First Steps toward Personalized Therapies for ABCA3 Deficiency

The ABCA3 (ATP-binding cassette transporter A3) protein transports phospholipids from the cytoplasm into lamellar bodies in alveolar type II cells (AEC2s) and is critical for pulmonary surfactant synthesis and function. Biallelic pathogenic *ABCA3* variants cause diverse pulmonary phenotypes, including lethal neonatal respiratory distress syndrome, childhood interstitial lung disease, and idiopathic pulmonary fibrosis (1–5). Current therapies, including steroids, hydroxychloroquine, and azithromycin, are nonspecific and have limited efficacy (3). Lung transplantation remains the definitive treatment option but is associated with ∼50% 5-year mortality and significant morbidities (6). The majority of more than 300 disease-associated *ABCA3* variants identified to date are private. Among the fewer than 10% of disease-associated variants that have been functionally characterized *in vitro*, two mechanistic classes, disruption of intracellular trafficking and disruption of phospholipid transport into the lamellar bodies, have been described (7, 8).

Although a genotype–phenotype correlation exists between biallelic frameshift or nonsense variants and neonatal respiratory distress syndrome (RDS) and death before 1 year of age without lung transplant, the disease presentation, severity, and course are difficult to predict for missense variants (2, 3). The similarities between ABCA3 and CFTR (encoded by *ABCC7*) and the clinical success of personalized therapies for patients with cystic fibrosis (9) support the potential for development of variant-specific therapies for the treatment of ABCA3 deficiency. A major barrier for developing such therapies for ABCA3 deficiency is the lack of a suitable *in vitro* system that mimics the biology and function of ABCA3 in AEC2s with a suitable readout for normal ABCA3 function amenable to high-throughput screening.

In this issue of the *Journal*, Forstner and colleagues (pp. 382–390) used a human pulmonary epithelial cell line (A549) and machine-learning algorithms to develop a phenotypic assay to detect morphologic differences between stably transfected cells expressing wild-type ABCA3 or missense variants (10). They next used this phenotypic assay to screen 1,280 U.S. Food and Drug Administration (FDA)-approved small molecules and identified cyclosporine as a corrector of several ABCA3 mutants that disrupt intracellular trafficking. The authors then validated the results of the phenotypic cell-based assay using established functional small format assays (7, 8). Importantly, this phenotypic screen was able to distinguish between mutants that disrupted intracellular trafficking and those that impaired phospholipid transport. Notably, cyclosporine treatment did not correct all trafficking mutants, demonstrating that, like CFTR, mutant-specific therapies may be necessary despite similar mechanisms of variant-encoded disruption of ABCA3.

Could cyclosporine, an immunosuppressive medication commonly used to prevent rejection of transplanted solid organs and FDA approved for more than 20 years, also be used to treat patients with ABCA3 deficiency? There are several potential limitations to this approach. First, it is not clear how many other *ABCA3* variants potentially could be corrected with cyclosporine treatment. As the authors note, there was a significant increase in the percentage of wild-type-like cells in the presence of cyclosporine for some, but not all, of the trafficking mutants. In addition, no effect was seen for the single functional phospholipid transport mutant (N568D) analyzed. Although there is no predominant *ABCA3* disease-causing variant, the most frequently encountered variant, p.Glu292Val, also impairs phospholipid transport. The majority of the disease-associated *ABCA3* variants have not been functionally characterized *in vitro*, and developing cell lines that stably express individual variants currently takes weeks to months in the research laboratory. Functional characterization of previously identified pathogenic *ABCA3* variants using the "landing pad" system may reduce the time for developing cell lines that express individual *ABCA3* variants (8). Second, while commonly used to study variants in surfactant genes (e.g., *ABCA3*, *SFTPC*), A549 cells are derived from adenocarcinoma alveolar basal epithelial cells and do not express the full repertoire of AEC2 machinery and cellular characteristics. Importantly, A549 cells do not make surfactant, and thus the functional effects of increasing the percentage of wild-type–like cells with cyclosporine treatment on surfactant production are unknown. Using AEC2 s generated from patient-derived induced pleuripotent stem cells (iPSCs) may provide information about production of functional surfactant in the presence of pharmacologic correctors (11). Third, because ABCA3 deficiency is a rare disease, and most variants are private, traditional clinical trials will be difficult. An N-of-1 clinical trial (12) (ClinicalTrials.gov: NCT04580368) for the study of variant-specific response to CFTR modulators using nasal epithelial brushings is underway. However, an easily accessible cell that expresses ABCA3 is not readily available for pharmacologic screening. Fourth, it is unlikely that cyclosporine would affect frameshift or nonsense variants that result in absence or truncation of the mature ABCA3 protein. Use of gene therapy or antisense oligonucleotides to reconstitute ABCA3 expression rather than a pharmacologic corrector may be an alternative approach for these variants (13). Fifth, cyclosporine appears to correct ABCA3 mutants through calcineurin-dependent and -independent mechanisms. Genetic deletion of the *Cnb1* (calcineurin b1) gene in respiratory epithelium of fetal mice disrupts structural maturation of peripheral lung, decreases ABCA3 synthesis, and causes respiratory failure (14). Investigation of its impact on lung development in

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ABCA3-deficient infants should be considered. Finally, many infants present at birth with severe respiratory distress syndrome (RDS), and confirmatory genetic testing may take weeks. By the time the diagnosis is established, significant lung injury from oxygen toxicity and ventilator-induced injury may be present, and whether this injury can be reversed is not known. The diverse phenotypes associated with *ABCA3* variants suggest disease mechanisms that range from lack of functional surfactant to chronic activation of intracellular stress pathways and may require different therapeutic approaches.

Given the extremely poor prognosis for many infants with ABCA3 deficiency and now widespread availability of genetic testing, families with or clinicians caring for infants and children newly diagnosed with ABCA3 deficiency may seek treatment with cyclosporine. Although cyclosporine is FDA approved, and may be prescribed off-label, it has significant side effects, including immunosuppression, nephrotoxicity, and hypertension (15, 16). In the absence of functional data for most *ABCA3* variants, its routine clinical use is premature, and its use should be evaluated rigorously in the context of a clinical trial. Despite these limitations, Forster and colleagues have developed an important phenotypic cell-based assay to identify small molecule–based, variant-specific therapies for patients with ABCA3 deficiency and suggest a path forward for the treatment of this severe and often life-limiting disorder. ■

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