care for patients with ABCA3 mutations.

KEYWORDS

ATP-binding cassette transporter subfamily A, Cystic fibrosis, Genetics, Interstitial lung diseases, Newborn, Respiratory distress syndrome

INTRODUCTION

Surfactant, a lipoprotein rich substrate produced by type II alveolar cells, lines the airways, reducing the surface tension and preventing lower airways collapse

during exhalation. Genetic mutations affecting surfactant production and metabolism result in severe respiratory disease. Mutations in the ATP-binding cassette subfamily A member 3 (ABCA3) were first recognized in 2004 as causing fatal surfactant deficiency in neonates who

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presented with severe respiratory disease.¹ Since then, *ABCA3* mutations have been linked to numerous clinical cases presenting with a range of pulmonary disease severity.

Members of the ATP-binding cassette (ABC) transporter superfamily function to allow transport across cellular membranes. Members of the ABC transporter family share structural similarities; two transmembrane regions and two ATP-binding domains (also called nucleotide binding domains). *ABCA3*, a 1 704 amino acid (AA) protein, is a member of the ABC super-family and is essential for lung surfactant metabolism, and therefore normal lung function.² Mutations in the encoding gene, *ABCA3* (NM_001089.2, OMIM 601615), lead to altered *ABCA3* function that, in turn, can lead to changes in surfactant composition.³ *ABCA3* appears to be necessary for the formation of lamellar bodies, which store and secrete surfactant into the alveolar space.⁴ Histologically, attenuation of *ABCA3* function results in changes in the lung parenchyma where the alveolar space is filled with periodic acid–Schiff (PAS) staining material as well as macrophages, as well as proliferation of type II alveolar cells.⁵

Another member of the ABC transporter family, the cystic fibrosis transmembrane regulator (CFTR), is a 1 480-AA membrane protein, which, when mutated, results in cystic fibrosis. CFTR (NP_000483.3) is unique among the ABC transporter proteins, as it is the only chloride channel. This chloride channel spans epithelial membranes of exocrine tissues throughout the body, and defects in the CFTR chloride channel result in elevated sweat chloride concentration, sinopulmonary inspissation, and blocked, atretic pancreatic ducts and vas deferens.⁶ Understanding the genetic defect underlying cystic fibrosis has significantly advanced the life expectancy over the past decade.⁶ One of the tools available in advancing CFTR research has been the Cystic Fibrosis Mutation Database.⁷ This loci database has collected and organized over 2 000 mutations. This work has led to the formation of the Clinical and Functional Translation of CFTR (CFTR2) database under the direction of the Cystic Fibrosis Foundation and Johns Hopkins School of Medicine. The database has allowed for the correlation of genetic mutations to clinical outcomes and treatments.⁸ The most common of these mutations have been organized into functional classes, based on defects in transcription, cellular processing, concentration and function.⁹ These classifications have helped clinicians understand and categorize defects on a molecular level and enabled the development of class-specific treatments. The most successful example of class-specific, molecular-targeted therapy in cystic fibrosis is ivacaftor, a chloride channel potentiator, which significantly improves lung function in patients afflicted with a mutation in CFTR that results in a non-

functional chloride channels at the cell membrane.^{10,11} The medical breakthrough of ivacaftor has established the precedence for successful, mutation-specific therapy.

Given that *ABCA3* and CFTR are both ABC transporters, and that classifying CFTR mutation has allowed significant advances in clinical care, we attempted to classify all *ABCA3* mutations that have been reported in the literature. Previously, severe presentations have been found to be associated with frameshift and nonsense *ABCA3* mutations, while missense mutations, splice site mutations and insertions/deletions were not associated with increased phenotypic severity.¹² Knowing that mutation type impacts phenotype, we classified the existing mutations, as this could help predict disease outcomes if the same mutations were found in other patients. However, there has been no attempt, to our knowledge, to organize the known mutations into general classes. The purpose of this study was to use the structure and the standards developed by CFTR research and apply them to reported *ABCA3* mutations. First, we identified sequence homology between *ABCA3* and CFTR. Then, using the CFTR classification system, we classified all the published, disease-causing *ABCA3* mutations. In particular, we focused on the nucleotide binding domains of CFTR and *ABCA3*, as these are the sites of action of the potential therapies that could be relevant for the treatment of disease associated with mutations in *ABCA3*. To our knowledge, this is the first attempt to genetically classify *ABCA3* mutations, and we hope that this will allow the progress of precision medicine as it pertains to disease related to *ABCA3* mutations.

METHODS

Sequence comparison

To establish the homology between the *ABCA3* and CFTR proteins, we aligned the complete protein sequences using NCBI Blastp.^{13,14} This alignment was performed with a standard initial BLASTP parameters. We then further refined our search by comparing specific locations within CFTR to similar locations in *ABCA3* including nucleotide binding domains and Walker A and B domains.

Article selection in order to define all the published mutations

A PubMed search was performed using the terms “*ABCA3*” and “mutation”, which generated 119 results. Broad, non-specific search terms were used in order to be more inclusive. The search was completed in November 2017, and contained only results that were available at this time. These results were manually filtered by reviewing the abstracts. Papers were finally selected that reported *ABCA3* mutations that were linked to specific patients and

TABLE 1 CFTR Classes¹²

Class	Outcome	Predominant Mutation Type	Domain Locations	Common Mutations	Phenotype Severity
I	Defective Protein Synthesis, No CFTR Protein. No Cl ⁻ Transport.	Nonsense, Frameshift, Splice site	NBD1, NBD2, TM1	G542X, W1282X, 621+1G>T, 394delTT, 1717-1G>A	High
II	Abnormal protein folding, processing and trafficking. No Cl ⁻ Transport	Missense	NBD1, NBD2	F508del, N1303K	High
III	Defective Regulation. Non-functional CFTR in membrane. No Cl ⁻ Transport	Missense	NBD1	G551D, R560T	High
IV	Decreased conductance. Minimal Cl ⁻ Transport.	Missense	TM1	R117H, R347P	Reduced
V	Reduced Synthesis. Minimal Cl ⁻ Transport.	Missense, Splice site	Intron	A455E, 3849+10kbC>T	Reduced

were written in English.

Classification criteria

CFTR mutations were grouped based on functional analysis, using accepted CFTR classification (Table 1); class I: defective protein synthesis, class II: abnormal protein folding and trafficking, class III: non-functional CFTR in the membrane, class IV: decreased chloride conductance and class V: reduced protein synthesis. Class VI was recently identified as having increased channel turnover. When the trafficking defect of the $\Delta F508$ CFTR mutation was corrected and the protein was successfully transported to the cell surface, this mutated molecule was found have increased turnover. Thus $\Delta F508$ CFTR mutation is both a class II and VI mutation.¹⁵

In order to classify the *ABCA3* mutations, published functional analyses were used to define functional effects of mutations.^{4,16-18} Of the 233 unique mutations collated, 14 had been experimentally classified. If only DNA mutations were reported, we attempted to find the protein mutation using MutationTaster.¹⁹ When the mutations effect on the protein was unknown, misfolding was assumed to be the default protein abnormality. The mutations were also classified by their mutation type: frameshift, nonsense, missense, splice site, insertion or deletion.

Using the *ABCA3* mutations that were experimentally classified by Matsumura et al¹⁷ and the location of each *ABCA3* mutation, missense mutations that had similar locations were assumed to have similar effects on protein function. Much of the classification work was an iterative process based on the experimental data that was available, the mutation type and location. For example, missense mutations in the *ABCA3* extracellular domain 1 (ECD1) location were classified as Type II mutations because specific *ABCA3* missense mutations in ECD1 were experimentally shown to lead

to the accumulation *ABCA3* protein in the endoplasmic reticulum.^{17,20} The same process was extended to mutations in extracellular domain 2 (ECD2). However, frameshift and nonsense mutations that were located in the nucleotide binding domain 1 (NBD1) were classified as Type I mutations as mutations of this type result in defective protein synthesis.¹⁷

RESULTS

Sequence homology

In order to compare specific regions, we first established the locations of conserved regions within each protein. As both CFTR and *ABCA3* are members of the ABC transporter family, albeit in different subfamilies, they share the same general structure (Figure 1). Both have four core domains, two transmembrane domains, and two nucleotide binding domains. The locations of the nucleotide binding domains of *ABCA3* and CFTR were established using the NCBI Protein Database.^{13,14} Both the *ABCA3* and CFTR nucleotide binding domains contain Walker A and Walker B motifs, which are conserved motifs commonly found in nucleotide binding proteins.²¹ However, two major differences were identified: *ABCA3* contains two extracellular domains, while CFTR contains an R domain, which plays an essential role in channel gating.

We identified the location of conserved domains within *ABCA3* and CFTR. NBD1 of *ABCA3* spans from AA 467 to 925. This is located between the end of the first transmembrane region (TM1) to the start of the second transmembrane region (TM2). The ATP-binding cassette domain, a functional region within the nucleotide-binding domain, spans from AA 530 to 751. Walker A domain is located from AA 566 to 573, and Walker B domain from AA 685 to 690. For the second nucleotide-

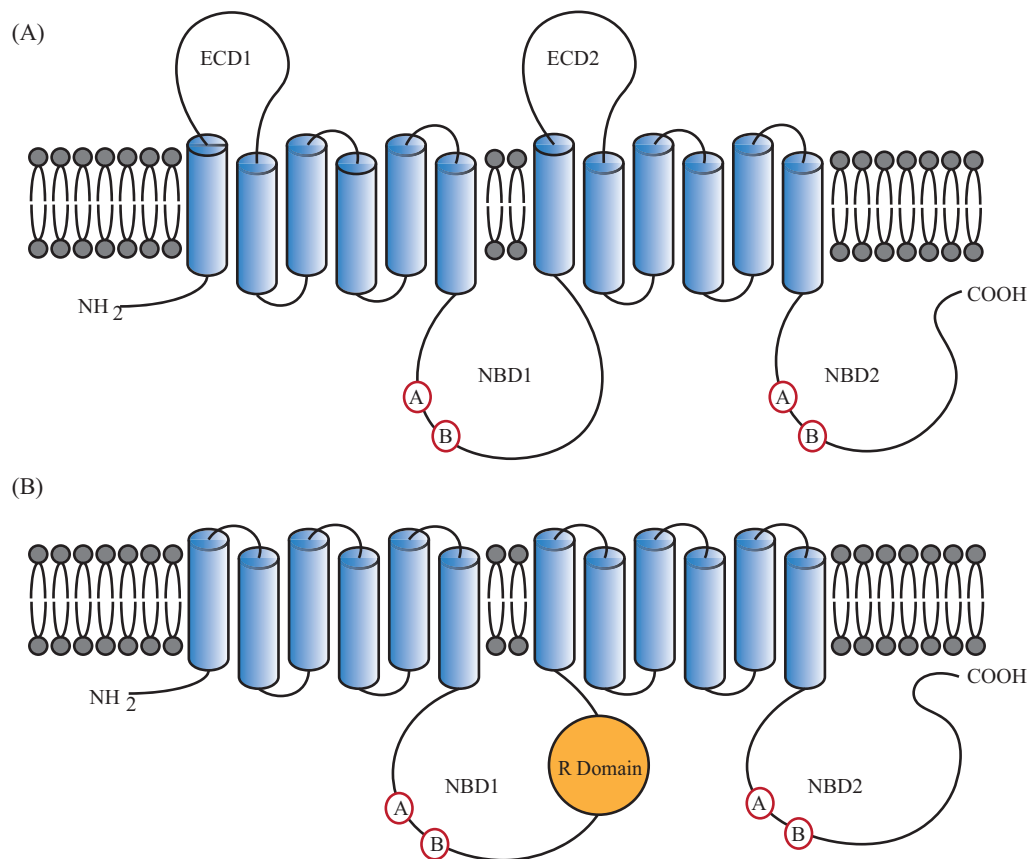


FIGURE 1 Schematic drawing of ABCA3 and CFTR proteins. ABCA3 and CFTR are both ABC transmembrane transporters and share structural similarities, outlined in this schematic drawing. NBD; nucleotide binding domain. ECD extracellular domain. A, Walker A motif; B, Walker B motif. Adopted from Dean et al.²

binding domain (NBD2) of ABCA3, the domain spans from AA 1 326 to the end of the protein, AA 1 704. The ATP-binding cassette domain is located between AA 1 381 and AA 1 602; Walker A between AA 566 and AA 573 and Walker B between AA 685 and AA 690. The CFTR locations were also established using the NCBI Protein Database. For CFTR: NBD1 spans from AA 350 to AA 860. The first ATP-binding cassette domain spans from AA 389 to AA 670; Walker A from AA 458 to AA 465 and Walker B from AA 568 to AA 573. NBD2 spans from AA 1 149 to AA 1 480; the second ATP-binding cassette domain from AA 1 208 to AA 1 480; Walker A from AA 1 244 to AA 1 251 and Walker B from AA 1 366 to AA 1 371.

When the complete protein sequences of ABCA3 and CFTR are aligned using NCBI Blastp, there is 19% identity shared between CFTR and ABCA3.¹³ Between the NBD1 domain of each molecule, there is a 22% shared identity, while there is no significant similarity between NBD2 of ABCA3 and CFTR. To further refine the alignment, we compared the region between the start of the CFTR Walker A motif to the end of the Walker B motif in NBD1 against the corresponding region in ABCA3 and

found that these regions shared 23% identity. This area is the highest area of sequence homology, which may have clinical implications as this area is targeted by the CFTR modulators (ivacaftor).¹⁰

Mutation classification

A PubMed search was performed using the terms “ABCA3” and “mutation”. The search was completed in November of 2017, and 119 papers were identified. All 119 papers had their abstracts reviewed and papers were selected for inclusion when the *ABCA3* mutations were linked to specific patients and were written in English. Fifty nine papers were reviewed in full. Of those 59 papers, 10 were not subject to further analysis. The reasons for excluding papers ranged from not containing patients with any genetic mutation, to only containing synonymous non-disease causing mutations. Twelve of the papers contained data that was also reported in Wambach et al.¹² For ease of access, and to avoid duplications, the data that was reported in the supplement of Wambach et al. was incorporated.¹² Supplement 1 contains a full list of papers, including notation on why specific papers were not included.

In total, mutations obtained from 292 patients were incorporated with 233 unique protein changes. Eighteen unique DNA mutations were identified that did not result in protein changes, as these mutations were located in introns or promoter regions. Of these mutations, the most common type of mutation was missense mutations, although nonsense, frameshift, splice site mutations, insertions, and deletions were reported (Table 2).

TABLE 2 Total mutations classified

Total Number of Patients	292
Total Unique Mutations	233
Mutation Type	Total
Missense	153
Frameshift	37
Nonsense	37
Insertion	5
Deletion	5
Splice Site	3
Insertion/Deletion	1

Whenever possible, experimentally-determined outcomes were used to classify *ABCA3* mutations (Table 3). Typically, frameshift or nonsense mutations resulted in abnormal protein synthesis and would be classified as a Type I (CFTR) mutations. Matsumura et al¹⁷ found that mutations in the extracellular domains (L101P and L982P) altered intracellular targeting of *ABCA3*. This finding is in accordance with the assumed role of these domains in intracellular trafficking. Thus missense mutations in these domains were classified as Type II (CFTR) mutations (Figure 2). Also the 4 mutations found in the NBD2 (Ins1518fs, L1553P, L1580P and Q1591P) were classified as Type II (CFTR) mutations as 3 of the mutations (Ins1518fs, L1553P, and Q1591P) had functional data that showed altered protein intracellular trafficking. As an exception to the above classification, the L1580P mutation altered ATP hydrolysis (a marker of *ABCA3* function). L1580P mutation is located in the N-terminal region. Due to the role of the N-terminal in directing proteins to the appropriate cell compartments, we proposed that missense

mutations in the N-Terminal would lead into abnormal intracellular localization and therefore classified them accordingly as Type II (CFTR) mutations. The L1580P was classified as Type II (CFTR) mutation as it altered protein trafficking.

Other mutations of *ABCA3* caused decreased ATP hydrolysis activity and had normal intracellular trafficking.^{16,17} Based on the experimental data of Matsumura et al¹⁷, a missense mutation in the NBD1 (N568D) significantly decreased ATP hydrolysis activity would be classified as a type III mutations in the CFTR classification system as final cellular location is correct and function is abolished. Thus this mutation and missense mutations in the NBD1 region were classified as Type III (CFTR) mutations (Figure 2). A recent paper by Wambach et. al confirmed this determination.¹⁸ That group described a mutation in the NBD1 domain, p.R1474W, that was found to have “normal trafficking and protein processing, but decreased ATPase activity.” This mutation would be correctly determined to be a Type III (CFTR) mutation using experimental data from Matsumura et al.^{16,17}

Missense mutation in the transmembrane region in CFTR are associated with reduced or absent chloride conductance and are generally classified as a Type III or IV mutation.⁹ In a milder phenotype of the *ABCA3* related disease, Matsumura et al found that a mutation in the TM2 (T1114) was associated with reduced lipid transport.¹⁶ We classified missense mutations in the transmembrane domains as Type IV (CFTR) mutations as they seem to be associated with a milder phenotype as some transport was preserved. Some of these mutations could fall into the CFTR Type III group if *ABCA3* function was abolished.

We did focus on mutations on the area between Walker A motif and Walker B motif in NBD1 as this area is targeted by the CFTR modulators.¹⁰ Only two mutations (L579P and L627H) were identified in this area.

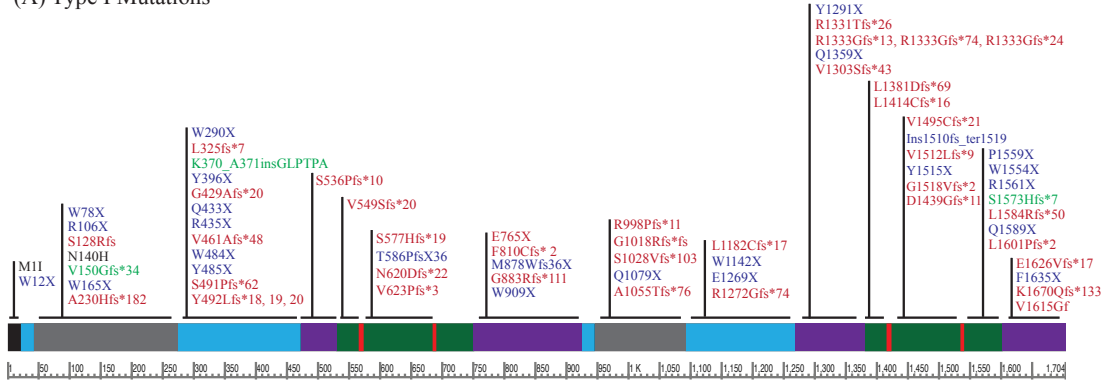
DISCUSSION

Complete classification of *ABCA3* mutations is important in order to take advantage of therapeutics under

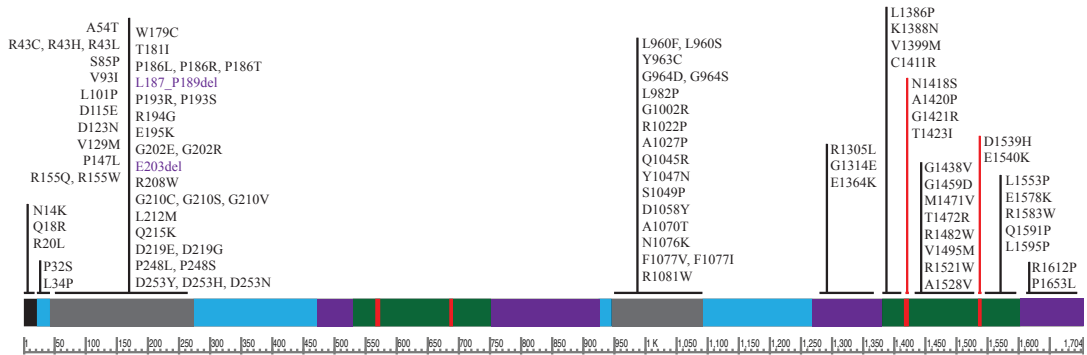
TABLE 3 Guidelines established for *ABCA3* classification

Class (CFTR Based)	Predominant Mutation Type	Predominant Location	Experimentally Determined Mutations	Phenotypic Severity
I	Frameshift, onsense	Unable to establish	p.G1518Vfs*2, p.W1142X, p.N140H	High ⁸
II	Missense	MSD1, MSD2, NBD2	p.Q1591P, p.L101P, p.L982P, p.L1553P	N/A
III	Missense	NBD1	p.N568D, p.E690K, p.R1474W	N/A
IV	Missense, Insertion, Deletion	MSD1, MSD2	p.E292V, p.G1221S, p.T1114M, p.T1173R	N/A
V	Splice Site	Intronic		N/A

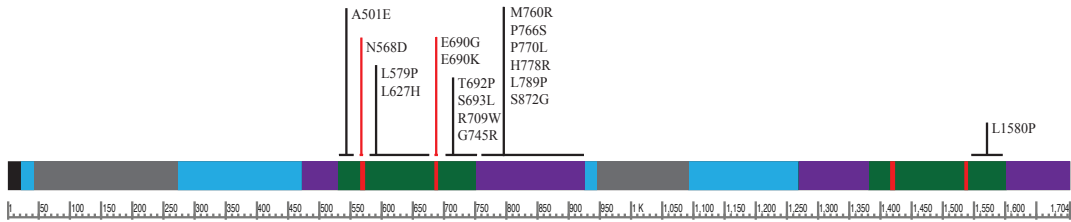
(A) Type I Mutations



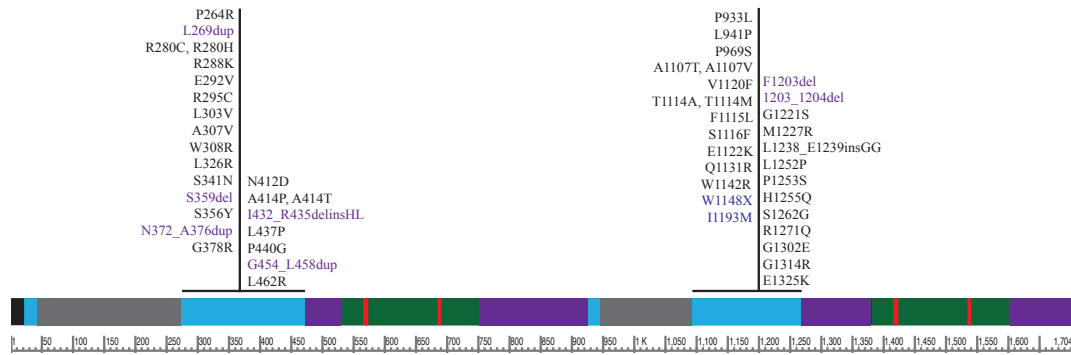
(B) Type II Mutations



(C) Type III Mutations



(D) Type IV Mutations



Key

Mutation Types	Gene Regions
■ Frameshift Mutations	■ Membrane Spanning Domain
■ Missense Mutations	■ ATP-binding Cassette Domain
■ Nonsense	■ Extra-Cellular Domain
■ Splice Site	■ Walker A and B
■ Deletion or Duplication	■ Nucleotide Binding Domain
	■ N-Terminal

FIGURE 2 Schematic Map of ABCA3 mutations.

development for other genetic diseases, such as cystic fibrosis. It is also important to understand the degree of homology when using another disease as a guide. Overall, the 19% overlap in identity between CFTR and ABCA3 is not impressive. However, between the NBD1 domain of each molecule there is a 22% shared identity; this was the highest area of genetic overlap. We focused on this area because it is the site of action for the potentiator class of cystic fibrosis drugs such as ivacaftor.^{11,22} Potentiators are a class of drugs that are designed to increase the time that the CFTR channel remains open when it is in the membrane.¹¹ They are approved to treat cystic fibrosis patients with the G551D mutation, which is a mutation that falls between the Walker A and Walker B motifs of the NBD1 of CFTR protein. G551D is the most prevalent Type III missense mutation, being found in 4% to 5% of people with cystic fibrosis. Potentiators are effective in treating G551D, because, as a Type III mutation, the CFTR protein localizes to the membrane, but is non-functional due to defects in the binding and the hydrolysis of ATP. Potentiators could potentially be used to treat ABCA3 mutations where they could increase the length of time that the channels are open, which could in turn aid phospholipid transport into the lamellar bodies. However, not enough is known about the role ABCA3 in surfactant homeostasis to know if keeping the channels open would have the desired effect. Furthermore, it is not clear that 23% sequence homology between this domain in CFTR and ABCA3 would be enough to successfully use CFTR-directed potentiators in ABCA3 disease.

Not only was the sequence homology of CFTR and ABCA3 in the region low, we only found 2 ABCA3 mutations (L579P and L627H) that fell in between the Walker A and B regions and were classified as a Type III mutations using the CFTR classification scheme. Both the L579P and L627H mutations were found in a patients who also carried a Type I frameshift mutation.¹² At this time it is difficult to conclude that CFTR-directed potentiators could target the ABCA3 mutations L579P and L627H like these drugs target G551D in CFTR. As a result, more research is needed on mutations in this region.

Using CFTR classification as a paradigm has its limitations. As previously stated, whenever possible experimentally determined information about the mutations and their effects was used to determine classification. However, of the 233 unique mutations, only 14 had experimentally determined functional classification (5.9% of the mutations). Therefore, it is important to note that most classifications were presumptive. Detailed understanding of the effects of ABCA3 mutations on protein processing is needed, and has important implications for patient care. As more research on the impact of specific mutations becomes available, this classification system may require modification. However, this system to classifying ABCA3 mutations will set a framework upon which additional data

can be set.

The classification of ABCA3 mutation types will facilitate further research. The data collected here represents what we believe to be the full extent of known pathogenic mutations in ABCA3. Previous papers have shown that in the case of frameshift and nonsense mutations, the type of mutation has a direct effect on the disease severity.¹² This newly described classification system could be used to further evaluate disease severity and differences in patient survival. Additionally, this new mutation classification may facilitate identification of novel therapeutics for ABCA3-related lung disease. In summary, this dataset represents an initial step in the organization and classification of ABCA3 mutations that will likely lead to clinically valuable insights and treatments.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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