

Unraveling the CFTR Function–Phenotype Connection for Precision Treatment in Cystic Fibrosis

The clinical phenotype of cystic fibrosis (CF), chronic infection, and inflammation leading to progressive obstructive lung disease and pancreatic insufficiency is caused by absence or dysfunction of the CFTR (cystic fibrosis transmembrane conductance regulator) protein (1). More than 2,000 variants of *CFTR* have been described, and these mutations are largely categorized based on the mechanism of impairment (2). Loss-of-function mutations (class I–III) result in little to no functional CFTR protein and are associated with a classic CF phenotype. Although there is a high degree of individual variation, people with at least one residual function mutation (class IV–VI) have some functional CFTR and more often have a clinical phenotype characterized by preserved pancreatic function and mild lung disease. Sweat chloride measurements have been used as a surrogate marker of CFTR function (3), with values observed to be higher in individuals homozygous for class I–III mutations than in heterozygotes with at least one residual function mutation (4). As lower sweat chloride values are associated with improved survival (4), these observations have led to the hypothesis that increasing CFTR function, even to a partial degree, would result in improved clinical phenotypes. CFTR modulators have been shown to increase CFTR function in genotype-specific patient populations, including many with class III (“gating”) mutations or residual function mutations, and for F508del homozygotes (5). With the advent of modulator therapies, the need to predict drug responsiveness and establish how CFTR functional improvements translate to phenotypic changes has gained new urgency.

In this issue of the *Journal*, McCague and colleagues (pp. 1116–1126) (6) attempt to address these questions using data collected for the Clinical and Functional Translation of CFTR (CFTR2) project (7). The vast CFTR2 database contains clinical and genetic information from 88,664 individuals with CF worldwide, as well as functional data associated with specific genetic variants. Leveraging *in vitro* studies that characterized genetic variant–specific responses to modulators, the investigators determined the percentage of CFTR activity relative to the wild-type in 54,671 individuals with 226 unique genotypes, and determined correlations with pancreatic function, lung disease, and sweat chloride. The investigators focused on individuals with CFTR function ranging from 0.85% to 50%, with 0.85% corresponding to those with one F508del mutation and a second mutation expected to result in no CFTR activity (NULL), and >50% indicating adequate CFTR to prevent disease. The investigators found a strong correlation between CFTR function and sweat chloride, and a less striking but still significant association with lung function and pancreatic status. Importantly, the relationship between CFTR function and clinical phenotype was observed to be logarithmic, suggesting that small increases in

individuals with little to no CFTR function is expected to result in larger changes in clinical status. Clinical phenotypes did not differ significantly between individuals with a given level of CFTR function for a lifetime and those who achieved similar CFTR function while on treatment with a CFTR modulator, suggesting that lung function may be recovered even later in the disease process.

A strength of this work is its use of the vast amount of data contained within the CFTR2 dataset, including functional data and clinical phenotyping. The functional data allowed the researchers to pool data for a subgroup of individuals with one NULL mutation expected to result in no CFTR activity, as many of these mutations are present in only a few known individuals worldwide. The benefits of developing national patient registries for rare diseases are now widely promoted, and this study highlights the potential uses and outcomes of broad disease-specific biorepositories.

Current U.S. Food and Drug Administration–approved CFTR modulators appear to increase CFTR function to levels consistent with a more mild disease phenotype in individuals with an ivacaftor-responsive mutation and to a lesser degree in those homozygous for the F508del mutation (8–10). Based on published phase 2 data, new “triple-combination” CFTR modulators for individuals with one copy of F508del and a second minimal-function mutation have the potential to improve lung function on par with the historic changes seen with ivacaftor treatment (11, 12), and phase 3 trials are currently underway. The study presented by McCague and colleagues provides hope that even small functional improvements in CFTR will translate into improved lung function and survival for individuals with CF, and importantly demonstrates that marked improvements are possible in those already in moderate to advanced stages of disease.

Although this work implies that increases in CFTR function could improve clinical outcomes across the CF population, there are limitations in applying these findings to individuals. Non-*CFTR* factors, including environmental factors and genetic modifiers, influence the CF clinical phenotype (13, 14). In addition, responses to therapeutics are known to be heterogeneous, with some individuals experiencing improvements and others having more limited responses (11, 12, 15). As indications for and the use of CFTR modulators expand in the CF population, these factors will continue to present challenges for prognostication. Future studies, including investigations of non-*CFTR* modifiers and pharmacogenomic variables, will be needed to enable more precise predictions of drug response. Both the presence of moderate to severe clinical disease in some individuals despite relatively preserved CFTR function and the variable response of individuals to modulator therapy raise a note of caution about individually prognosticating outcomes with modulator treatment.

McCague and colleagues have leveraged a vast amount of cohort data, at genotype-specific levels, to address fundamental questions related to CF disease and treatment. Their work provides important benchmarks for CFTR function that is needed to ameliorate disease and offers genotype-driven reference thresholds that may inform clinical conversations and care. These efforts, as well as the underlying development of CFTR2, may also have implications as a model system for other rare diseases. In the realm

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of CF, therapeutics that increase CFTR function to reduce and ultimately delay the onset and progression of CF lung disease appear to be within sight, providing great hope for people living with CF. ■

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⊗ The Challenging Road of Moving from Association to Causation for Microbiome Research in Idiopathic Pulmonary Fibrosis

In patients with idiopathic pulmonary fibrosis (IPF), respiratory infections are devastating events from which they often do not recover (1). Over the past decade, we have moved away from the use of immunosuppressive therapy and into an era of antifibrotic therapy. Although this has undoubtedly had a positive impact on the risk of acute infection in patients, the role of bacteria in the pathogenesis and progression of IPF does not end there. Over the past 5 years, there have been a number of studies highlighting the role of the bacterial communities (the microbiome) in the airways in IPF (2–5). Bacteria are persistent, present outside the context of an overt infection, and have been hypothesized to act as a continued driver of epithelial injury. A higher bacterial burden,

disordered bacterial communities, and increased numbers of potentially pathogenic organisms have now all been associated with disease progression in IPF. The key word here, of course, is “association”: Despite elegant correlations with peripheral blood transcriptome signatures, inflammatory profiles, or genotypes, these studies have all boiled down to observations and correlations made in patients with this condition, albeit with plausible mechanisms underlying their conclusions. Causal inference requires many key elements that are frequently difficult to accomplish when studying human microbiota. Under the framework established by the Bradford Hill criteria, nine principles should be used to establish causation: strength, consistency, specificity, biological gradient, plausibility, coherence, analogy, temporality, and experiment (6). In this issue of the *Journal*, O’Dwyer and colleagues (pp. 1127–1138) try to tackle some of these remaining criteria through the use of a series of very elegant studies performed in preclinical models in an attempt to move us from association to causation (7).

The largest cohort study of the microbiome in IPF, published in the *Journal* in 2014, demonstrated that the overall bacterial load is

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