PGx testing: recommended alleles for *CYP2C19* panels

Elizabeth Silverman

August 2018—After more than a year of gathering information and deliberating, members of the Association for Molecular Pathology Pharmacogenomics Working Group have issued the first in what will be a series of recommendations to standardize pharmacogenetic testing.

The group's first set of recommendations identify a minimum set of alleles for inclusion in clinical *CYP2C19* genotyping panels. Published in the May issue of the *Journal of Molecular Diagnostics* (Pratt VM, et al. 2018;20[3]:269–276), the recommendations were designed to complement and fill gaps in existing pharmacogenomics guidelines.

The working group, a subgroup of the AMP's Clinical Practice Committee, came together after the 10th Genome Medicine conference of the National Human Genome Research Institute, in 2017, called for assay standardization, says group member Karen E. Weck, MD, a past chair of the CAP/American College of Medical Genetics and Genomics Biochemical and Molecular Genetics Resource Committee. An additional impetus was a survey of pharmacogenetic panels used to characterize cell lines for reference materials, conducted by the Centers for Disease Control and Prevention's Genetic Testing Reference Material Program. It found that the variants tested by laboratories were not consistent and that no two tests that examined any of the 28 pharmacogenetic genes in the study were designed to detect the exact same set of variants. "It seemed as if there was a need for better standardization in this space," Dr. Weck said in an AMP webinar in June, noting that Victoria Pratt, PhD, of the Department of Medical and Molecular Genetics, Indiana University School of Medicine, led the effort. "We identified where there might be heterogeneity or lack of concordance and gaps in current practice," Dr. Weck tells CAP TODAY. She is a professor of pathology and laboratory medicine and genetics and director of the molecular genetics laboratory, University of North Carolina School of Medicine.

CYP2C19 is one of the cytochrome P450 enzymes in the liver that is important for phase one metabolism of many drugs. Together, CYP2C19, CYP2C9, CYP2D6, CYP3A4, and CYP3A5 are responsible for the metabolism of more than 80 percent of drugs prescribed today, Dr. Weck said. The cytochrome genes are also highly polymorphic and contain many variants or single nucleotide polymorphisms that can affect enzymatic activity and alter drug metabolism. In particular, the substrates of CYP2C19 include clopidogrel, voriconazole, proton pump inhibitors, SSRIs, and some tricyclic antidepressants. In addition to the normal *CYP2C19*1* allele, the *CYP2C19* gene can have up to 35 different alleles (named in order of discovery), many of which are associated with altered enzyme function, resulting in poor, intermediate, rapid, or ultra-rapid drug metabolism. Failure to test for an allele that alters drug metabolism results in an incorrect classification to the default *CYP2C19*1* normal function allele. "If you're not including the SNP," Dr. Weck said, "you're not identifying the SNP."

Dr. Weck

Because *CYP2C19* affects the metabolism of so many drugs, it is one of the most frequently tested for genes. Much of the testing has also been driven by the FDA's 2010 black-box warning for clopidogrel that two common variant *CYP2C19* alleles, *CYP2C19*2* and *CYP2C19*3*, result in poor metabolism of clopidogrel and increase the risk of adverse cardiovascular events. Also driving *CYP2C19* testing is the publication of guidelines by the Clinical Pharmacogenetics Implementation Consortium and the Dutch Pharmacogenetics Working Group for *CYP2C19* for multiple drugs. "I would say that the CPIC and DPWG dosing guidelines have been extremely important in helping

to recommend pharmacogenetic action based on pharmacogenetic testing," Dr. Weck said. In her experience, "this has also produced a big uptick in the number of places that are doing pharmacogenetic testing for *CYP2C19* and for other genes as well."

She noted two caveats about pharmacogenomics. First, allele function derived from in vitro models may not directly translate to a clinical phenotype and metabolizer status. "And much of the pharmacogenetic characterization of certain allele function is done by looking at enzymatic activity in in vitro models. So we took into consideration both what's known about functional activity in these in vitro models and what's been shown to be clinically relevant in terms of drug response."

Second, allele function for pharmacogenetic alleles can be substrate and/or drug concentration dependent. "So there may be certain alleles that have been shown to be very important for some drugs or certain dosages of some drugs that haven't borne out in other cases." This further complicates analysis of the clinical importance of pharmacogenetic alleles, she said.

The working group members first defined the attributes that a recommended must-test allele should have. Their framework for evaluation consisted of the functional status of the *CYP2C19* alleles, their frequency in a multiethnic population, and whether reference materials are available. They also examined what commercial genotyping platforms are available and which alleles are on those platforms.

With respect to the variants themselves, the group reviewed the literature, testing resources, reference materials, which tests are being offered now, and the availability of CAP proficiency testing and other quality assessment programs.

Their recommendations are divided into a tier-one minimum set of alleles and a tier-two set of alleles for extended panels. To be included in tier one, a variant allele needs to meet all three of the following criteria; for tier two, at least one criterion.

First, the function of the variant allele and its clinical effect on drug metabolism must be well characterized, and the functional SNP must be known so it's clear which SNP should be included on the genotyping platform. Second, the allele must have an appreciable frequency in the patient population or a major ethnic subpopulation. Third, reference materials must be available.

The alleles recommended for inclusion in tier one for clinical *CYP2C19* genotyping panels are *CYP2C19*2* and *CYP2C19*3*, which are loss-of-enzymatic-function alleles, and *CYP2C19*17*, which is associated with increased function of the enzyme. In the case of clopidogrel, which is a prodrug, loss-of-function alleles result in a lack of enzymatic cleavage to the drug's active form and a clinically inadequate drug response. Individuals with *CYP2C19*17* have the opposite response, which may lead to abnormally high active drug levels and elevated bleeding risk. Although more work has been published on the adverse cardiovascular events and the higher risk of clotting with clopidogrel in patients with **2* and **3* alleles than on the risk of bleeding in patients with the **17* allele, *CYP2C19*17* is important for other drugs such as proton pump inhibitors and SSRIs. In these cases, the *CYP2C19*17* allele is associated with a too rapid clearance metabolism and difficulty in reaching and maintaining therapeutic drug levels. "But those studies haven't been as well defined and the evidence isn't as strong," Dr. Weck said. "So many laboratories initially developed *CYP2C19* genotyping just for **2* and **3* for clopidogrel response." The group emphasized the importance of including *CYP2C19*17* as a recommended tier-one allele on genotyping panels because *CYP2C19* genotyping performed for one drug indication may be stored in the electronic medical record and used to make treatment decisions for other drugs, and because it's present at a high frequency in the population.

The three tier-one *CYP2C19* alleles are all common in the population, though their frequencies vary by ethnic group. The *CYP2C19*2* allele—a splicing variant that knocks out enzymatic activity—is common in most major ethnic groups but highest in Asians. *CYP2C19*3*, a G to A nonsense variant that is also a loss-of-function allele, has a 10 to 15 percent allele frequency in East Asians and is relatively common in African-Americans but is less common in Caucasians. *The CYP2C19*17* allele, a promoter polymorphism, is found in a number of ethnicities but

is highest in Caucasian and African populations. Together, the three recommended alleles account for 40 percent to more than 90 percent of the alleles in most racial and ethnic groups. “So by genotyping for just three alleles,” Dr. Weck said, “you’re going to be testing for most of the poor function or increased function metabolism alleles in the majority of ethnic groups.”

The group’s recommended tier-two *CYP2C19* alleles are **4A, *4B, *5, *6, *7, *8, *9, *10,* and **35*. This list will be reviewed periodically, and as more information becomes available, these alleles may be promoted to tier one. The group considered that all of the alleles on its tier-two list are important but they lacked at least one of the tier-one attributes. *CYP2C19*4, *5, *6, *7,* and **8* have low population frequencies. *CYP2C19*9* and **10* are decreased-function alleles (associated with intermediate metabolism) that are relatively common but their effect is less well characterized clinically. *CYP2C19*35* is common in African-Americans, where it has an allele frequency of up to three percent, but it is less well characterized and, like **5* and **7*, suffers from a lack of reference material. The *CYP2C19*4B* allele was not included in tier one even though it includes a loss-of-function SNP that can be seen on the same haplotype as **17* and overrides the rapid metabolism effect of the **17* allele. This is because of its overall low population frequency and because it can be technically difficult to determine whether the loss-of-function SNP is in cis or trans with the **17 SNP.*

“The *CYP2C19*4B* allele is more common in certain ethnic subpopulations, such as Ashkenazi Jewish individuals, so it is important for laboratories that are doing genotyping to know what the ethnic groups are that are commonly tested in their laboratories,” Dr. Weck said, “and take this into consideration when designing genotyping panels.”

Although inclusion on commercially available genotyping platforms was not a required criterion, the group found that all of the recommended tier-one alleles were included on available commercial platforms, as were many of the tier-two alleles. “So we think these recommendations will be very practical for laboratories to implement,” Dr. Weck said.

The group stresses the need for a high degree of transparency in pharmacogenomic testing. This includes not only making sure the star alleles and variants included on the panel are listed in reports but also communicating the limitations of each assay. Says Dr. Weck: “In the example of *CYP2C19*2* and **10*, the **10* variant SNP is c.680C>T, which is directly adjacent to the **2* variant SNP, c.681G>A. So if you have both of these alleles present, with any genotyping assays that incorporate specific probes to the **2* SNP, the presence of the **10* SNP on the other allele will interfere with the assay and cause lack of binding of the wild-type probe.” Therefore, for any individual who is typed by her laboratory’s TaqMan genotyping assay as a *CYP2C19*2* homozygote, the report has a caveat that individuals who are compound heterozygous for **2* and **10* will be reported as *CYP2C19*2* homozygous in this assay due to interference of the **10* allele. Since this is a limitation of the assay, Dr. Weck’s laboratory includes this on the report even though *CYP2C19*2/*10* compound heterozygosity is rare.

“No matter how big your panel is,” Dr. Weck said, “it’s important to be transparent about exactly what SNPs and alleles you’re testing for, what your platform might miss, and to be aware of the frequency of each of those alleles.”

The increasing availability of high-throughput DNA sequencing argues against a must-test recommended list of alleles, Dr. Weck acknowledged. However, many pharmacogenetic genes have homologous genes and pseudogenes, she said, “and that can greatly complicate the interpretation of DNA sequencing and the development of bioinformatic algorithms to correctly call alleles.” Still, the recommended panel may lose relevance if more laboratories begin to do whole gene sequencing—and the group is ready. “We certainly have plans to periodically reassess the field and to determine whether the recommended panel should be expanded or changed based on what the current practice of testing is,” Dr. Weck said.

Elizabeth Silverman, of New York, NY, is a writer who covers genomics.