



# Genetic Testing in Pain Medicine

ANDREA M. TRESBOT, MD

Director  
The Pain Center  
Eagle River, Alaska

Dr. Tresbot is medical director at Pinnacle Laboratory Services and is on the medical advisory board of Proove Biosciences.



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*If it were not for the great variability among individuals, medicine might as well be a science and not an art.*  
—William Osler (1892)

Clinicians who treat pain have noted that the response to opioids varies widely among patients, with opioid dose requirements varying in the clinical setting by as much as 40-fold.<sup>1</sup>

Differences in the degree of pain stimulation (a fractured femur compared with a splinter in the toe) and pain sensitivity (Figure 1), weight and age differences, prior opioid use and tolerance, and the differences in bioavailability of various opioid formulations have been cited as causes for the wide variability in analgesia seen with opioids. However, even measuring blood levels of opioids does not predict analgesia.<sup>2</sup> Just as there are differences in hair and eye color, there are differences in response to pain and to analgesic medications. We are beginning to recognize that, as is seen in much of medicine in the 21st century, genetics may explain the variability of responses and predict more effective (or less dangerous) medication choices.

By identifying the genetic risks and the most effective analgesic for an individual patient, the clinician (at least theoretically) could improve the efficacy of the pain medication and decrease the risk for iatrogenically induced overdose, addiction, and death.

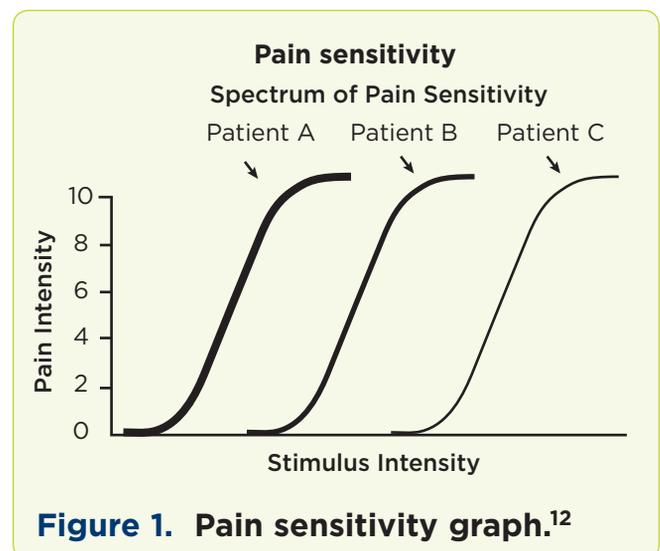
## Genetic Versus Environmental Factors

In the classic “nature versus nurture” scenario, investigators use twin pairs, both identical and fraternal, reared together or apart, to evaluate the heritability of a feature or a condition. Several twin studies have looked at pain conditions, and concluded that migraines have a 39% to 58% genetic contribution<sup>3-5</sup>; low back pain carries a 21% to 67% genetic contribution<sup>6-8</sup>; and menstrual pain has a 55% contribution.<sup>9</sup> In general, significant familial effects account for 24% to 32% of the

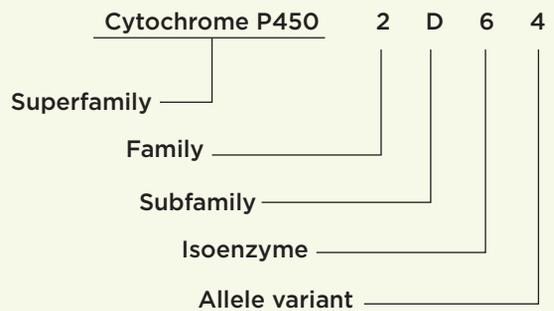
observed variance detected for heat and cold pressor pain thresholds and opioid-mediated elevation in cold pressor pain tolerance.<sup>10</sup>

## Genetics of Analgesia

When we give an opioid for pain relief, there is a continuum of responses, from good analgesia and improvement in function, to poor analgesia, tolerance, physical dependence, and addiction.<sup>11</sup> There are several ways that genetics can influence analgesic response, including drug metabolism enzymes, drug transporters,



**Figure 1. Pain sensitivity graph.<sup>12</sup>**



Nomenclature system for designating enzymes and alleles of cytochrome P450

**Figure 2. Cytochrome naming system.<sup>22</sup>**

opioid or other pain medication receptors, and structures involved in the perception and processing of pain. There are 2 specific genetic issues involving analgesia. The first is the genetic contribution of a variety of different pain types, because if a genetic basis underlies how pain is expressed, including the varying mechanisms of nociceptive, neuropathic, and visceral pain, then the potential exists for new analgesic targets. The second is the genetic influence on drug effectiveness and safety.<sup>12</sup>

### Genetic Hypoanalgesia

There are several well-studied hereditary disorders of insensitivity to pain, including “hereditary insensitivity to pain with anhidrosis,”<sup>13</sup> familial dysautonomia (Riley-Day syndrome,<sup>14</sup> Lesch-Nyhan syndrome,<sup>15,16</sup> de Lange syndrome,<sup>17</sup> and Tourette syndrome<sup>18</sup>). More than 200 candidate genes have been identified that may be involved in pain processing.

### Drug Actions

Drug *pharmacokinetics* describes a patient’s metabolic status, or the individual’s ability to metabolize certain drugs. For example, a patient with impaired metabolism may be unable to activate a prodrug such as codeine into the active morphine metabolite. *Pharmacodynamics* describes a patient’s ability to respond to a drug at the level of the drug target or receptor. Here, an example would be a patient who has a nonfunctional receptor for a certain drug who will be unable to respond to that drug regardless of the dosage. *Pharmacogenetics* describes the genetic influence on both the pharmacokinetics and pharmacodynamics. Polymorphic genes that encode the drug-metabolizing enzymes, drug transporters, drug receptors, and other proteins can serve as valuable markers, predictive of the efficacy and adverse responses in human subjects. *Pharmacogenomics* is the science that examines the inherited variations in genes that dictate drug response, predicting whether a patient will have a good or bad response to a drug or no response

at all. So, pharmacogenetics refers to the study of inherited differences in drug metabolism and response, whereas pharmacogenomics refers to the general study of the many genes that determine drug behavior. The distinction between the two terms is considered arbitrary and they can be used interchangeably.

Today, many of the complexities of human drug response are sufficiently well understood to transform the field of pharmacogenetics from a descriptive to a predictive science, leading to safer and more effective prescribing and dosing.<sup>12</sup> This kind of testing is being used more frequently in cancer treatment (eg, *BRCA1* in breast cancer) and internal medicine (eg, *VKORC1* for warfarin metabolism), but only very recently in pain medicine.

### Pain Conditions

Allele-based association studies are expected to shed light on the medical mystery of why pain persists in some patients but not others, despite seemingly identical traumas.

In other words, why do some patients with diabetes develop only numbness as the manifestation of their peripheral neuropathy, whereas others with the same blood sugar fluctuations develop a painful peripheral neuropathy? Why do only some patients with shingles develop postherpetic neuralgia? Why don’t all of the victims of a car accident develop the same whiplash pain?

Part of the issue may be “piss poor protoplasm,” a term that many young doctors learned as part of their medical training. In a study of Chinese volunteers, investigators found that an allele (*COL9A2*), which codes for a chain of collagen, was associated with a 4-fold increase in the risk for developing annular tears in individuals aged 30 to 39, and a 2.4-fold increase in the risk for developing degenerative disk disease and end-plate herniations in people aged 40 to 49 years old.<sup>19</sup>

### Drug Interactions

There are 3 major types of enzyme interactions. A *substrate* is any medication metabolized by that enzyme. An *inhibitor* is a medication that slows the metabolism of another medication, which may result in excessively high blood levels, extended effect, and related toxicity; however, if this is a drug that has to be activated (a *prodrug*), there may be decreased effect. An *inducer* is a medication that boosts the metabolism of another medication, which may result in accelerated breakdown, increased clearance, shortened duration, subtherapeutic levels, or withdrawal; it also may cause increased activity in a prodrug.

### Clinical Potential for Disaster

There are potentially many drug interactions, and the risk increases with increased numbers of medications being used. Glintborg et al<sup>20</sup> looked at 200 patients discharged from the hospital; the average age of the patients was 75, and the median number of drugs used was 8 (with a range of 1-24). They calculated a potential of 476 drug interactions in 63% of the

**Table 1. Common Substrates of CYP Enzymes<sup>23,24</sup>**

1A2	2B6	2C9	2C19	2D6	3A4
Acetaminophen	Bupropion	Celecoxib	Amitriptyline	Amitriptyline	Alprazolam
Amitriptyline	Ketamine	Ibuprofen	Barbiturates	Amphetamines	Amiodarone
Caffeine	Methadone	Piroxicam	Carisoprodol	Codeine	Atorvastatin
Clozapine	Testosterone	Valproic Acid	Citalopram	Dextromethorphan	Buprenorphine
Cyclobenzaprine		Warfarin	Clomipramine	Doxepin	Cyclosporine
Desipramine			Clopidogrel	Duloxetine	Digoxin
Duloxetine			Diazepam	Hydrocodone	Erythromycin
Fluvoxamine			Imipramine	Meperidine	Fentanyl
Imipramine			Phenytoin	Metoclopramide	Indinavir
Lidocaine			Sertraline	Nortriptyline	Lovastatin
Nabumetone			Topiramate	Oxycodone	Methadone
Melatonin				Propranolol	Midazolam
Theophylline				Tamoxifen	Sildenafil
Tizanidine				Tramadol	Trazodone
Warfarin				Venlafaxine	Verapamil

CYP, cytochrome P450

patients. In another study, patients who were taking 3 to 5 drugs had a 29% risk for interactions, whereas patients who were taking 11 or more drugs had a 96% risk for interactions. Only 1% of patients were aware of the potential for drug-drug interactions.<sup>21</sup>

### Cytochrome P450 Enzymes

The cytochrome P450 (CYP) enzyme system is a heme-containing, microsomal drug-metabolism superfamily involved in biosynthesis and degradation of endogenous compounds, chemicals, toxins, and medications. There have been 57 enzymes identified in humans, and they are divided into family, subfamily, isoenzymes, and allele variants (Figure 2).<sup>22</sup> Metabolism of most currently used drugs occurs by about 7 clinically relevant enzymes: CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4, all of which have different (but partially overlapping) catalytic activities. Many of the medicines commonly used are substrates (Table 1), inducers (Table 2), or inhibitors (Table 3) of medicines used in pain treatments.

There also are multiple potential interactions between herbs, supplements, or foods and prescription medications. For instance, St. John's wort, commonly taken for depression, induces CYP1A2, 2C9, and 3A4 enzymes. The induction of CYP1A2 and CYP2C9 can increase warfarin metabolism, leading to lower

blood levels and, therefore, an increase in the risk for clotting. St. John's wort can decrease verapamil, statins, methadone, digoxin, and HIV medication levels (via CYP3A4), tricyclic antidepressant and tizanidine levels (via CYP1A2), as well as potentially causing a serotonin syndrome with serotonin reuptake inhibitors. Intestinal CYP3A4 concentration can be decreased by 47% within 4 hours of grapefruit consumption.<sup>25</sup> Smoking is a potent inducer of CYP1A2, leading to decreased caffeine levels (which may be the cause of the increased agitation seen with smoking cessation, as caffeine levels increase when the induction stops). In a study comparing smokers with nonsmokers, the smokers had higher pain scores, and took larger doses of hydrocodone, but had significantly lower serum levels of hydrocodone.<sup>26</sup>

### Why Consider Genetic Testing?

There are several potential reasons to consider genetic testing. Drugs are metabolized slowly in individuals carrying a genetic polymorphism that causes absent or decreased enzyme activity, and these individuals are at an increased risk for adverse drug reactions (ADRs) or therapeutic failure. However, drug therapy could be ineffective if the drug is metabolized too quickly because of a genetic polymorphism. Knowledge of these polymorphisms before beginning a drug therapy

**Table 2. Common Inducers of CYP Enzymes<sup>23,24</sup>**

1A2	2C9	2C19	2D6	3A4
Carbamazepine	Barbiturates	Carbamazepine	Carbamazepine	Butalbital
Griseofulvin	Rifampin	Ginkgo	Dexamethasone	Carbamazepine
Lansoprazole	Ritonavir	Rifampin	Phenobarbital	Modafinil
Omeprazole	St. John's wort		Phenytoin	Nevirapine
Ritonavir			Rifampin	Phenytoin
St. John's wort				St. John's wort
Tobacco				Topiramate

CYP, cytochrome P450

**Table 3. Common Inhibitors of CYP Enzymes<sup>23,24</sup>**

1A2	2C9	2C19	2D6	3A4
Caffeine	Amiodarone	Fluoxetine	Amiodarone	Diltiazem
Ciprofloxacin	Fluvoxamine	Fluvoxamine	Bupropion	Erythromycin
Fluvoxamine	Modafinil	Modafinil	Celecoxib	Grapefruit juice
Grapefruit juice	Paroxetine	Oral contraceptives	Chlorpromazine	Indinavir
Mexiletine	Tamoxifen	Paroxetine	Cimetidine	Ketoconazole
Verapamil		Topiramate	Duloxetine	Mifepristone
			Fluoxetine	Nefazodone
			Haloperidol	Ritonavir
			Methadone	Verapamil
			Metoclopramide	
			Paroxetine	
			Quinidine	
			Ritonavir	
			Sertraline	

CYP, cytochrome P450

could help in choosing the right agent at a safe dosage, especially those drugs with a narrow therapeutic index and a high risk for the development of ADRs.<sup>27</sup> In a literature review of ADRs from 1995 to 2000, more than 50% of the drugs cited are metabolized by at least one enzyme with known poor-functioning alleles.<sup>28</sup>

### Types of Metabolizers

Patients can be classified by how effectively they metabolize a medication, which is based on how many copies of normal or abnormal alleles they inherited

(Table 4). An *extensive metabolizer* (EM) has 2 normal, or “wild-type,” alleles and is considered “normal.” An *intermediate metabolizer* (IM) has 1 normal and 1 reduced allele or 2 partially deficient alleles. A *poor metabolizer* (PM) has 2 mutant alleles leading to a very limited or complete loss of activity, whereas the *ultra-rapid metabolizer* (UM) has multiple copies of functional alleles leading to excess activity.

There also is an ethnic distribution of this polymorphism. Approximately 7% to 10% of whites are *CYP2D6* deficient (PM), but only 1% to 2% of Asians and

2% to 4% of blacks are PMs. However, approximately 30% of Asians and blacks have intermediate metabolism of CYP2D6. On the other hand, approximately 29% of Ethiopians, 10% of Southern Europeans, and 1% to 2% of Northern Europeans are UMs.<sup>29</sup> In psychiatry, 52% of the psychiatric and 62% of antidepressant or antipsychotic drugs are metabolized by CYP2D6.<sup>30</sup> A prospective 1-year clinical study of 100 psychiatric inpatients suggested a trend toward longer hospital stays and higher treatment costs for UMs and PMs of CYP2D6.<sup>31</sup> Tamoxifen must be metabolized via CYP2D6 to endoxifen to be effective; therefore, a PM might be at risk for failure of breast cancer treatment.<sup>32</sup> And, as is seen shortly, CYP2D6 activity can have substantial influence on the opioids that are commonly used in pain management.

These alternate genes, known as single-nucleotide polymorphisms (SNPs), are identified by letters or numbers. For example, normal functional activity alleles of the *CYP2D6* gene are designated CYP2D6\*1 and CYP2D6\*2. The four most common mutant alleles are CYP2D6\*3, CYP2D6\*4, CYP2D6\*5, and CYP2D6\*6 and account for 93% to 97% of the PM phenotypes in the white population.

### CYP2D6 Influence on Opioids

*Codeine* is an inactive compound (prodrug), metabolized by CYP2D6 into its active form, *morphine*. It only has a weak affinity for the  $\mu$ -receptor, 300 times less than morphine.<sup>33</sup> Therefore, CYP2D6 PM patients and patients taking CYP2D6 inhibitors (see Table 3) who are given Tylenol #3 (codeine/acetaminophen) are really being given only acetaminophen, whereas UM patients may have dangerously high levels of morphine after standard doses.<sup>34</sup> Tramadol is metabolized by CYP2D6 to its M1 metabolite, which is at least 6 times more potent than the parent compound.<sup>35</sup> *Hydrocodone* displays weak binding capacity for the  $\mu$ -receptor, but the CYP2D6 enzyme demethylates it into *hydromorphone*, which has much stronger  $\mu$  binding than hydrocodone.<sup>36</sup> Otton et al found that individuals identified as EM reported more “good opiate effects” and fewer “bad opiate effects” than PM or EM patients pretreated with quinidine (a potent CYP2D6 inhibitor). They concluded that activity of CYP2D6 might limit the abuse liability of hydrocodone.<sup>37</sup> A study looking at 25,200 urine samples from patients taking only hydrocodone showed a 60-fold variability in hydrocodone/hydromorphone ratios. They identified 0.6% UM and 4% PM, with a 134-fold between-subject variability.<sup>38</sup> *Oxycodone* is metabolized by glucuronidation to noroxycodone (which has less than 1% of the analgesia potency of oxycodone), and by 2D6 to *oxymorphone*. Oxycodone is an analgesic, not a prodrug; however, oxymorphone is an active metabolite of oxycodone, and may have significant effects on analgesia.

Because oxycodone is dependent on the 2D6 pathway for clearance, it is possible that toxicity and overdose can occur with 2D6 inhibitors.<sup>39</sup>

Drugs of abuse also are metabolized by CYP2D6.

**Table 4. Population Distribution of Isoenzymes**

Gene	PM	IM	EM	UM
<i>CYP2C9</i>	2%-4%	>35%	60%	NA
<i>CYP2C19</i>	2%-20%	24%-36%	14%-44%	30%
<i>CYP2D6</i>	10%	35%	48%	7%

EM, extensive metabolizers; IM, intermediate metabolizers; PM, poor metabolizers; UM, ultra-rapid metabolizers

Methamphetamine acts as both a substrate and a competitive inhibitor of *CYP2D6*, whereas methylenedioxy-*N*-methylamphetamine (MDMA) acts as a high-affinity substrate and potent inhibitor of the enzyme, so that methamphetamine and MDMA users, regardless of their genotype, act as poor metabolizers of CYP2D6.<sup>40</sup>

CYP3A4 also is involved in opioid metabolism. Fentanyl and buprenorphine are excreted via CYP3A4, and blood levels would be expected to rise in PM patients or those receiving CYP3A4 inhibitors.<sup>41</sup> Methadone has been widely reported to be metabolized by CYP3A4,<sup>42,43</sup> although some evidence suggests that it is primarily metabolized by CYP2B6<sup>44</sup> and patients who are homozygous for the variant *CYP3B6*\*6 gene required lower doses of methadone than the heterozygotes or noncarriers.<sup>45</sup>

### Genotype-Based Dose Adjustments

Standard dose adjustments look at the differences in pharmacokinetic parameters, such as clearance and area under the curve (AUC). Genotype-based dose adjustments would suggest a standard dose (eg, 2 tablets of medication X) for an EM; however, a PM might need only 1 tablet, an IM might need 1.5 tablets, and an UM might need 3 or more tablets of the same medication to get the same effect.<sup>46</sup> In a study of antidepressant drugs, it was calculated that, for a CYP2D6 PM patient taking nortriptyline, the therapeutic dose would be 50 mg, whereas a UM patient would need a dose of 500 mg.<sup>46</sup>

As another example, amitriptyline is metabolized by CYP2C19 to nortriptyline, which is then metabolized and excreted by CYP2D6. Genetic testing of CYP2D6 and CYP2C19 can identify patients at low or high risk for side effects of amitriptyline therapy. Carriers of 2 functional CYP2D6 alleles had a significantly lower risk for side effects than carriers of only 1 functional allele, with the lowest risk seen for carriers of 2 functional CYP2D6 alleles combined with only 1 functional CYP2C19 allele.<sup>47</sup> The authors noted that 65% of patients (normal CYP2D6 and normal to poor CYP2C19) could receive standard doses of amitriptyline (which is very inexpensive) with little or no side effects, but those patients with normal CYP2C19 and poor CYP2D6 were at very high risk for

Test	Test Outcome	Measured Results	Creatinine normalize
Codeine	Negative		-
Morphine	Negative		-
Hydrocodone	Positive	530	640
Norhydrocodone	Positive	494	595
Hydromorphone	Positive	78	95
Oxycodone	Positive	8,388	10,119
Noroxycodone	Positive	2,358	2,845
Oxymorphone	Positive	899	1,085

**Figure 3. Urine drug screen showing poor conversion of hydrocodone to hydromorphone and oxycodone to oxymorphone.**

Image courtesy of Andrea Trescot, MD.

Test	Test Outcome	Measured Results	Creatinine Normalized
<b>Natural and Semi-synthetic Opioids</b>			
Codeine	Negative	16,077	-
Morphine	Negative		-
Hydrocodone	Positive		29,718
Norhydrocodone	Negative		-
Hydromorphone	Negative		-
Oxycodone	Negative		-
Noroxycodone	Negative		-
Oxymorphone	Negative		-
<b>Synthetic Opioids</b>			
Fentanyl	Negative	16,502	-
Norfentanyl	Negative		-
Methadone	Positive		30,503
EDDP (methadone metabolite)	Negative		-

**Figure 4. Lack of urine metabolites, consistent with adulteration.**

Image courtesy of Andrea Trescot, MD

anticholinergic and mental side effects, and should be treated with newer (and more expensive) medications.

### Urine Drug Screening

Many urine drug screens (UDS), especially office “point-of-service” dipsticks, give a simple “positive” or “negative” result. But some quantitative UDS report opioid metabolites (Figure 3), which can give clues as to the genetic makeup of a patient. In this example, there is poor conversion of hydrocodone to hydromorphone, as well as poor conversion of oxycodone to oxymorphone, suggesting a CYP2D6 deficiency or inhibition. If this patient had complaints of poor analgesia, changing to hydromorphone or oxymorphone would be expected to bypass the CYP2D6 enzyme and provide better pain relief. Most normetabolites (such as norhydrocodone

and noroxycodone) have longer elimination half-lives than the parent drugs, so that urine samples that test negative for the parent compound still can be positive for the normetabolite.<sup>48</sup> Checking for metabolites in the urine also can uncover adulterations such as those seen in Figure 4, where the dipstick was positive for hydrocodone and methadone, as was prescribed, but the UDS showed a complete lack of metabolites, consistent with scraping the pills into the urine (which this patient admitted to when confronted by the results).

### DNA Testing

The use of oral samples or buccal swabs for specific genetic testing recently has become economically feasible, given a dramatic decrease in pricing. Several SNPs are readily available, providing information

on CYP enzymes 2D6, 2C9, 2C19 as well as VKORC1 (reflecting the metabolism of warfarin). Additional testing for CYP3A4 also is available. However, intriguing information regarding potential risk for addiction and misuse also may be available through genetic testing.

### How Do We Use Genetic Testing?

Genetic testing can be used to explain and confirm ineffective or high opioid use. For example, patients with CYP2D6 deficiencies would be expected to have poor (or relatively poor) relief from tramadol, codeine, hydrocodone, and oxycodone, whereas patients with CYP2D6 UM might be at risk for unexpectedly high levels of morphine from codeine.<sup>49</sup> Switching to an opioid not metabolized by that enzyme (such as fentanyl or morphine) might be much more effective or less risky. Patients with poor opioid efficacy from an inactive OPRM1 allele might benefit from a  $\kappa$ -agonist such as buprenorphine instead of a  $\mu$ -agonist such as morphine.

There is less evidence for (but a great deal of interest in) the predictive value of genetic testing. Can it be used to predict those patients who are likely to participate in risky behaviors or those patients more likely to abuse opioids? Can it predict the patients more likely to develop post-traumatic stress disorder after a motor vehicle collision or more likely to fail antidepressants?

### Future Therapies

Knowledge of genetic issues is allowing more effective screening of drugs for inflammatory and neuropathic pain treatment.<sup>50</sup> Currently, each patient is given a trial-and-error analgesic trial. However, in the near future, pharmacogenetic approaches may be implemented to best predict which medicine may be most appropriate for an individual, providing the therapy with the most sustained efficacy and the best side-effect profile.<sup>51</sup> Dronney and colleagues maintain that “integration of genetic analysis in clinical studies with carefully defined outcome measures will increase the likelihood of identifying clinical and genetic factors which can be used to predict opioid response.”<sup>52</sup>

### Conclusion

Patient care may be improved by genotyping and following drug concentration levels.<sup>53</sup> Pharmacogenetics and therapeutic drug monitoring can potentially minimize adverse events, while maximizing efficacy.<sup>54</sup> Integration of genetic analysis in clinical studies will increase the likelihood of identifying clinical and genetic factors that can be used to predict opioid responses.<sup>52</sup> With knowledge of a patient’s potential for beneficial response to a given opioid, a physician is armed with critical information that can guide therapeutic decisions. Incorporation of such biomarkers are occurring on the forefront of personalized medicine, and have the potential to dramatically improve the utility and efficacy of both current and future pain management strategies.

### Tips for Clinicians

- Take a medication history of prior adverse effects or inadequate effects (eg, “What has worked well for you in the past?” “What hasn’t helped?” “Are you sensitive to medications or do you need larger than normal doses of medications?”).
- Check for common potential interactions with opioids, especially CYP2D6 inhibitors.
- When starting new medications, check the metabolic pathway for activation or excretion issues.
- Be aware of potential drug-drug interactions when adding new medications.
- Use Uniform Data system quantitative metabolite results to evaluate potential drug interactions.
- Consider formal genetic testing to evaluate appropriate opioid choices and potentially to predict opioid risks.

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