From Genetics to Precision Therapy: Finding a Path through the Scientific Valley of Death

In this issue of the *Journal*, Ahmadi and coworkers (pp. 755–764) apply genetic epidemiology data derived from genome-wide association studies (GWASs) to begin to define a potential target for treatment of cystic fibrosis (CF) (1). Recent developments regarding combinations of corrector and potentiator drugs to restore CFTR (CF transmembrane conductance regulator) function have brought new hope to patients with CF and their families. However, there remain many patients who will continue to be severely affected because of imperfect efficacy, lack of efficacy, or delayed availability of the new drugs (2). Therefore, there is a need to continue to study agents that could further improve outcomes.

In moving genetic discoveries forward toward therapeutic relevance, Ahmadi and coworkers have gone a good way down the path through the scientific/translational "valley of death"—the unfortunate place where many scientific discoveries die before they can be translated to benefit patients (3) (allusion from Psalm 23). The trailhead of this path is normally genetic epidemiology: is a gene variant associated with a disease outcome? The next milepost is the anatomy and histology of the expression of the gene: specifically, is the encoded protein expressed in the right location, in the right cell type, in the relevant organ, and/or in a relevant upstream location? The next milepost normally involves sorting out the cell biology and physiology: does expression of the gene variant modify protein function and lead to cellular dysfunction relevant to the disease? The next, related milepost is to understand the biochemistry: are the metabolic substrates and products of the expressed gene product present in relevant concentrations in the cell and/or tissue? At the end of this path comes what is normally the most difficult terrain: can the protein of interest be targeted to affect the originally observed disease outcome? Ahmadi and coworkers provide an excellent template—with a few caveats—for the journey.

Genetic Epidemiology

GWASs had shown that variants in *SLC6A14*—a gene encoding a $2Na^+/Cl^-$-dependent amino acid transporter—were associated with several adverse CF phenotypes, including meconium ileus, early-onset lung disease, and early *Pseudomonas aeruginosa* lung infection (1, 4). The encoded transporter was known to be required for cellular uptake of cationic amino acids such as L-arginine. This information provided the critical starting point. Note that future discoveries based on genetic epidemiology are less likely to be guided by GWAS analyses and more likely to involve exomic or whole-genome analyses.

Anatomy and Histology

Importantly, Ahmadi and coworkers began by establishing that the *SLC6A14* gene product is expressed in airway epithelial cells from humans with and without CF, that it is expressed apically in the epithelium, and that it actively transports arginine from the apex (luminal) side of the cell. The localization was confirmed in an overexpression system.

One important caveat is that this is not normally the polarity (from airway lumen into the cell) one might expect in a mechanism relevant to amino acid uptake, although it could be quite useful if inhaled arginine were to be used for CF treatment.

Cell Biology and Physiology

The authors went on to show that in the presence of arginine, expression of the *SLC6A14* gene product in primary human airway epithelial cells *in vitro* increased airway surface liquid depth, and increased airway epithelial CFTR function in Ussing chamber experiments. This was true for cultures with both normal CTFR and homozygosity for F508Del CFTR.

Biochemistry

Ahmadi and colleagues then showed that the effect of the *SLC6A4* gene product to increase F508Del CFTR function could, at least in part, be dependent on signaling by nitric oxide synthase (NOS). They used the NOS inhibitor 1400W. This is classically considered to be an inducible NOS inhibitor, although it also inhibits endothelial NOS more transiently (5). The CF airway epithelium expresses little inducible NOS (6, 7), but arginine may be a substrate for apical endothelial NOS in the CF airway (7, 8), which can increase F508Del CFTR maturation (7). Note that the apical sodium and 100 μM arginine required may be rate limiting for this effect in the CF airway at baseline, but certainly can be achieved pharmacologically.

The authors considered another biochemical issue as well: the type of NOS product that could increase F508Del CFTR expression and function. They used S-nitrosoglutathione (GSNO), a molecule produced by NOS isoforms (9, 10) that recapitulates the effect of the *SLC6A4* gene product to increase CFTR function. This choice was important because the nanomolar levels of nitric oxide (NO) present in the normal airway are too low to affect CFTR (10–12), whereas GSNO levels in the normal airway could augment CFTR maturation (10–12). Note that GSNO levels are below normal in the CF airway (13) and might be enhanced in the presence of an optimally functional *SLC6A4* product. Increased GSNO in the airway increases fractional exhaled NO (14), and increased fractional exhaled NO can be a marker for a beneficial increase in nitrogen oxides that are more relevant than NO in the CF airway.

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Precision Medicine

This work suggests that overriding or bypassing an epithelial defect in arginine transport by using inhaled arginine (15) and NaCl—or inhaled GSNO (14)—could be beneficial, specifically in patients with CF with an *SLC6A4* allele associated with decreased function of the *SLC6A4* gene product. This sort of therapeutic targeting is at the heart of precision medicine. The common-sense next step would be to try these interventions in the genetically identified target population. Here, government or philanthropic sponsors often need to step in to aid in completing the journey to the other side of the valley. Clinical trials are expensive. Investors in small companies are often looking for the largest market, not for a small, precise subpopulation.

The guides on this path will be scientists and physicians. Much of contemporary clinical medicine can be performed—with considerable cost savings—by nonphysician professionals who do not have, and do not need to have, an extensive background in science. The role of scientists and physicians will become, as it always should have been, to guide the translation of basic science discoveries into personalized patient care. ■

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