PGx in transplant medicine: tacrolimus as foundation

Charna Albert

August 2021—Pharmacogenetic testing is not standard of care at most transplant centers, nor do the FDA or others mandate it. But “transplant medicine is ripe for observing the benefits of pharmacogenetics,” said Gwendolyn A. McMillin, PhD, D(ABCC), in an AACC virtual session last year.

“Commonly used immunosuppressants exhibit tremendous variability in metabolism and elimination, and these molecules target a wide range of signaling pathways,” said Dr. McMillin, medical director of pharmacogenomics, clinical toxicology, and mass spectrometry, ARUP Laboratories, and professor of clinical pathology, University of Utah School of Medicine.

Dr. McMillin

Although there are well-characterized pharmacogenetic (PGx) applications in transplant medicine, she said, lack of relevant tests with good content for the patient population and reasonable logistics such as turnaround time and cost are barriers to adoption. PGx tends to be used preemptively for transplant patients with a known history of difficulty in responding to medications or a family history of adverse reactions such as graft rejection or toxicity. Some patients are tested retrospectively because they’ve been unable to achieve therapeutic concentrations of prescribed drugs or because of signs of graft rejection or toxicity.

In the U.S., the FDA is the gatekeeper of much PGx information and an advocate for pharmacogenetics, she said, and it has sought opportunities to provide pre- and post-market information in drug labeling. As of June 2020, it included PGx biomarker information in the labeling for almost 300 drugs. “While that may sound like a lot, consider that there are more than 20,000 approved prescription medications today, so overall PGx is not that common,” Dr. McMillin said. But it applies to most medical specialties and therefore is poised to benefit a wide range of patients. “And there will be more and more pharmacogenetics incorporated into drug labeling and routine practice in the coming years.”

The FDA in 2020 published a series of tables of pharmacogenetic associations: one for which the data support therapeutic management recommendations, another for which the data indicate a potential impact on safety or response, and a third for which the data demonstrate a potential impact on pharmacokinetic properties only. The tables are organized by drug and identify the gene, affected subgroups, and the gene-drug interaction. But the FDA resources do not directly address the immunosuppressants used in solid organ transplant, Dr. McMillin said, “so we have to go somewhere else.” And that is the NIH-funded PharmGKB (www.pharmgkb.org/), which she calls “the most comprehensive and best respected public noncommercial PGx database that’s available and contributed to internationally.” As of October 2020, it listed a combined 295 clinical associations for the immunosuppressants cyclosporine, tacrolimus, sirolimus, everolimus, mycophenolic acid, methotrexate, and azathioprine.

“And these associations relate to both pharmacokinetics and pharmacodynamics,” she said. “So this is a rich place to start to understand what’s known, but also to see what kind of research is occurring for immunosuppressants as well as other drugs.”

PharmGKB also publishes pharmacogenetic-based drug dosing guidelines authored by professional organizations such as the Clinical Pharmacogenetics Implementation Consortium. On the site now are clinical practice guidelines

for three immunosuppressants: tacrolimus, methotrexate, and azathioprine. “It’s important to recognize that while these organizations provide guidance regarding what to do with PGx information, none of the groups specifically recommend or mandate testing,” she said. The decision to test is left to the relevant regulatory agencies and clinical providers, “though the guidelines are trustworthy, evidence-based, and updated regularly.”

Tacrolimus is metabolized by the gastrointestinal and hepatic cytochrome P450 (CYP) 3A enzymes, Dr. McMillin said. The *CYP3A* family is a cluster of four genes that metabolize about 50 percent of all drugs. “This family of genes is responsible for about 30 percent of the total amount of hepatic CYP450 protein, so it’s a pretty big deal.” Tacrolimus is converted to inactive metabolites by the reactions mediated through CYP3A4 and CYP3A5. When CYP3A5 is expressed, it is the predominant enzyme responsible for this reaction. And CYP3A5 expression, she said, is determined mainly by genetics. All three of the published gene-based dosing guidelines for tacrolimus cite *CYP3A5*, and one also cites *CYP3A4*.

CYP3A5 enzymes are expressed in the small intestines, liver, and kidney. Considerable presystemic or “first-pass” metabolism occurs in the intestine and liver, limiting the bioavailability of the active drug. “And much of the bioavailability is influenced by genetic variability,” she said, which is common in *CYP3A5*. “More often than not, *CYP3A5* is variant. That’s one reason the PGx has been extensively studied and is the predominant component of the gene-based dosing guidelines for this drug to date.”

CYP3A5, along with the other *CYP* genes, is classified by core (usually positive) variants or combinations of variants, described as *alleles. The **1* allele, considered wild type or normal, exhibits no known variants and tends to be most common. “Most labs report **1* when none of the variants in the targeted assay are detected,” Dr. McMillin said, so when interpreting a **1* result, it is critical to know which variants the test is designed to detect. “It’s entirely possible that a **1* [result] does contain variants that are simply not detected by the assay performed.” This may be the case when a patient’s phenotype doesn’t match the genotype. “And in those scenarios you should consider more comprehensive testing, including potentially full-gene sequencing.”

In *CYP3A5*, however, the **3* variant allele, defined by a splicing defect in intron 3 that results in the absence of functional protein, is most common in all populations except for the African American population. The **3* allele is considered a nonexpressive allele, with a predicted non-function phenotype. Many laboratories design their assay to detect only the **3* allele because it is so common, she said, noting that it is observed in 92.4 percent of Europeans, 77 percent of Latinos, 74.6 percent of East Asians, and 31.6 percent of African Americans. But the **6* and **7* variant alleles, too, are associated with a lack of CYP3A5 expression and are significant in African American and Latino populations. “So again it’s important to verify that a lab test that has been ordered can detect the alleles relevant to your specific patient population.”

Metabolic phenotypes, Dr. McMillin said, are predicted based on diplotypes, which are determined based on the combination of alleles detected. Normal metabolizers carry two functional (**1*) alleles. Intermediate metabolizers carry one functional and one nonfunction allele (**1/*3*), and poor metabolizers carry two nonfunction alleles (**3/*3*). Two functional alleles is associated with the expression of the protein in normal metabolism. Historically, she noted, normal metabolism has been called extensive metabolism, “so you see a switch in the nomenclature between different sources of information, but they mean the same thing.”

About 80 percent of people have impaired CYP3A5 expression and therefore impaired metabolism, she said. “So roughly 85 percent would be classified as either intermediate or poor metabolizers in a mixed population.” The consequences of the reduced metabolic phenotype include a prolonged half-life, reduced time to steady-state concentrations of the drug, and overall lower dose requirements. “As you might imagine, for a person who has impaired CYP3A5 expression, CYP3A4 is going to become more important for tacrolimus dosing and dose optimization because CYP3A4 then becomes a predominant player in the metabolism and inactivation of tacrolimus.” But loss-of-function alleles, she said, are not common in *CYP3A4*. The most well-characterized variant allele detected thus far is the *CYP3A4*22* allele, which is associated with decreased function and occurs only in about five percent of Europeans and mixed Americans. “So that’s part of the reason studies thus far have poor statistical significance for this gene and relatively low levels of evidence.”

Yet it is noteworthy that the activity of CYP3A4 is modulated by other proteins, she said, such as P450 oxidoreductase and peroxisome proliferator-activated receptor alpha. “This makes the picture more complicated and suggests additional work is necessary.” *POR*28* and *PPARA* are known to modulate CYP3A4 expression. And CYP3A4 is also subject to drug and food interactions, of which the most well known is inhibition by grapefruit juice. “So that further complicates the research contributing significance of this gene to routine dosing guidelines.”

There are data to suggest that *CYP3A4* and *CYP3A5* are clinically important when variants are present, she said. Scheibner, et al., reported in 2018 on tacrolimus elimination in four kidney transplant recipients who carry the rare genotype combination *CYP3A5*3/*3* and *CYP3A4*22/*22* (Scheibner A, et al. *Pharmacotherapy*. 2018;38[7]:e46–e52). It is common for Caucasians to carry these variant alleles, the authors write, but rarely are they homozygous for both *CYP3A5*3* and *CYP3A4*22*. The four transplant recipients were identified from a larger cohort of 1,366 Caucasian kidney transplant recipients. To understand the significance of the genotype combination *CYP3A5*3/*3* and *CYP3A4*22/*22* on tacrolimus troughs and doses, the authors compared the four patients to recipients without this combination.

Patients homozygous for both variants are at risk for profound reductions in metabolism of *CYP3A* substrates, Scheibner, et al., write. A 342 percent and a 90.6 percent increase in the median dose-normalized trough was observed when the *CYP3A5*3/*3* and *CYP3A4*22/*22* genotype combination was compared with the *CYP3A5*1/*1* and *CYP3A4*1/*1* genotype combination and the *CYP3A5*3/*3* and *CYP3A4*1/*1* genotype combination, respectively. The four recipients with the rare genotype combination required on average 2.5 mg/day of tacrolimus. In contrast, the median dose required for those patients with no variant alleles (*CYP3A5*1/*1* and *CYP3A4*1/*1*) was 8 mg/day. There was a clear relationship between metabolic capacity and trough concentrations, Dr. McMillin said.

This suggests that normal metabolizers require higher dosing “due to the extensive metabolism of tacrolimus in those patients. The patients with no variants—the normal metabolizers—took longer to reach a stable dose and that stable dose was much higher than that of the poor or intermediate metabolizers. As such, the normal metabolizers are the population most likely to benefit from nonstandard dosing.” Achieving target concentrations in the therapeutic range more quickly could reduce the risk of graft rejection from underexposure and toxicity from overexposure. These data suggest the benefit of PGx is best realized when *CYP3A4* and *CYP3A5* genotypes are known pretherapeutically, she said. “So this requires a little planning.”

In that planning, who should be tested—the donor, recipient, or both? The available data suggest it depends on the organ being transplanted. “And this makes sense,” she said, because the metabolism of tacrolimus occurs primarily in the intestines and liver, and though some metabolism does occur in the kidney, most patients receive only one kidney transplant at a time. The current recommendation is to pretherapeutically test kidney, heart, lung, and hematopoietic stem cell transplant recipients to achieve target blood concentrations sooner. For liver transplant patients, the recommendation is to pretherapeutically test both donor and recipient and use the guidelines at tacrolimus initiation only if donor and recipient genotypes are identical. The literature varies on when “donor genotype and associated proteins kick in,” she said, “but it is known that donor genotype can impact concentrations anywhere from the first week post-transplant to several months after.” If pretherapeutic testing is not performed but the patient struggles to achieve therapeutic blood concentrations, she said, then reactive PGx testing to troubleshoot may be indicated.

The dosing guideline of the Clinical Pharmacogenetics Implementation Consortium for tacrolimus based on CYP3A5 phenotype is as follows:

- For normal and intermediate metabolizers, increase starting dose 1.5 to two times the standard recommended starting dose. Total starting dose should not exceed 0.3 mg/kg/day. “Dose, just as with any other patient, would then be refined based on the results of therapeutic drug monitoring

to achieve the target steady-state concentration."

- CYP3A5 nonexpressors, or poor metabolizers, should receive the standard recommended dose at initiation, and therapeutic drug monitoring should be used to guide adjustments.

"The *CYP3A4* and *CYP3A5* example with tacrolimus serves as a foundation upon which other gene-drug associations can be compared," Dr. McMillin said, noting the hundreds of gene-drug associations for immunosuppressants. "These associations vary in relevance to specific clinical indications and patient populations. As such they are not created equally, and you must consider the levels of evidence associated with each clinical association for the indication and patient population of interest." And PGx testing in no way replaces clinical and therapeutic drug monitoring. "They're complementary," she said.

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