Building the case for PGx testing

William Check, PhD

January 2013—Mammals have a striking range of gestation periods, from the 12 days and 31 days of the opossum and rabbit to the 266 days and 360 days of the human and whale. Laboratory tests, too, take shorter or longer amounts of time to be delivered into routine clinical practice, with pharmacogenomics beginning to look like the elephant—more than 600 days' gestation—of laboratory testing. Our first major discussion of this topic was in 2005, and the clinical pathology world had been "expecting" its arrival for some time before that.

Perhaps it's finally time to hang the stork sign on the laboratory door. In a plenary session at the Association for Molecular Pathology 2012 Annual Meeting on Genomic Medicine, Michael Laposata, MD, PhD, the Edward and Nancy Fody professor of pathology and a professor of medicine at Vanderbilt University School of Medicine, spoke on "Making the Case for Pharmacogenomics Testing: Integration into a Healthcare System." In his talk, Dr. Laposata, who is also pathologist-in-chief at Vanderbilt University Hospital, described Vanderbilt's pharmacogenomics program, called PREDICT—Pharmacogenomic Resource for Enhanced Decisions in Care and Treatment.

As Dr. Laposata presented it, the case for pharmacogenomics is self-evident and seemingly incontrovertible. "Would it not be great," he asked attendees, "if we could select the right antihypertensive or the right antidepressant or the right antiplatelet agent immediately—rather than using the trial and error method and having a poor patient outcome until the drug that works is identified?" PREDICT seeks to achieve this goal for four analytes: warfarin, clopidogrel, simvastatin, and azathioprine. Tamoxifen, abacavir, and tacrolimus are in the works for 2013.

While prior attempts to initiate pharmacogenomics testing centered on warfarin, PREDICT focuses on clopidogrel (Plavix). "It was unfortunate that pharmacogenomics started with warfarin," Dr. Laposata said in an interview with CAP TODAY. "That experience put a cloud over the whole pharmacogenomics story." With clopidogrel, on the other hand, "This is where it really matters," he says. "The case for [PGx for] Plavix is far more compelling."

Even so, Vanderbilt administrators are not yet seeking payment or reimbursement for pharmacogenomics testing. "We at Vanderbilt do more [pharmacogenomics] because we're willing to do it without being paid for it," Dr. Laposata told CAP TODAY. "The key to marketing pharmacogenomics, like any test, is to show how you would use it for taking care of patients. If you try to move government to pay for something that sounds good but they can't see any concrete benefit, it's never going to happen. We need to show benefit first." To this end, Dr. Laposata and his colleagues are collecting clinical experience and conducting a single-center trial.

Dr. Allan

A similar approach and attitude are prevalent at the University of North Carolina, where genotyping of CYP 2C19 alleles, which determine clopidogrel metabolism, is part of routine care. "Our cardiology group is very proactive doing genotyping in their percutaneous coronary intervention patients receiving stents since the FDA's black-box warning about poor metabolism [of Plavix] increasing the risk of death due to thrombotic events," says Karen Weck, MD, professor of pathology and laboratory medicine and genetics at UNC and director of molecular genetics. "That test has had the biggest clinical uptake of any pharmacogenomics test here at UNC." Dr. Weck is associate director of the UNC Institute for Pharmacogenomics and Individualized Therapy.

Like the Vanderbilt group, Dr. Weck and her colleagues have been taking an evidence-based approach. “We have been involved in several clinical trials of pharmacogenomics,” she says. “We have finished a trial for warfarin and are now evaluating the data.” They also did a trial of PGx testing for tamoxifen metabolism and a dose-escalation trial with clopidogrel.

Also like Vanderbilt, UNC is not yet heavily emphasizing reimbursement. “We are charging insurance, since it is a clinical test,” Dr. Weck says. “But I don’t have the data
for how often we are being reimbursed.”

At the University of Florida College of Medicine, the pathology, pharmacy, and information technology departments collaborated to set up pharmacogenomics testing for clopidogrel. “We believe it will become a big part of future medicine,” says Michael Clare-Salzler, MD, chair and Stetson professor in experimental pathology and director of the Center for Immunology and Transplantation. “We incorporated it as part of our development of molecular medicine. It is not accepted by everyone yet, but it has pretty strong acceptance in the pharmacogenomics community.”

“We see pharmacogenomics for CYP 2C19 as a pilot project,” says Robert Allan, MD, associate professor of pathology and medical director of UF PathLabs. “It is a good prototype to set up the lab protocol and get the chip validated.”

They are also doing a study, in this case to collect data on several other pharmacogenomic applications. “The cost to do genotyping on 256 SNPs [on one chip] is no more than just doing CYP 2C19,” Julie Johnson, PharmD, distinguished professor of pharmacy and medicine, says. “We will generate lots of data, then figure out which genotypes are clinically actionable and make them available.”

Dr. Johnson

Despite all this activity, it is still possible that pharmacogenomics will be an example of “The elephant labored and brought forth a mouse.” For one thing, Dr. Weck notes, “I hear a lot of controversy about whether CYP 2C19 testing should be done. There is some conflicting literature about whether poor metabolizers [of clopidogrel] have an increase in adverse events.” And, of course, we are still awaiting direct evidence that testing for CYP 2C19 will prevent the adverse events.

But a larger issue looms over pharmacogenomics. It may simply disappear as a distinct individual field of testing, subsumed under the 500-pound gorilla in the room—next-generation sequencing. During his AMP talk, Dr. Laposata showed the number of adverse events expected at his hospital from six drugs that are or will be in the PREDICT program. “With next-generation sequencing we get all of these at once,” he said.

Dr. Weck and her colleagues have been awarded a grant to do whole exome sequencing on patients with five medical conditions likely to have a genetic etiology (“Whole exome sequencing and pharmacogenomics,” page 30.) As a side benefit, she says, “We will be getting pharmacogenomic information on all these patients.” She’s particularly interested in the pharmacogenomic component because she thinks it could have the most usefulness in terms of medical treatment.

In his talk, Dr. Laposata reviewed the “unenthusiastic” reception given to pharmacogenomics for warfarin. As a valuable anticlotting agent, warfarin is used in millions of patients each year. “If I have a clot, someone’s going to give me a shot of Lovenox and a pill of Coumadin,” Dr. Laposata said. However, because warfarin has a narrow

therapeutic window, it must be dosed accurately, with an INR between 2.0 and 3.0 or 2.5 and 3.5, depending on the indication. Risk of intracranial hemorrhage increases 11-fold for an INR greater than 4.9 and 18-fold for an INR greater than 6.6. Genotyping for CYP C29 and vitamin K epoxide reductase (VKORC1) can help bring the INR into the therapeutic range more often.

However, he noted, physicians who use warfarin said, "I don't think I need to do this." He admits: The INR is "a pretty simple way to monitor these patients."

"We've had 20-plus years of experience adjusting people's Coumadin dose based on INR," he tells CAP TODAY. "Many doctors know how to do it, including primary care doctors." When the pharmacogenomic result came back in three to seven days, the INR was often already adjusted into the therapeutic range. "When we told the doctor a patient was hypersensitive to warfarin, the doctor said, 'I know. I'm down to 2.5 mg per day of warfarin by following the INR.'"

"Clinical studies so far have not showed clinical benefit [of PGx for warfarin]," he adds.

Clopidogrel is another matter. "The Plavix story is much more convincing because there is no INR-type test," he told his AMP audience. It has been known since 2005 that some patients don't respond to clopidogrel (Serebruany VL, et al. *J Am Coll Cardiol*. 2005;45:246–251; Hochholzer W, et al. *Circulation*. 2005; 111:2560–2564). Among the lowest quartile of Plavix responders, there is a 40 percent risk of recurrent cardiovascular events at six months, compared with zero risk in the lowest two quartiles (Matetzky S, et al. *Circulation*. 2004;109:3171–3175). Clopidogrel is metabolized by CYP 2C19; by measuring this enzyme one can identify patients who do not have an adequate response and switch them to an alternative antiplatelet agent (Simon T, et al. *N Engl J Med*. 2009;360:363–375).

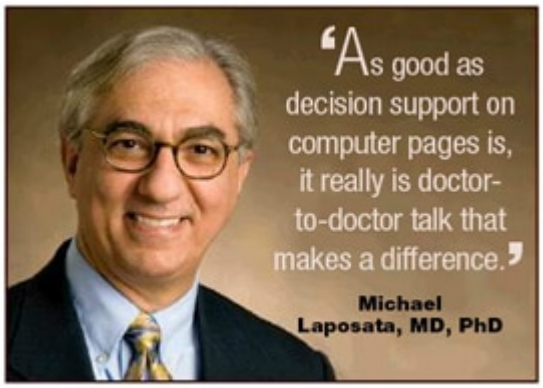
At Vanderbilt, testing for CYP 2C19 is done in all patients who are receiving a coronary artery stent by interventional cardiology, as well as patients seen in primary care who are expected to require a coronary artery stent. "Patients chosen for this testing qualify by a complex formula based on clinical and laboratory findings," Dr. Laposata said. A switch to prasugrel or ticagrelor is recommended in patients homozygous or heterozygous for loss-of-function (LOF) alleles, the most common of which is *2. However, at least 10 alleles of CYP 2C19 are now known, several of which are rare but cause loss of function."This is more complicated than it started out," Dr. Laposata said.

To help the physician implement the pharmacogenomic result for CYP 2C19 into patient management, the Vanderbilt program provides support in the form of a pharmacy resident with a fact sheet, supplemented by a "content expert" whom the pharmacy resident can consult. Decision support is provided in the laboratory report, but it quickly became clear this was not adequate. Some physicians were confused by the "*" nomenclature. When Dr. Laposata said, "That's an allele," some said, "What's an allele?" Faced with heterozygous results, some physicians interpreted them like sickle cell, in which only those who are homozygous for the mutant allele have the disease. They didn't see the need to switch patients from clopidogrel.

Dr. Laposata doesn't view this lack of familiarity with genetics in a negative way.

"If you're a proceduralist, you should be spending your time getting better at your procedure," he says. "I see my role as helping them understand. For a decision this big, most practitioners want to talk to an expert in the field rather than depend on a decision support printout. They want to talk to a colleague who does this for a living and ask, 'What would you do if you were me?'"

Dr. Laposata shared an incident that demonstrated the potential value of pharmacogenomic testing. A patient received a stent during a two-week period when pharmacogenomic testing for Plavix was unavailable for technical reasons. During that time, the patient had an in-stent thrombosis. Subsequent testing showed that this patient was homozygous for LOF alleles. "This convinced many interventional cardiologists at Vanderbilt of the value of pharmacogenomic testing [for clopidogrel]," he said.



While anecdotes are emotionally powerful, only rigorous data are ultimately convincing, particularly for reimbursement purposes. Dr. Laposata and his colleagues are conducting a clinical study with two outcome measures. First, to determine the number of prescription changes away from Plavix when loss of function alleles are detected. Second, to determine the number of in-stent thromboses or major adverse coronary events in patients originally treated with Plavix and then switched to a different antiplatelet agent.

From March 2011 to February 2012, pharmacogenomic testing for clopidogrel was done in 3,312 patients; 149 had an actionable genotype and a drug-eluting stent. Of these, 131 received a recommendation for a medication change. Only 49 changes were actually made. "We don't yet have a complete understanding why not everybody is changing medication," Dr. Laposata says. "I think more changes would have been made if there had been more interaction between cardiologist and coagulation expert." This interaction is now taking place. "As good as decision support on computer pages is, it really is doctor-to-doctor talk that makes a difference," he says.

Dr. Laposata calls the second outcome measure, reduction in coronary events, "the big enchilada."

"We are continuing to collect that data. I suspect we are going to get enough numbers over a year. We realize we need to prove it works to get paid."

For the third analyte on the pharmacogenomic panel, genotyping of the SLCO1B1 gene may help reduce myopathy from the 80-mg dose of simvastatin. Evaluation of thiopurine methyltransferase (TPMT) genotype is the most recent introduction to the PREDICT program. Severe TPMT enzyme deficiency can sometimes cause life-threatening myelosuppression in patients on azathioprine.

Adding new analytes is not technically difficult because, like the University of Florida/Shands group, Dr. Laposata is working with a highly multiplexed assay platform, Illumina's VeraCode ADME Core Panel assay, which genotypes 184 common polymorphisms in 34 genes associated with drug absorption distribution, metabolism, and excretion. Performing all assays on one chip does raise questions. All pharmacogenomic requests include all alleles on the panel, so, for example, a physician can order the test for clopidogrel and get a result for simvastatin. "We report out Plavix status when we get a request for simvastatin," Dr. Laposata said in his AMP talk. "Ethical challenges abound in this area," he noted, adding, "We can't go backward." In the interview with CAP TODAY, he posed the ultimate question: "What are you going to do when everybody gets a whole genome sequence?"

As for reimbursement, Dr. Laposata told the AMP attendees, "For many advances in medicine, if you have to ask that question first, you'll never get to question No. 2."

"Some say, 'We are not going to start unless you show how we will pay for this upfront,'" he tells CAP TODAY. "Others say, 'We understand you will not pay money upfront. We will look for clinical value then, and if there is clinical value, we will approach you to get paid for this work.'" Vanderbilt has taken the latter stance.

Readmission cost is a major financial consideration. "If people thrombose their stent, as we understand the rules, we are not going to be paid for that readmission. So spending $150 in reagents and supplies works out to our advantage. The cost of the test is dwarfed by the cost of readmission."

Dr. Laposata cites another cost aspect: Clopidogrel became generic in 2012. “This makes pharmacogenomics more important,” he says. “Some people were saying that the way to avoid adverse outcomes from Plavix resistance was to switch everybody to prasugrel or ticagrelor. That decision now has major financial consequences because of the higher cost of these two newer drugs.”

At the University of North Carolina, it was the clinicians who initiated pharmacogenomic testing for clopidogrel. “The cardiologists came to us and asked us to provide testing,” Dr. Weck says. “They were well aware of the FDA black-box warning.” Along with the Institute for Pharmacogenomics and Individualized Therapy, Dr. Weck set up a dose-escalation clinical trial with the cardiology group to look at whether doubling the normal dose of clopidogrel in heterozygous patients with a single LOF allele could be effective. “The results are being published now,” Dr. Weck says. Doubling the dose from 75 to 150 mg in heterozygous individuals resulted in normalization of platelet inhibition, showing that those patients do not require a medication switch. “This provided indirect evidence that pharmacogenomics testing would have clinical utility,” Dr. Weck says.

Now Dr. Weck’s laboratory does daily CYP 2C19 genotyping for percutaneous coronary intervention patients receiving a stent using a laboratory-developed PCR assay for the three most common variants (*2, *3, and *17). Turnaround time is 24 hours. “In most cases doctors have the information before the patient is discharged,” she says. An alert system is in place in which a pharmacist working with the cardiologists is paged with the result.

Dr. Weck

Patients are started on prasugrel or ticagrelor. If the patient has a normal CYP 2C19 genotype, he or she is switched to clopidogrel. This is a turnaround from the past few years when patients were started on clopidogrel and switched to an alternative drug based on pharmacogenomic information. Now that clopidogrel is generic, this algorithm is more expensive. “Maybe the cardiologists have adopted this more conservative approach partly because they are worried that treating patients with clopidogrel, even for a short time, might not be safe and effective if they have a reduced metabolism allele,” Dr. Weck says.

“Not all institutions or all physicians are doing this routinely,” she says. “There is still some controversy whether the level of evidence is enough to use pharmacogenomics clinically for CYP 2C19.”

Dr. Weck’s laboratory is not doing pharmacogenomic testing for simvastatin. “The general consensus is that there is not enough evidence that clinical decisions will be changed based on that result,” she says. “We haven’t had any requests for it.”

Based on the results of their clinical trial of pharmacogenomic testing for warfarin, Dr. Weck says, “It seems as though using a PGx-guided dosing algorithm did not result in any decrease to time to therapeutic range.” Nor was there any significant reduction in adverse events. “To have statistically significant results we would need more patients,” she says. She considers the results of the ongoing National Heart, Lung and Blood Institute trial of PGx for warfarin to be important. “My guess is that it will be several years before it’s finished, in part because the number of adverse events is rather small. That’s because the algorithm focuses on common variants that have a smaller effect.” In her view, the emphasis should be on studying rarer alleles that have greater effects on response. “In many studies rare variants are not even interrogated,” she says. Coding region variants in VKORC1, several of which are associated with extreme resistance to warfarin, are not included in dosing algorithms, she notes.

Dr. Weck and her colleagues also did a tamoxifen dose-escalation trial using genotyping of CYP 2D6. “We showed

that increasing tamoxifen dose in intermediate metabolizers normalized plasma levels of the active metabolite endoxifen. But we really are going to need outcomes trials to show that pharmacogenomic dosing has clinical utility to have greater uptake."

At the University of Florida, pharmacogenomic testing for clopidogrel is offered for all patients evaluated for coronary artery disease, not just those getting a stent. "There is going to be a fair proportion who will not have a stent placed," Dr. Johnson, the distinguished professor of pharmacy and medicine, says. "Having a genotype available is valuable, since they may require a stent at some time in their life." She calls this a"preemptive genotype."

Pharmacogenomic testing is done using Life Technologies' OpenArray on the QuantStudio PCR system with a custom-designed chip carrying 256 SNPs. Hui-Jia Dong, PhD, assistant professor of pathology and technical director for molecular pathology, says they test for the *2, *3, *4, *5, *6, *8, *10, and *17 alleles. All except *17 are nonfunctional or poor metabolizers and trigger a pop-up alert in the medical record, both in homozygous and heterozygous configurations. The laboratory report generates a specific recommendation with regard to Plavix use.

Any nonfunctional or low-function allele in combination with either *1 or *17 has this interpretation: "This patient has predicted *impaired metabolism* via the CYP 2C19 drug metabolizing enzyme." For any two nonfunctional alleles in combination, the wording is "*very impaired metabolism*." The clinical interpretation for all these combinations is: "This patient *will not* effectively convert clopidogrel to its active metabolite. Therapeutic alternatives are recommended."

Testing for clopidogrel uses eight of the chip's slots. "On the remaining 248, we are collecting research informed consent data," Dr. Johnson says. "We are asking people to allow us to use other SNPs potentially in the future clinically in the medical record, and to use genomic data for research purposes going into the medical record." As they validate additional genes, they will move them into clinical use.

Charging for this assay is awaiting clarification of new CPT codes for 2013. "New molecular codes have been published," Dr. Clare-Salzler says. "But we don't yet know the reimbursement amount. That still has to be set by CMS and will guide what we can charge for the test."

"Not all insurance companies cover this," he adds, "but at least for CYP 2C19 some do."

Widespread adoption of pharmacogenomic testing and routine reimbursement await further validation of clinical utility. To Dr. Weck, the value and impact of data for clinical utility are underscored by one area that she says "has really taken off" in her laboratory: detecting somatic mutations in cancers, mutations such as EGFR, KRAS, and BRAF that guide the selection of specific drugs. "Most tests in our lab in the last few years have been in this area," she says. "There has been an explosion of molecularly targeted drugs that have led to routine molecular testing. I still think of it as pharmacogenomics," she says. They are genotyping a cancer cell to find variants that can be predictive of drug response. One big difference is that the value to patients of the tests in conjunction with the therapeutic agents has been well established—companies doing phase three trials on oncologic drugs now routinely incorporate tests for genomic markers, thus validating the drug and the biomarker test simultaneously. Pharmacogenomics outside the realm of oncology still has a way to go to generate proof that is this compelling.

William Check is a writer in Ft. Lauderdale, Fla.

Whole exome sequencing and pharmacogenomics

A striking example of the interrelation between pharmacogenomics and next-generation sequencing is provided by a Clinical Sequencing Exploratory Research (CSER) grant awarded to Karen Weck, MD, and her colleagues at the University of North Carolina, one of six such grants awarded by the National Human Genome Research Institute. In this work, whole exome sequencing will be done on 750 patients with a likely genetic etiology to their disease to look for diagnostic results. The conditions to be included are cardiomyopathy, seizures, neuromuscular disorders, inherited or familial cancer, and microencephaly and developmental delay.

“The study is designed so we can study characteristics of genomic information that patients want and study its impact on medical treatment and behavior,” says Dr. Weck, professor of pathology and laboratory medicine and genetics at UNC and director of molecular genetics. “If you do whole genome sequencing or whole exome sequencing, what information do you find that is useful to patients?

“We will also be returning so-called incidental information not related to that patient’s disease,” Dr. Weck says. Study subjects are randomized to receive only diagnostic information or diagnostic information with the choice to receive incidental information. “I am particularly excited about pharmacogenomic information that we will glean from this study, which is a big part of personalized medicine,” Dr. Weck says. Patients can decide whether they want pharmacogenomic information along with information about their risk of future disease. “We will have the opportunity to study how patients and physicians deal with pharmacogenomic information and whether they find it useful, and to see what effect it has on medical treatment and diseases,” Dr. Weck says. “This is a huge issue. That’s why we designed the study this way.” They have divided incidental genomic information into different risk categories or “bins,” she says, based on the potential harms and benefits to the patient, including whether information is medically actionable.

Information about pharmacogenomics is considered fairly low risk, while information about diseases such as Huntington’s is fairly high risk. Information about the apolipoprotein E gene, which affects the risk for Alzheimer’s disease, would be considered intermediate risk.

For high-risk information, patients go through a series of counseling steps. “Our approach is to have a conversation with the patient upfront,” Dr. Weck says. “We will tell them, ‘This is the type of information we might get.’ And to have the patient be the driver. My guess is that most patients will say, Yes, I want to know it all, but that they haven’t thought about all the ramifications. So having a serious conversation about that is very important.”

“If information is potentially life-threatening and treatable or medically actionable, we will return it to people even in the control arm,” Dr. Weck says. A genetic variant for long QT syndrome falls into this category. It is associated with an increased risk of sudden death, and there is something you can do about it. “That’s why I think pharmacogenomic variants will be of extreme interest to patients,” she says. “By definition it will affect their response to a drug if the patient ever needs that drug.”

—*William Check, PhD*