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Pharmacogenetics: Personalizing Pain Medicine

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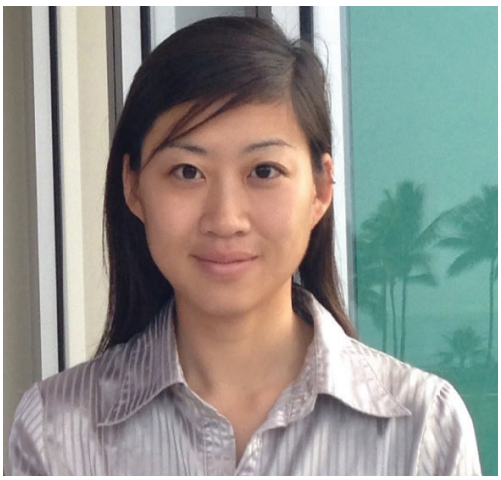

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Clinical Update

Pharmacogenetics: Personalizing Pain Medicine

Introduction

In an era of health care reform, personalized medicine represents a promising avenue for optimizing patient outcomes and improving quality of life while lowering health care costs. The trial-and-error approach to medication prescribing currently used is demonstrably costly and may put patients at risk for undue suffering that results from delays in effective care, inappropriate or unnecessary medication use, as well as adverse drug reactions and drug-drug interactions.[1] Among the tools available with which to implement a more personalized approach that has greater precision, pharmacogenetics has emerged as a practical means of determining interindividual differences in the efficacy and toxicity of medications that result from inherited genetic variation in drug-metabolizing enzymes, transporters, receptors, and other drug targets.[2]

To allow a better understanding of how pharmacogenetics can be applied to daily practice, this *Clinical Update* will discuss the science of pharmacogenetics with a focus on pain medications—the most commonly prescribed medications in the United States[3] and the most widely used class of medications that have pharmacogenetic tests associated with them (Figure 1, page 4).[4-6] The following discussion will cover the impact of variations in cytochrome P450 (CYP450) metabolism, how to incorporate CYP450 test results into treatment management for acute and chronic pain, and how and when to test for genetic polymorphisms in CYP450 enzymes.

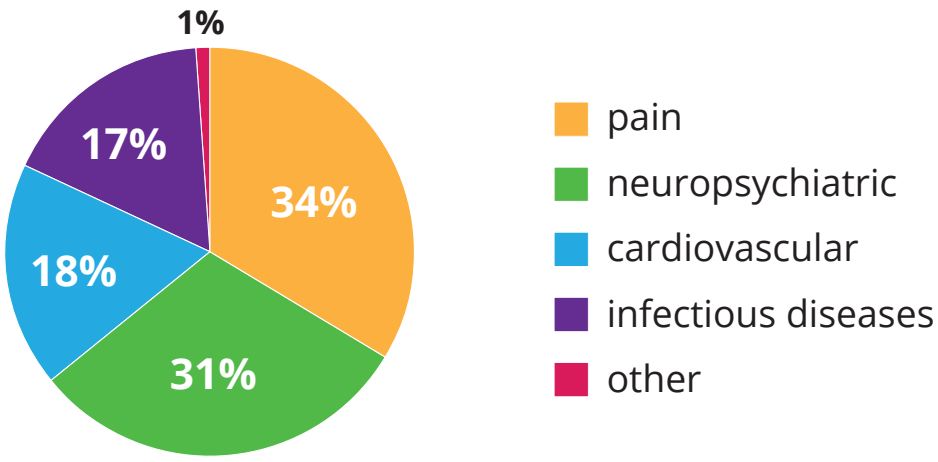

Figure 1. The top four therapeutic areas ranked by the estimated number of prescriptions during 2010-2012 for drugs with pharmacogenetic information on the label.[4-6]

Brief History of Pharmacogenetics

In the early 1950s, a genetic basis was established for the occurrence of hemolysis in patients given antimalarial drugs who had a deficiency in glucose-6-phosphate dehydrogenase (G6PD), an enzyme in red blood cells that protects erythrocytes from hemolysis caused by oxidative stress.[2,7,8] Other early examples of inherited differences in drug effects include prolonged muscle relaxation with use of suxamethonium in patients with an inherited deficiency of plasma cholinesterase, and peripheral neuropathy resulting from use of isoniazid that was associated with inherited differences in acetylation of this medication.[9-11]

Subsequently, researchers discovered that pretreatment genetic testing in HIV and hepatitis C virus management is effective in identifying which medication will work best for an individual and in preventing use of agents that will cause severe adverse reactions.[12-16] In cancer research, marked advances were made in tumor genetics such that currently a significant number of somatic, tumor-based genetic variants have been tied to the effects of specific drugs such as imatinib. These discoveries in viral and tumor genomics have established important precedents for the use of genetic testing to predict the efficacy and toxicity of other medications.[17] In these fields, use of genetic testing to guide treatment is linked to substantial cost savings in addition to improved outcomes.[18,19]

Pharmacogenetic testing is now being applied to some of the most commonly prescribed medications. The U.S. Food and Drug Administration has approved the addition of pharmacogenetic information to more than 150 medication labels. Furthermore, international pharmacogenetic guideline committees have been established in the United States, Europe, and Canada, to guide the implementation of decisions that follow from such pharmacogenomic testing. Medications with well-established clinical guidelines include abacavir, codeine, clopidogrel, azathioprine, and simvastatin.[20-24]

Overview of Pharmacogenetics In Pain Management

Pharmacogenetic science allows health care providers to use a patient's genetic makeup to predict a given medication's pharmacokinetics (ability to metabolize, transport, and eliminate the drug) and pharmacodynamics (response to a drug at the level of the drug target or receptor).[25] Pharmacokinetic parameters describe the concentrations of a drug in the blood, and help clinicians decide what dose of a drug is needed to reach effective concentration at its target. In contrast, pharmacodynamic measurements are used to describe the relationship between the resulting plasma concentration and physiologic effects. This relationship may be affected by differences in the way a patient's system absorbs, distributes, metabolizes, and/or eliminates the drug, all of which influence pharmacokinetics, or by transporter- and receptor-mediated effects among other factors that influence pharmacodynamics and, ultimately, clinical outcomes (Figure 2).[25,26]

Management of acute and chronic pain is complex for a variety of reasons. First, treatment often involves the concurrent use of several medications that have different mechanisms of action to target different pain pathways. This approach inevitably exposes patients to a greater risk for drug-drug interactions. In addition, the vast variability in patient response

to medications makes it challenging to determine in advance which prescribed medications actually will be effective in any individual. Second, a variety of factors affect drug metabolism and disposition, making it hard to predict how much drug will get into the body and at what rate it will be eliminated. As a result, it is often difficult to anticipate the ideal dose for an individual patient and to know whether a patient will respond to a given medication. Third, prescription drug misuse and risk for overdose are a concern with all pain medications but particularly the most potent: the opioid class of drugs.

Genetic variation is believed to account for up to 40% of individual differences in drug metabolism and response.[27] The body recognizes many medications as toxins and metabolizes them in order to break down and eliminate them. Many drugs, including most pain medications, are metabolized in the liver and gastrointestinal tract by enzymes that are located there to provide a barrier against dangerous xenobiotics ingested in food. Among the many enzymes located there, the CYP450 enzymes are particularly important, and are both essential and rate-limiting for the metabolism of many medications. Of the more than 50 CYP450 enzymes currently known, seven—CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5—are responsible for metabolizing approximately 90% of currently used medications.[28,29] Table 1, page 6, shows the known genetic polymorphisms in CYP450 enzymes that are involved in metabolism of pain medications.[30-33]

Variations in CYP450 Metabolism

Each CYP450 enzyme is coded for DNA by a specific gene. One genetic allele is inherited from each parent, and these alleles are categorized as wild type (most common in the general population) or variant.[34] The wild-type alleles are generally active while the variant alleles may be dysfunctional. When a variant allele replaces one or both wild-type alleles, this is known as a polymorphism because the population distribution curve of metabolic activity has more than one shape or peak.[34]

Pharmacogenetic testing for variant and wild-type alleles of CYP450 enzymes is able to categorize patients into 1 of 4 possible phenotypes (Table 2, page 6): ultra-rapid, extensive, intermediate, and poor metabolizers.[34-36] On one end of the spectrum are ultra-rapid metabolizers who have faster enzyme activity that clears certain pain medications too quickly, often resulting in the need for high doses of the medication to achieve pain relief. On the other end of the spectrum are poor metabolizers who will have markedly decreased ability to clear a medication, which may lead to a high serum concentration and toxicity.[34,35]

Importantly, a patient who is a poor metabolizer may be unable to convert pro-opioids into the active metabolites that provide pain relief (ie, codeine into morphine, hydrocodone into hydromorphone, dihydrocodeine into morphine, and tramadol into O-desmethyltramadol). A patient who is an ultra-rapid metabolizer will quickly metabolize these medications into their metabolites.

The rate at which CYP450 enzymes catalyze drug metabolism also may be

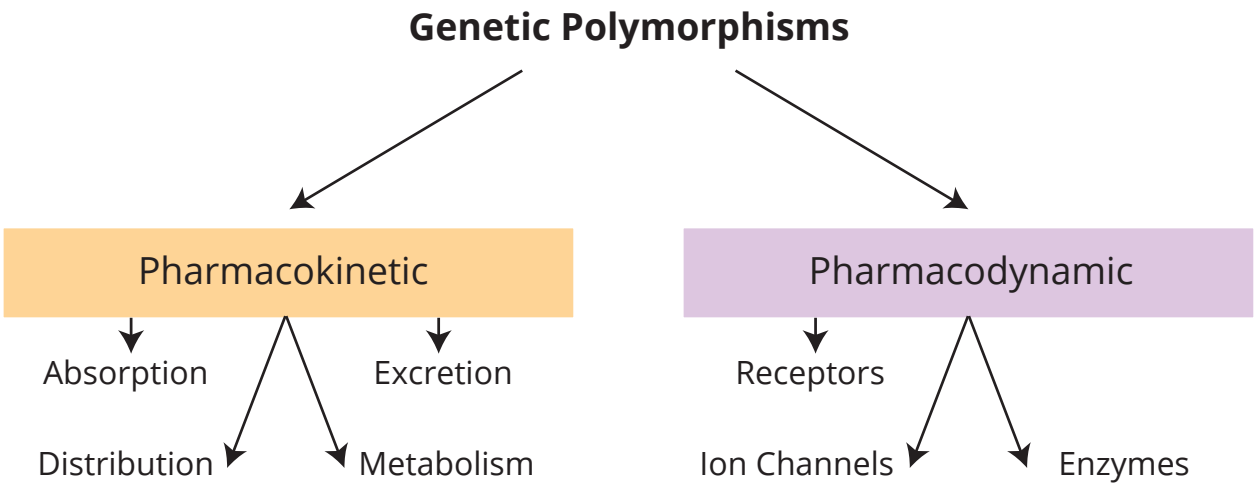

Figure 2. Pharmacokinetic and pharmacodynamic parameters.[25,26]

Table 1. The Principal Polymorphic CYP450 Enzymes Involved in the Metabolism Of Drugs Commonly Used in Pain Management

Enzymes	Important Gene Variants	Influenced Drug(s)	Reference
CYP2B6	516G > T 785A > G 983T > C 1459C > T SV1 splice variant	Opioid (methadone)	30
CYP2C9	430C > T 1075A > C	Coxib (celecoxib)	31
CYP2C19	681G > A 636G > A – 806C > T	SSRI (citalopram)	32
CYP2D6	2549 Del A 1846G > A 100C > T 1707 Del T 1023C > T 1659G >A 3183G >A 2988G >A	Opioids (codeine, hydrocodone, tramadol) TCAs (amitriptyline, nortriptyline, imipramine, and desipramine) SSRIs (fluoxetine, paroxetine, and citalopram) SNRI (venlafaxine)	33

SNRI, serotonin-norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant

Table 2. Clinical Implications of CYP450 Phenotypes[34-36]

Phenotype	Characteristics	Clinical Implications
Ultra-rapid metabolizer	Has multiple copies (ie, more than 2) of wild-type/functional alleles and metabolizes drugs faster than a patient with normal enzyme activity (ie, extensive metabolizer).	The overactive enzyme activity makes the active opiate metabolize more quickly and may cause patients to experience unanticipated toxicity while they are taking regular doses. In addition, long-acting agents for which the parent drug is active may not last as long as intended.
Extensive metabolizer	Has 2 copies of wild-type/functional alleles and normal enzyme activity.	Patient has a typical response to standard medication doses.
Intermediate metabolizer	Has 1 wild-type/functional allele and one variant allele, resulting in decreased enzymatic activity and, thus, a decreased ability to metabolize a given medication through the enzymatic pathway tested.	Patient may have a diminished response to a medication that requires metabolism to an active metabolite for optimum activity. In addition, higher than usual serum concentrations of the parent drug may result in unanticipated toxicity.
Poor metabolizer	Has 2 copies of variant alleles, making the enzyme involved have little or no ability to metabolize medication through the enzymatic pathway involved.	Patients taking prodrugs such as codeine may experience little analgesic benefit since little active metabolite will be present. In addition, long acting medicines may become toxic relatively quickly with repeated dosing as the result of high serum concentrations.

Table 3. CYP2D6 Inhibitors Commonly Prescribed Concomitantly in Patients in Pain[37,38]

CYP2D6 Potency	Medication
Strong CYP2D6 inhibitor	Bupropion
	Fluoxetine
	Paroxetine
Weak CYP2D6 inhibitor	Citalopram
	Diphenhydramine
	Sertraline

affected by drug interactions, including inhibition or induction of CYP450 enzymes that may reduce medication efficacy or increase the risk of side effects. Using codeine as an example, drugs that are inhibitors of CYP2D6 are known to reduce the metabolism of codeine to morphine.[37] Table 3 shows commonly used strong and weak CYP2D6 inhibitors.[37,38] The stronger the CYP2D6 inhibitor used concomitantly with codeine, the lower the amount of morphine generated and the lower the morphine-to-codeine ratio, with CYP2D6 poor metabolizers having the lowest morphine-to-codeine ratio.[37] Thus, it is important to consider the potential for both drug-drug interactions and a patient's CYP450 genotype.

In addition, herbal agents and some foods also may affect the rate at which CYP450 enzymes perform. For example, St. John's wort results in enhanced activity of some, but not all, CYP450 enzymes and concomitant use of grapefruit can inhibit the activity of CYP3A with clinical consequences.[34,36,39]

Thus, understanding a patient's overall CYP450 metabolism phenotype can help optimize the treatment plan and reduce the risk for treatment failure, toxicity, and drug-drug interactions.

Examples of Relevant Clinical Outcomes With Cytochrome P450 Genotyping

The following examples of CYP450 testing on patients taking hydrocodone, codeine, and methadone are designed to help the reader gain a better understanding of the clinical use and implications of CYP450 genotyping.

Hydrocodone

Hydrocodone is metabolized to hydromorphone by CYP2D6 enzymes. Hydromorphone has a 10- to 33-fold greater affinity for binding to μ-opioid receptors as compared with the parent drug hydrocodone.[24] Pain relief has been correlated with plasma concentrations of hydromorphone in patients, and not with hydrocodone.[40] In a CYP2D6 poor metabolizer, hydrocodone may not be metabolized effectively into hydromorphone, and therefore the patient may have altered analgesic response.[40] In contrast, in an ultra-rapid CYP2D6 metabolizer, hydrocodone is rapidly metabolized into hydromorphone. Because hydromorphone is a more potent opioid, ultra-rapid CYP2D6 metabolizers may have an increased response to hydrocodone, but also will require close monitoring for the emergence of adverse events. CYP2D6 genetic variants that result in altered metabolism of hydrocodone to hydromorphone should alert the clinician to consider alternative medications for managing pain.

Codeine

Codeine is metabolized into morphine almost exclusively by the CYP2D6 enzyme. Since codeine itself is a weak opiate, most of the analgesic activity is believed to be derived from codeine's much more potent metabolite: morphine. Individual patients who are poor CYP2D6 metabolizers may therefore experience markedly less pain relief when codeine is used to treat their pain.[24]

In 2012, the Clinical Pharmacogenetics Implementation Consortium released guidelines to aid clinicians in interpreting CYP2D6 genotype test results when they prescribe codeine therapy (Figure 3, page 8).[24] As described in the guidelines, a poor metabolizer may have inadequate pain relief due to limited generation of active morphine from inactive codeine, and another opiate may need to be chosen. In an ultra-rapid metabolizer, codeine is quickly metabolized to morphine and morphine concentrations may reach toxic levels, necessitating a change of medication or a reduction in dose.

Another consideration is use of codeine in nursing mothers. In 2007, the FDA issued a warning regarding use of codeine products in nursing mothers based on postmarket drug safety information. The FDA noted that "nursing infants may be at increased risk of morphine overdose if their mothers are taking codeine and are ultra-rapid metabolizers of the drug."[41] In addition, researchers have reported cases of fatalities resulting from codeine use in children undergoing tonsillectomy who were ultra-rapid metabolizers of CYP2D6.[42] The FDA cites that the risk for being an ultra-rapid metabolizer of codeine varies depending on patient race/ethnicity with a wide range that extends

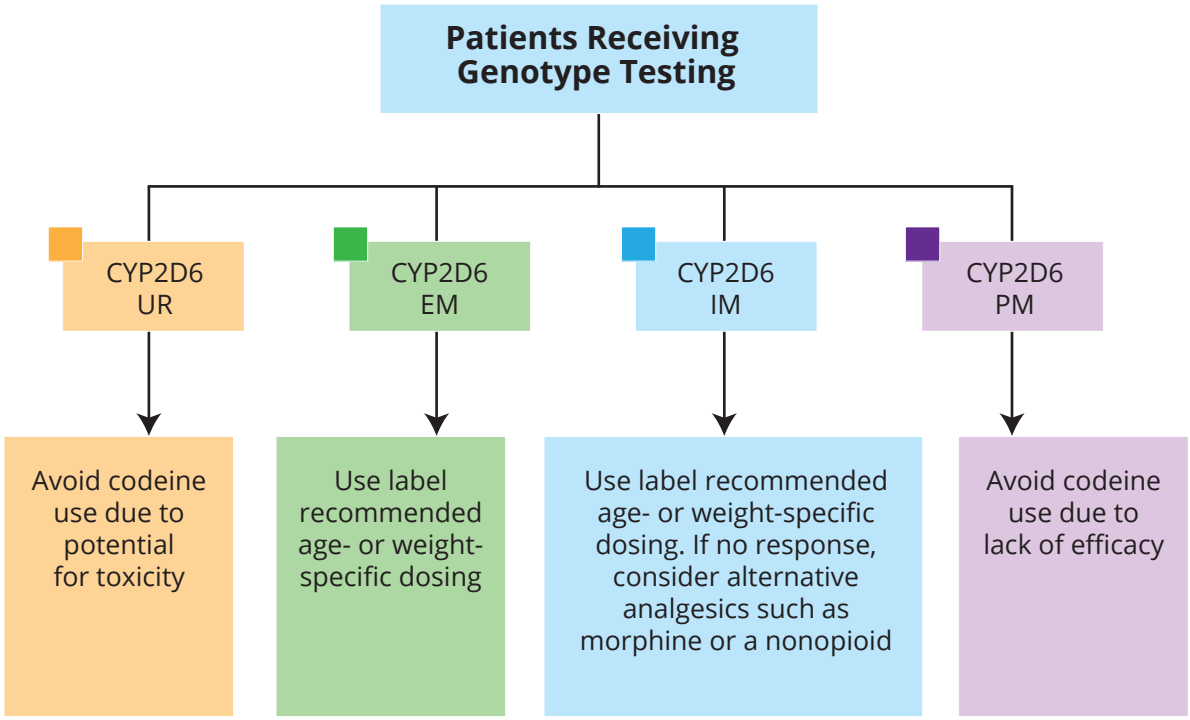

Figure 3. Algorithm for prescribing codeine therapy using CYP2D6 phenotype based on the Clinical Pharmacogenetics Implementation Consortium guidelines.[24]
EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; UR, ultra-rapid metabolizer

from less than 1 per 100 people in some populations to 28 per 100 people in others.[41]

More recently, researchers have found that genetic testing for CYP2D6 and ABCB1 genotypes predict 81% of cases of codeine-induced central nervous system depression in breastfed infants and their mothers.[43] When this genetic testing was combined with clinical factors—such as codeine dose—the accuracy rose to 87% with a sensitivity of 80% and a specificity of 87%. These researchers believe that the findings are relevant to all patient populations.

Methadone

Methadone is administered as a racemic drug metabolized primarily by CYP3A4 and CYP2B6 enzymes, and to a lesser extent by CYP19 and by CYP2D6 enzymes.[44] Methadone is of particular value to patients who require long-term treatment of chronic pain. However, methadone is frustratingly difficult to use effectively. The drug is subject to highly variable pharmacokinetics that include up to 100-fold variability in clearance,[45-47] and as a result initial dosing requirements and subsequent adjustments are difficult to anticipate.

Pharmacogenetic studies have shown that genetic polymorphisms in the CYP2B6 gene may be indicators of the degree of metabolism and clearance, and the plasma concentration of one of the enantiomers, S-methadone.[48,49] However, R-methadone is the primary active enantiomer at the μ-opioid receptor and thus contributes most to the pain-relief effect of this drug. More pharmacogenetic research is needed regarding other contributing CYP450 enzymes before clinical pharmacogenetic testing for methadone could be recommended.

Clinical Guidelines On Pharmacogenetics

Organized and consistent pharmacogenetic testing may become more widely adopted in clinical practice given the recent publication of guidelines on this topic. In 2013, the Office of Public Health Genomics at the Centers for Disease Control and Prevention released a continuously updated table of genomic tests sorted by level of evidence to help guide clinical decision making in the treatment of specific conditions.[50,51] The tests are classified into 3 color-coded classifications based on the level of evidence supporting their use: Tier 1/green indicates that evidence supports use in practice; Tier 2/yellow indicates that evidence is insufficient to support use in practice but may help guide use of testing in selective situations; Tier 3/red indicates that evidence discourages use of testing or there is a lack of relevant synthesized evidence.[50,51]

Table 4. Common Combination Products Containing Codeine and Hydrocodone[57,58]

Type	Generic	Brand Examples	Indication
Codeine Combination Agents	acetaminophen-codeine	Tylenol with codeine Tylenol No. 3 Tylenol No. 4	analgesic
	codeine-guaifenesin	Bitex Brontex Cheracol with codeine Cheratussin Guiatuss AC Mytussin AC Robitussin AC	cough suppressant and expectorant
Hydrocodone Combination Agents	acetaminophen-hydrocodone	Lorcet Lorcet Plus Lortab Vicodin Vicodin ES Vicodin HP	analgesic
	ibuprofen-hydrocodone	Vicoprofen	analgesic

As already discussed, the Clinical Pharmacogenetics Implementation Consortium released guidelines on codeine use based on CYP2D6 genotype test results as did the Canadian Pharmacogenomics Network for Drug Safety.[24,52] In 2011, the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy established pharmacogenetics-based therapeutic (dose) recommendations for 53 medications, including codeine, oxycodone, and tramadol.[53] In 2010, the National Academy of Clinical Biochemistry (NACB) published the first set of laboratory medicine practice guidelines for pharmacogenetics.[54] Furthermore, pharmacogenetic guidelines were issued by the European Medicines Agency in 2011 after extensive discussion at the European Science Foundation-University of Barcelona (ESF-UB) Conference in Biomedicine on Pharmacogenetics and Pharmacogenomics in 2010.[55,56]

Why CYP450 Test Results May Guide Treatment Strategies For Pain Management

Effective pain management is widely recognized as inadequate in many settings and this is particularly true in settings where the most potent drugs are used to treat the patients with the greatest severity of pain. Opiates are the most widely used potent analgesics in both the acute and chronic care settings, and their use is widespread in multiple clinical care settings, including in the post-operative setting and in nursing homes and chronic care facilities. The wide availability of CYP450 pharmacogenetic testing makes it clear that there are few practical impediments to obtaining testing: the challenge, therefore, is how to best use the available tools to improve pain control.

It is important to use pharmacogenetic testing for medications that have evidence-based guidelines demonstrating clinical utility. In the pain management field, pharmacogenetic testing guidelines that discuss the use of codeine, tramadol, hydrocodone, and oxycodone are available.[24,53] Importantly, codeine and hydrocodone are used in a wide variety of combination products (see Table 4).[57,58]

How and When to Use CYP450 Testing for Pain Management

Testing may be most useful in patients who need long-term pain therapy for chronic conditions and are not responding to treatment or are having side effects.[52] Testing also is recommended for young children before initiation of codeine.[52] Testing also may be beneficial in select situations such as in patients with a family history of drug sensitivity or hyperreactivity to medications, or patients who have experienced poor response to medication in the past, patients with complex conditions, or patients who are taking multiple medications.[59,60]

Table 5. Patient Education Materials on Pharmacogenetics

Organization	Title	Web Site
Mayo Clinic	Personalized Medicine and Pharmacogenomics	http://www.mayoclinic.org/healthy-living/consumer-health/in-depth/personalized-medicine/art-20044300?pg=1
National Genetics and Genomics Education Centre	Predicting the Effects of Drugs	http://www.geneticseducation.nhs.uk/genomics-in-health/predict-drug-effects
National Human Genome Research Institute	Frequently Asked Questions About Pharmacogenomics	http://www.genome.gov/27530645#al-3
National Institute of General Medical Sciences	Frequently Asked Questions about Pharmacogenomics	http://www.nigms.nih.gov/Research/SpecificAreas/PGRN/Background/Pages/pgrn_faq.aspx
National Institute of General Medical Sciences	Medicines For You	http://publications.nigms.nih.gov/medsforyou/index.html
University of Utah Health Sciences	Pharmacogenomics	http://learn.genetics.utah.edu/content/pharma/
U.S. Food and Drug Administration	Personalized Medicine Will Fit You Like a Glove	http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm317362.htm

Steps to Take Before Testing

Prior to pharmacogenetic testing, it is important to educate patients on the purpose of testing and the role of genes in drug response. This education can include how testing may be used to guide drug selection and/or adjust doses. In addition, it is important to discuss the risks, benefits, and limitation of testing.[61] For example, patients should understand that positive results are not an absolute predictor of non-response or risk for toxicity. Likewise, a negative result does not rule out the risk for non-response or adverse events as current tests only include known variants in known genes; other variants and clinical factors are also involved in the risk for these negative outcomes of treatment.[61] Patient education materials are available online, and it may be helpful to disseminate them to patients who have access to the internet (Table 5).[61]

Communicating Test Findings

Once pharmacogenetic test results are available, it is important to communicate the results in an effective and patient-appropriate manner. It has been proposed that patients may better respond to the significance of the results, such as that the patient has a slower than normal ability to metabolize—or break down—a drug in their body, than to information about their specific genotype. Use of words that may be perceived as negative by the patient, such as "poor" metabolizer or "mutant" should be avoided.

It is important to carefully discuss what changes to the patient's medication regimen are necessary based on test results and why the changes are being made. To help patients better understand the clinical significance of the results and to encourage patients to share the results with other health care providers, it is valuable for physicians to provide patients with a follow-up summary letter describing the test results in layman's language.[61]

Frequency of Testing

A discreet advantage of pharmacogenetic testing is that one need only test once. A result of pharmacogenetic testing could, in theory, be that more frequent monitoring will be instigated, but this would not involve repeated testing for a given genetic variant.

Given that many of the most commonly prescribed medications are influenced by a handful of genes, use of a single pharmacogenetic test result may be used to guide current as well as future treatment decisions. The test results should be shared with other health care providers to help reduce the need for repeated testing and to encourage use of the test results. In the future, increased use of electronic health records will facilitate dissemination of this information.[61]

Reimbursement Issues

Reimbursement for testing of this nature will be drug- and patient-specific. In general, insurance providers have adopted a reimbursement approach that involves the satisfaction of criteria for analytical and clinical validity and also

for clinical utility. A test is considered analytically valid if it is repeatable within and between labs. It is considered clinically valid if it can reproducibly predict a pharmacokinetic or dynamic parameter, such as the concentration of a drug in the serum or a patient's blood pressure. Clinical utility is achieved when a test can reproducibly predict a clinical outcome that would lead a health care provider to change their therapy such that a real change in outcome is achieved. Such a change in therapy might reasonably be a change in drug, a change in dose, or the institution of more careful monitoring.

While these definitions are clear, their interpretation remains inevitably fluid since the definition of a valuable clinical outcome can be viewed subjectively by different insurers. In addition, a number of states are currently adjudicating lawsuits in which either patients or states themselves are challenging non-coverage decisions by insurers. These considerations mean that while reimbursement for pharmacogenetic testing often occurs without impediment, this is not universal, and so it behooves each provider to pay attention to reimbursement for these tests and follow closely what happens on behalf of their patients.

Conclusion

Pharmacogenetic testing may help optimize the choice of effective medication therapy while minimizing side effects and drug-drug interactions. It should be considered one of the significant clinical variables that routinely guide therapeutic decisions. Pharmacogenetic testing is appropriate in patients who are refractory to routine doses of potent pain medications, and in patients who have a family history consistent with opiate toxicity or lack of efficacy.

As in all cases, good physician-patient communication is important to relay test findings to patients and educate patients on why certain treatments are being selected or why medication changes may be necessary. In the future, discovery of additional genes involved in drug disposition and effect may lead to new genetic tests that further personalize medication prescribing.

Reference List

1. Latif A, Rawat N, Pustavoitau A, Pronovost PJ, Pham JC. National study on the distribution, causes, and consequences of voluntarily reported medication errors between the ICU and non-ICU settings. *Crit Care Med.* 2013;41(2):389-398.
2. Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. *Science.* 1999;286(5439): 487-491.
3. IMS Institute for Healthcare Informatics. *Medicine Use and Shifting Costs of Healthcare: A Review of the Use of Medicines in the United States in 2013.* April 2014.
4. Bartholow M. Top 200 Drugs of 2012. Pharmacy Times. Accessed June 9, 2014. Available at: http://www.pharmacytimes.com/publications/issue/2013/July2013/Top-200-Drugs-of-2012. 2013.
5. Casey T. Prescription Volume, Spending Increasing in 2011. First Report Managed Care. Accessed June 9, 2014. Available at: http://www.firstreportnow.com/articles/prescription-volume-spending-increasing-2011.
6. IMS Institute for Healthcare Informatics. Declining medicine use and costs: for better or worse? A review of the use of medicines in the United States in 2012. May 2013. Accessed June 9, 2014. Available at: http://www.imshealth.com/portal/site/imshealth/menuitem.762a961826aad98f53c753c71ad8c22a/?vgnextoid=5b21ee0a8e631410VgnVCM10000076192ca2RCRD&vgnextchannel=736de5fda6370410VgnVCM10000076192ca2RCRD&vgnextfmt=default.
7. Alving AS, Carson PE, Flanagan CL, Ickes CE. Enzymatic deficiency in primaquine-sensitive erythrocytes. *Science.* 1956;124(3220):484-485.
8. Ronquist G, Theodorsson E. Inherited, non-spherocytic haemolysis due to deficiency of glucose-6-phosphate dehydrogenase. *Scand J Clin Lab Invest.* 2007;67(1):105-111.
9. Kalow W. Familial incidence of low pseudocholinesterase level. *Lancet.* 1956;2:576-577.
10. Hughes HB, Biehl JP, Jones AP, Schmidt LH. Metabolism of isoniazid in man as related to the occurrence of peripheral neuritis. *Am Rev Tuberc.* 1954;70(2):266-273.
11. Evans DA, Manley KA, Mc KV. Genetic control of isoniazid metabolism in man. *Br Med J.* Aug 13 1960;2(5197):485-491.
12. Hirsch MS, Conway B, D'Aquila RT, et al. Antiretroviral drug resistance testing in adults with HIV infection: implications for clinical management. International AIDS Society--USA Panel. *JAMA.* 1998;279(24):1984-1991.
13. Martin AM, Nolan D, Gaudieri S, et al. Predisposition to abacavir hypersensitivity conferred by HLA-B*5701 and a haplotypic Hsp70-Hom variant. *Proc Natl Acad Sci U S A.* 2004;101(12):4180-4185.
14. Hughes DA, Vilar FJ, Ward CC, et al. Cost-effectiveness analysis of HLA B*5701 genotyping in preventing abacavir hypersensitivity. *Pharmacogenetics.* 2004;14(6):335-342.
15. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature.* 2009;461(7262):399-401.
16. Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature.* 2009;461(7265):798-801.
17. Vivona D, Bueno CT, Lima LT, et al. ABCB1 haplotype is associated with major molecular response in chronic myeloid leukemia patients treated with standard-dose of imatinib. *Blood Cells Mol Dis.* 2012;48(2):132-136.
18. Parker JL, Lushina N, Bal PS, et al. Impact of biomarkers on clinical trial risk in breast cancer. *Breast Cancer Res Treat.* 2012;136(1):179-185.
19. Kauf TL, Farkouh RA, Earnshaw SR, et al. Economic efficiency of genetic screening to inform the use of abacavir sulfate in the treatment of HIV. *Pharmacoeconomics.* 2010;28(11):1025-1039.
20. Scott SA, Sangkuhl K, Stein CM, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for CYP2C19 genotype and clopidogrel therapy: 2013 update. *Clin Pharmacol Ther.* 2013;94(3):317-323.
21. Martin MA, Hoffman JM, Freimuth RR, et al. Clinical pharmacogenetics implementation consortium guidelines for hla-B genotype and abacavir dosing: 2014 update. *Clin Pharmacol Ther.* 2014;95(5):499-500.
22. Relling MV, Gardner EE, Sandborn WJ, et al. Clinical pharmacogenetics implementation consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing: 2013 update. *Clin Pharmacol Ther.* 2013;93(4):324-325.
23. Wilke RA, Ramsey LB, Johnson SG, et al. The clinical pharmacogenomics implementation consortium: CPIC guideline for SLCO1B1 and simvastatin-induced myopathy. *Clin Pharmacol Ther.* 2012;92(1):112-117.
24. Crews KR, Gaedigk A, Dunnenberger HM, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for cytochrome P450 2D6 genotype and codeine therapy: 2014 update. *Clin Pharmacol Ther.* 2014;95(4):376-382.
25. Adams JU. Pharmacogenomics and Personalized Medicine. *Nature Education.* 2008;1(1):194.
26. Pirmohamed M, Park BK. Genetic susceptibility to adverse drug reactions. *Trends Pharmacol Sci.* 2001;22(6):298-305.
27. Ingelman-Sundberg M. Pharmacogenetics: an opportunity for a safer and more efficient pharmacotherapy. *J Intern Med.* 2001;250(3):186-200.
28. Wilkinson GR. Drug metabolism and variability among patients in drug response. *N Engl J Med.* 2005;352(21):2211-2221.
29. Slaughter RL, Edwards DJ. Recent advances: the cytochrome P450 enzymes. *Ann Pharmacother.* 1995;29(6):619-624.
30. Thorn CF, Lamba JK, Lamba V, Klein TE, Altman RB. PharmGKB summary: very important pharmacogene information for CYP2B6. *Pharmacogenet Genomics.* 2010;20(8):520-523.
31. Van Booven D, Marsh S, McLeod H, et al. Cytochrome P450 2C9-CYP2C9. *Pharmacogenet Genomics.* 2010;20(4):277-281.
32. Scott SA, Sangkuhl K, Shuldiner AR, et al. PharmGKB summary: very important pharmacogene information for cytochrome P450, family 2, subfamily C, polypeptide 19. *Pharmacogenet Genomics.* 2012;22(2):159-165.
33. Owen RP, Sangkuhl K, Klein TE, Altman RB. Cytochrome P450 2D6. *Pharmacogenet Genomics.* 2009;19(7):559-562.
34. Lynch T, Price A. The effect of cytochrome P450 metabolism on drug response, interactions, and adverse effects. *Am Fam Physician.* 2007;76(3):391-396.
35. Tennant F. Cytochrome P450 testing in high-dose opioid patients. *Pract Pain Manage.* 2012.
36. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol Ther.* 2013;138(1):103-141.
37. Lam J, Woodall KL, Solbeck P, et al. Codeine-related deaths: The role of pharmacogenetics and drug interactions. *Forensic Sci Int.* 2014;239:50-56.
38. Hemeryck A, Belpaire FM. Selective serotonin reuptake inhibitors and cytochrome P-450 mediated drug-drug interactions: an update. *Curr Drug Metab.* 2002;3(1):13-37.
39. Michalets EL. Update: clinically significant cytochrome P-450 drug interactions. *Pharmacotherapy.* 1998;18(1):84-112.
40. Stauble ME, Moore AW, Langman LJ, et al. Hydrocodone in postoperative personalized pain management: pro-drug or drug? *Clin Chim Acta.* 2014;429:26-29.
41. U.S. Food and Drug Adminstration. FDA Warning on Codeine Use by Nursing Mothers May Increase Chance of Serious Side Effects in Infants. August 17, 2007. Available at: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2007/ucm108968.htm.
42. Kelly LE, Rieder M, van den Anker J, et al. More codeine fatalities after tonsillectomy in North American children. *Pediatrics.* 2012;129(5):e1343-1347.
43. Sistonen J, Madadi P, Ross CJ, et al. Prediction of codeine toxicity in infants and their mothers using a novel combination of maternal genetic markers. *Clin Pharmacol Ther.* 2012;91(4):692-699.
44. Lu WJ, Thong N, Flockhart DA. Reduced methadone clearance during aromatase inhibition. *J Clin Psychopharmacol.* 2012;32(4):511-517.
45. Weschules DJ, Bain KT, Richeimer S. Actual and potential drug interactions associated with methadone. *Pain Med.* 2008;9(3):315-344.
46. Totah RA, Sheffels P, Roberts T, et al.

Role of CYP2B6 in stereoselective human methadone metabolism. *Anesthesiology.* 2008;108(3):363-374.

47. Eap CB, Buclin T, Baumann P. Interindividual variability of the clinical pharmacokinetics of methadone: implications for the treatment of opioid dependence. *Clin Pharmacokinet.* 2002;41(14):1153-1193.
48. Crettol S, Deglon JJ, Besson J, et al. Methadone enantiomer plasma levels, CYP2B6, CYP2C19, and CYP2C9 genotypes, and response to treatment. *Clin Pharmacol Ther.* 2005;78(6):593-604.
49. Wang SC, Ho IK, Tsou HH, et al. CYP2B6 polymorphisms influence the plasma concentration and clearance of the methadone S-enantiomer. *J Clin Psychopharmacol.* 2011;31(4):463-469.
50. Dotson WD, Douglas MP, Kolor K, et al. Prioritizing genomic applications for action by level of evidence: a horizon-scanning method. *Clin Pharmacol Ther.* 2014;95(4):394-402.
51. Centers for Disease Control and Prevention. Genetic Testing: Genomic Tests and Family History by Levels of Evidence. Accessed May 14, 2014. Available at: http://www.cdc.gov/genomics/gtesting/tier.htm.
52. Madadi P, Amstutz U, Rieder M, et al. Clinical practice guideline: CYP2D6 genotyping for safe and efficacious codeine therapy. *J Popul Ther Clin Pharmacol.* 2013;20(3):e369-396.
53. Swen JJ, Nijenhuis M, de Boer A, et al. Pharmacogenetics: from bench to byte—an update of guidelines. *Clin Pharmacol Ther.* 2011;89(5):662-673.
54. The National Academy of Clinical Biochemistry. Laboratory Medicine Practice Guidelines: Laboratory Analysis and Application of Pharmacogenetics to Clinical Practice. Accessed May 14, 2014. Available at: http://www.aacc.org/members/nacb/LMPG/OnlineGuide/PublishedGuidelines/LAACP/Pages/default.aspx#. 2010.
55. Becquemont L, Alfirevic A, Amstutz U, et al. Practical recommendations for pharmacogenomics-based prescription: 2010 ESF-UB Conference on Pharmacogenetics and Pharmacogenomics. *Pharmacogenomics.* Jan 2011;12(1):113-124.
56. European Medicines Agency. Guideline on the Use of Pharmacogenetic Methodologies in the Pharmacokinetic Evaluation of Medicinal Products. August 2012; 1-23. Available at: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000411.jsp&mid=WC0b01ac058002958e.
57. Medline Plus. Hydrocodone. Accessed June 9, 2014. Available at: http://www.nlm.nih.gov/medlineplus/druginfo/meds/a601006.html.
58. Medline Plus. Codeine. Accessed June 9, 2014. Available at: http://www.nlm.nih.gov/medlineplus/druginfo/meds/a682065.html.
59. Tennant F. Making practical sense of cytochrome P450. *Pract Pain Manage.* 2010;10(4):12-19.
60. Fishbain DA, Fishbain D, Lewis J, et al. Genetic testing for enzymes of drug metabolism: does it have clinical utility for pain medicine at the present time? A structured review. *Pain Med.* 2004;5(1):81-93.
61. Mills R, Voora D, Peyser B, Haga SB. Delivering pharmacogenetic testing in a primary care setting. *Pharmgenomics Pers Med.* 2013;6:105-112.

Notes

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