CHAPTER 8

AN INTRODUCTION TO PHARMACOGENOMICS

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Advances in health care have led to a significant improvement in patient survival in the past three decades. The introduction of more selective and potent therapeutic agents and optimal patient-care services have affected patient survival and quality of life significantly, with life expectancy rising from 70.9 years in 1970 (U.S. Department of Health, Education, and Welfare, 1974) to 77.7 years in 2006 (Arias, 2010). Drug therapy is often the most challenging aspect of care. Optimal drug treatment requires selection of the best possible agents with close monitoring of pharmacokinetics, pharmacodynamics, adverse drug reactions, and the cost of different agents. This chapter focuses on the pharmacogenomic influences on drug therapy. Adverse drug reactions (ADRs) are discussed in depth in Chapter 5, although this chapter will also discuss ADRs related to genetic polymorphisms.

Pharmacogenomics is the branch of science concerned with the identification of the genetic attributes of an individual that lead to variable responses to drugs. Interestingly, the science has evolved to also consider patterns of inherited alterations in defined populations, such as specific ethnicities, that account for variability in pharmacotherapeutic responses. For the purposes of this chapter, the term *pharmacogenomics* is used more generally to refer to genetic polymorphisms that occur in a patient population—for instance, in an ethnic group—as opposed to individual patients.

Until recently, the ultimate goal of pharmacogenomics had been the development of prediction models to forecast debilitating adverse events in specific individuals and, more recently, across populations based on similarities in age, gender, or more commonly, race or ethnicity, as contrasted with the rest of the population. However, in spite of this newer usage, pharmacogenomics may predict the extreme deviation of some patients from predictable pharmacokinetic and pharmacodynamic responses: the idiosyncratic response.

In recent practice, pharmacogenomic tools coupled with proteomics and other advanced molecular diagnostics are emerging as the cornerstone of individualized patient therapy, especially when differential genetic responses to xenobiotics are considered across specific ethnicities. For instance, a 2010 *New York Times* article described the cutting-edge genetic characterization of a patient's cells to identify the specific aberrant oncogenes responsible for cancer in this individual, who was subsequently administered chemotherapy specific to the identified altered molecular pathway (Kolata, 2012). This is the new and changing face of pharmacogenomics in the present century—enabling patient-centered and patient-specific pharmaceutical care.

Pharmacogenomics seeks to identify patterns of genetic variation that are subsequently employed to guide the design of optimal medication regimens for individual patients. Historically,

the approach to drug therapy has been largely empiric and based on clinical studies that determined the maximally tolerated dose and reasonable toxicity in a narrowly defined population. This approach typically leads to the safe and effective administration of drugs for most individuals. However, with empiric therapy, interindividual (allotypic) variation in drug response occurs—with patient outcomes varying from a complete absence of therapeutic response to potentially life-threatening adverse drug reactions (ADRs).

Genetic differences may account in part for some of the well-documented variability in response to drug therapy. Obviously, many factors other than genetics—such as age, sex, other drugs administered, and underlying disease states—also contribute to variation in drug response. However, inherited differences in the metabolism and disposition of drugs and genetic polymorphisms in the targets of drug therapy (e.g., metabolizing enzymes or protein receptors) can have an even greater influence on the efficacy and toxicity of medications. Interestingly, age, gender, and endemic geographical differences may themselves emerge as phenotypic consequences of differential epigenetic control. This implies that heterogeneity in the control of gene expression based upon age, gender, and geographic location is itself a life-long changing process that is under the control of molecular "epigenetic" switches that either activate or inhibit groups of genes as a unit. Specific identification of these epigenetic controls in special populations, for instance differences in pediatric or geriatric protein expression in immune cells when compared to the general adult population, can provide valuable clues to how special populations based on age, gender, pregnancy, and even geographical location respond differentially to specific drugs. This information can then be incorporated in optimal therapeutic design.

With the publication in 2001 of Lander's and Venter's description of their groundbreaking effort to map the entire human genome, the Human Genome Project (HGP), about 95% of the sequence of all human DNA was established, resulting in the identification of Open Reading Frames (ORF) for many human proteins. A more recent development has been the discovery of single-nucleotide polymorphisms (SNPs), genetic differences that account for allotypic phenotype variations. About 1.4 million SNPs in humans have been identified through a mass effort by the SNP Consortium, which was funded by multiple pharmaceutical companies. The existence of the SNP Consortium is an excellent reminder of the significance of SNPs to drug companies, since SNPs may account for some of the differences in drug responses seen in pharmacotherapy in the population at large (Howe, 2009). With the identification of the individual SNPs, our understanding of pharmacogenetics and pharmacogenomics has exploded.

A study published in 2011 by Li, Zhang, Zhou, Stoneking, and Tang on the heterogeneity in drug-metabolizing genes in globally defined populations has provided profound insights and ever stronger evidence for the significance and relevance of SNP-induced variation in drug metabolism. This study compared differences in 283 drug-metabolizing enzymes and transporter genes across 62 globally distributed ethnic groups and demonstrated that patterns of emergence of SNPs in specific populations spread out across the world indicate positive selection at work and that these differences in SNPs importantly account for the differential drugs response in any given population (Li et al, 2011). Not only does this work support and explain the origin of genetic polymorphism in drug-metabolizing enzymes, it purports to provide an evolutionary rationale for such differences across ethnicities.

GENETICS REVISITED

An individual's genetic makeup (or genotype) is derived as a result of genetic recombination or "mixing" of genes from that individual's parents. All the DNA contained in any individual cell is known as the *genome* of the individual, a word formed by the combination of "gene" and "chromosome," and thus represents all the genes that individual can express. Interestingly, even though two unrelated people share about 99.9% of the same DNA sequences, the less than 0.1% difference between them translates into a difference of 3 million nucleotides. These variants, introduced above, are the SNPs (pronounced "snips") (Howe, 2009). The variability of the genome at these various SNPs accounts for nearly all of the phenotypic differences we see in each other.

The Human Genome Project has sought not only to identify and correlate SNPs with phenotypic differences but also to record and map haplotypes as well (Nebert, Zhang, & Vesell, 2008). Haplotypes are large portions of genetic material (around 25,000 base pairs) that tend to travel together. Understanding how SNPs and haplotypes make humans genetically unique is the current focus of much genetic research (Nebert et al, 2008). The completion of the Human Genome Project, as well as the mapping of SNPs and haplotypes, has allowed the field of pharmacogenomics to understand the variability of drug metabolism seen across individuals and populations. Box 8-1

BOX 8–1 DEFINITIONS

Genetic polymorphism: multiple differences of a DNA sequence found in at least 1% of the population
Genetics: the study of heredity and its variations
Genomics: the study of the complete set of genetic information present in a cell, an organism, or species
Pharmacogenetics: the study of the influence of hereditary factors on the response of individual organisms to drugs (Venes, 2005); the study of variations of DNA and RNA characteristics as related to drug response (U.S. Food and Drug Administration, 2010b)
Pharmacogenomics: the study of the effects of genetic differences among people and the impact that these differences have on the uptake, effectiveness, toxicity, and metabolism of drugs
SNP: single-nucleotide polymorphism

Source: Venes, D. (2005). *Taber's cyclopedic medical dictionary* (21st ed.). Philadelphia: FA Davis; U.S. Food and Drug Administration. (2010b). Table of valid genomic biomarkers in the context of approved drug labels. Retrieved from http://www.fda.gov/RegulatoryInformation/Guidances/ucm129286.htm

provides definitions of terms used in pharmacogenetics and pharmacogenomics.

Another interesting aspect of this discussion is the frequency with which the "mutant" gene copy is expressed. If the variant copy of a gene, such as is common for genes encoding Drug Metabolizing Enzymes (DME), is expressed in the equivalent of 1% or more of the population, the genetic variation is referred to as a polymorphic variation.

Standard adopted nomenclature is used in pharmacogenomics and pharmacogenetics. Of the various mutant variants of a specific gene, each variant is numerically and sequentially named starting with the "wild-type" or normal or nonmutated copy of the gene. Thus, for instance, *CYP2D6* written in italics refers to the normal copy of the gene, whereas *CYP2D6*1* (pronounced "star 1") refers to the first identified natural variant (mutant) copy of this gene.

HISTORY OF PHARMACOGENETICS

The Greek philosopher and mathematician Pythagoras recorded the first interindividual difference of drug administration in 510 BCE when he noted that some patients developed hemolytic anemia after ingesting the fava bean (Nebert et al, 2008). The term *pharmacogenetics* was first coined by Vogel in 1959, but not until1962 was pharmacogenetics defined as the study of heredity and the response to drugs by Kalow (Nebert et al, 2008). Since 1962, the term has been used to refer to the effects of genetic differences on a person's response to drugs.

Interest in pharmacogenetics emerged in the 1950s in response to the discovery of an abnormal butyrylcholinesterase enzyme in psychiatric patients who exhibited prolonged muscular paralysis after administration of succinylcholine before electroconvulsive therapy (Meyer, 2004). Also in the 1950s a connection was established between the development of hemolysis in African American males treated for malaria with primaquine and glucose-6-phosphate dehydrogenase deficiency (Beutler, 1959). Other seminal pharmacogenetic findings include the identification of the proportion of slow acetylators in certain ethnic groups, including 10% of the Japanese and Eskimos; 20% of the Chinese; and 60% Caucasians, blacks, and South Indians (Ellard, 1976), and attribution of peripheral neuropathy to slow acetylation of isoniazid in some patients treated for tuberculosis due to genetic diversity in the enzyme *N*-acetyltransferase (Fig. 8-1) (Yamamoto, Subue, Mukoyama, Matsuoka, & Mitsuma, 1999). "The rate of acetylation of a drugs such as isoniazid is clinically relevant because it determines the rate of elimination of the drug from the body. Thus, individuals known as slow acetylators will metabolize the drug slowly, allowing greater residence time in the body and enhanced toxicity. It is pharmacogenomic variation which is responsible for slow or fast acetylators as explained below."

PHARMACOGENOMICS

The ultimate promise of pharmacogenomics is the possibility that knowledge of the patient's DNA sequence might be used to enhance drug therapy to maximize efficacy, to target drugs only to those patients who are likely to respond, and to avoid ADRs. Increasing the number of patients who respond to a therapeutic regimen with a concomitant decrease in the incidence of ADRs is the promise of pharmacogenomic information. The long-term expected benefits of pharmacogenomics are selective and potent drugs, more accurate methods of determining appropriate drug dosages, advanced screening for disease, and a decrease in the overall cost to the health-care system in the United States caused by ineffective drug therapy.

GENETIC DIFFERENCES IN DRUG METABOLISM

Genetic differences in metabolism were first realized by the observation that sometimes very low or very high concentrations of drug were found in some patients despite their having been given the same amount of drug. Most genetic differences in drug metabolism have been found to be "monogenic" genetic polymorphisms, meaning that they arise from the variation in one gene (Nebert et al, 2008).

Genetic Polymorphism

A genetic polymorphism occurs when a difference in the allele(s) responsible for the variation is a common occurrence. An allele is an alternative form of a gene. A gene is called polymorphic when allelic variations occur throughout a given population at a stable rate of less than 1% (Howe, 2009). Under such circumstances, mutant genes will exist somewhat frequently alongside wild-type genes. The mutant genes will encode for the production of mutant proteins in these populations. The mutant proteins will, in turn, interact with drugs in different manners, sometimes slight, sometimes significant. Monogenic traits by themselves cannot explain the complexity of drug metabolism (Nebert et al, 2008). Genes interact on a complex level, yielding different responses depending on which genes are wild-type and which show mutant phenotypes. Sometimes these interactions can be very difficult to elucidate and may in fact be the source of seemingly unexplainable drug reactions. Figure 8-1 illustrates the relationship between genetic polymorphisms in drug metabolism and at drug receptors.

Four different phenotypes categorize the effects that genetic polymorphisms have on individuals: poor metabolizers (PMs) lack a working enzyme; intermediate metabolizers (IMs) are heterogeneous for one working, wild-type allele and one mutant allele (or two reduced-function alleles); extensive metabolizers (EMs) have two normally functioning alleles; and ultrarapid metabolizers (UMs) have more than one functioning copy of a certain enzyme (Belle & Singh, 2008). See Table 8-1 for the clinical implications of genetic polymorphisms.

Phase I and Phase II Metabolism

Drug metabolism generally involves the conversion of lipophilic substances and metabolites into more easily excretable water-soluble forms. Drug metabolism takes place

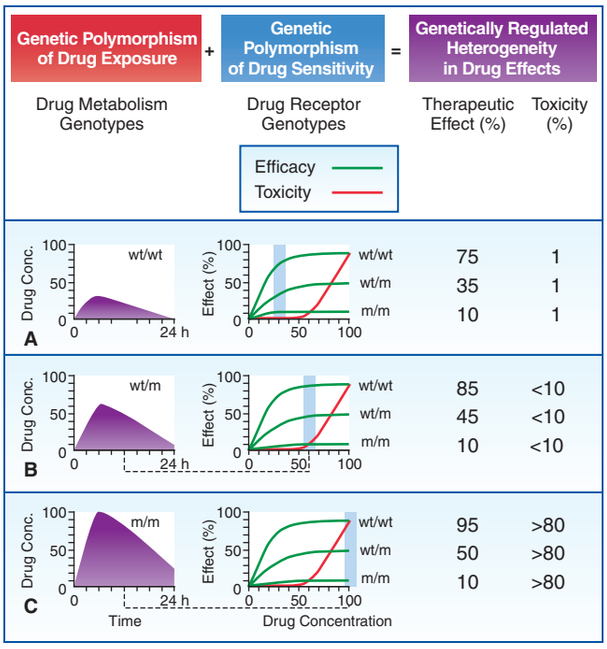

Figure 8–1. Genetic polymorphisms and drug metabolism/receptors.

Table 8–1 Clinical Implications of Genetic Polymorphisms

Metabolizer Phenotype	Effect on Drug Metabolism	Clinical Implications
Poor to intermediate metabolizers	Slow	Prodrug will be metabolized slowly into active drug metabolite. May have accumulation of prodrug. Active drug will be metabolized slowly into inactive metabolite. Potential for accumulation of active drug. Patient requires lower dosage of medication.
Ultrarapid metabolizers	Fast	Prodrug rapidly metabolized into active drug. No dosage adjustment needed. Active drug rapidly metabolized into inactive metabolites leading to potential therapeutic failure. Patient requires higher dosage of active drug.

mostly in the liver and is divided into two major categories, phase I (oxidation, reduction, and hydrolysis reactions) and phase II metabolism (conjugation reactions). A hallmark experiment in pharmacogenomics, diagrammed in Figure 8-2, illustrates how differences in the rates of the phase II metabolizing enzyme *N*-acetyltransferase (NAT-2) can affect the half-life and plasma concentration of drugs that are subject to NAT-2 metabolism (Meyer, 2004).

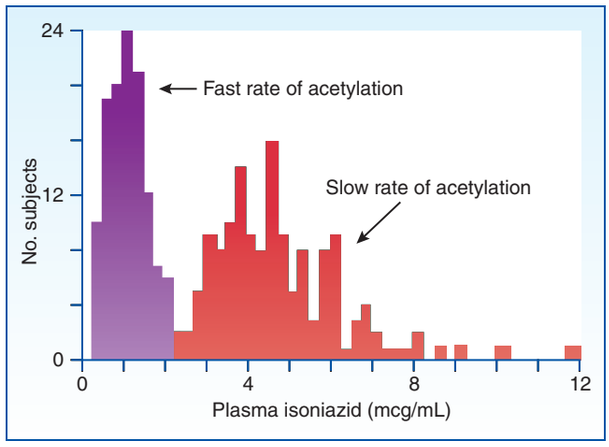

Figure 8–2. Pharmacogenomics of acetylation in isoniazid. Plasma isoniazid concentrations in 267 patients measured 6 hours postdose. The bimodal distribution shows the effect of an *NAT-2* genetic polymorphism.

Phase I metabolism enzymes are responsible for approximately 59% of the adverse drug reactions cited in the literature (Phillips, Veenstra, Oren, Lee, & Sadee, 2001). In terms of evolution, the cytochrome P450 (CYP450) enzyme system was one of the first biocatalytic machineries to emerge on earth. These ubiquitous enzymes contain an iron-porphyrin ring center that is essential to the chemical reaction they catalyze. During this oxygenation reaction, the oxidative state of iron in the porphyrin ring changes, resulting in spectrophotometric absorption maxima observed at 450 nm, which contributed to their naming.

CYPs are generally located in the endoplasmic reticulum (ER) and the mitochondria in human cells, of which the ER isoforms are of particular importance to the field of drug metabolism. In terms of their organ distribution, they are found in greater amounts in the liver and the intestine and to a somewhat lesser extent in other organs, such as the skin, brain, lungs, and kidneys. Hepatic, renal, and intestinal ER CYPs are involved in the biotransformation of a plethora of drugs and endogenous substrates in humans mainly by oxygenation of the target substrate molecule and mediated by differential oxidation states of the central iron atom in the enzyme. Due to this oxygenation reaction, CYPs are classified as monooxygenases. The high genetic variability of the cytochrome *P450* enzymes constitutes the most important of the phase I metabolizing enzymes, with a total of 57 genes encoding for CYP450 enzymes. Of these, CYP2D6, CYP2C9, and CYP2C19 are highly polymorphic and account for upward of about 40% of hepatic phase I metabolism (Phillips et al, 2001) (Fig. 8-3 and Table 8-2).

Specific CYP450 Enzymes

CYP2D6

Up to 25% of drugs are metabolized via CYP2D6 (Belle & Singh, 2008). Phenotypic variations between some enzymes can have an astounding outcome on drug therapy. For example, a 1,000-fold difference has been observed in the rate of

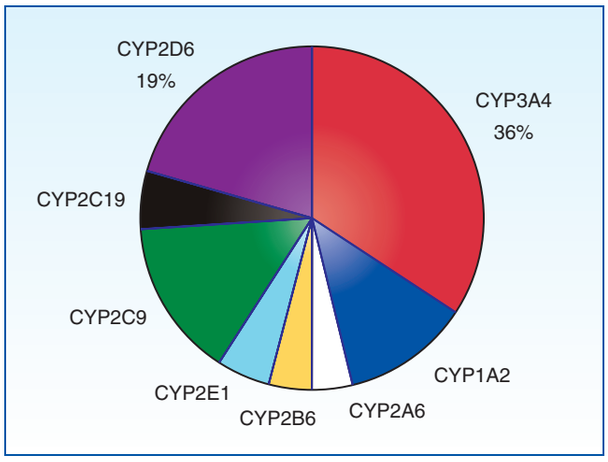

Figure 8–3. Proportion of drugs metabolized by CYP450 isoenzymes.

metabolism of some substrates due to differences in CYP2D6 isozymes. Figure 8-4 illustrates this difference within the European population for the CYP2D6 substrate nortriptyline.

Pharmacogenomic Variance of *CYP2D6*

CYP2D6 is a well-studied instance of a drug-metabolizing enzyme (DME) coding gene that exhibits polymorphism. The CYP2D6 gene product acts on many xenobiotics, including many common prescription drugs (Table 8-3) such as the selective serotonin reuptake inhibitors (SSRIs) fluoxetine, tricyclic antidepressants (TCAs), beta blockers (metoprolol), calcium channel blockers (diltiazem), theophylline (Phillips et al, 2001), and tamoxifen. Research has shown that while approximately 10% of Caucasians, up to 7% of African Americans, and 4.8% of Asians have the "poor metabolizer" (PM) phenotype, 5% of Caucasians and 4.9% of African Americans have the "ultrarapid metabolizer" (UM) phenotype. For Asians, the percentage of CYP2D6 ultrarapid metabolizers shoots up to 21%, perhaps leading to the therapeutic failure of or the need for increased therapeutic dosages of drugs such as SSRIs in these target populations (Belle & Singh, 2008). Thirty-five percent of the population carries a nonfunctional 2D6 allele. This nonfunctional allele may increase the risk of ADRs, especially in patients with polypharmacy. Interestingly, of the 43 alleles of the CYP2D6 gene, about 5 alleles account for the poor metabolic phenotype.

Among the many reasons for genetic variations, an interesting one that specifically applies to CYP2D6 is gene duplication. As the name suggests, in some ethnicities duplication of the allele coding for 2D6 may result in increased protein expression and therefore ultrarapid metabolism and markedly reduced activity of some drugs. The percentage of population that shows gene duplication for CYP2D6 across different countries is shown in Figure 8-5.

CYP2D6 and Tamoxifen

Recently, the ameliorative effects that variable isoforms of CYP2D6 have on the metabolism and therapeutic efficacy of tamoxifen in some patients have received much attention. With reference to tamoxifen, the role of CYP2D6 is not so much the metabolism of this drug as it is to activate it by conversion to endoxifen inside the cell. Pharmacogenetic variation in CYP2D6 has been shown in clinical trials conducted in the United Kingdom and Germany to lead to variable therapeutic outcomes to tamoxifen treatment of estrogen-sensitive cancers (Schroth et al, 2009, 2010; Thompson et al, 2011).

Table 8–2 Medications and Their Receptors

Gene	Medications	Drug Effect Linked to Polymorphism
Drug-Metabolizing Enzymes		
CYP2C9	Tolbutamide, warfarin, phenytoin, NSAIDS	Anticoagulant effect of warfarin
CYP2D6	Beta blockers, antidepressants, antipsychotics, codeine, debrisoquine, dextromethorphan, encainide, flecainide, guanoxan, methoxyamphetamine, *N*-propylamaline, perhexiline, phenacetin, phenformin, propafenone, sparteine	Tardive dyskinesia from antipsychotics; narcotic side effects, efficacy, and dependence: imipramine dose requirement; beta blocker effect
Dihydropyrimidine dehydrogenase	Fluorouracil	Fluorouracil neurotoxicity
Thiopurine methyltransferase	Mercaptopurine, thioguanine, azathioprine	Thiopurine toxicity and efficacy; risk of second cancers
Drug Targets		
ACE	Enalapril, lisinopril, captopril	Renoprotective effects, cardiac indices, blood pressure, immunoglobulin A nephropathy
Potassium channels	Quinidine	Drug-induced long QT syndrome
HERG	Cisapride	Drug-induced torsade de pointes
KvLQT1	Terfenadine, disopyramide, mefloquine	Drug-induced long QT syndrome
hKCNE2	Clarithromycin	Drug-induced arrhythmia

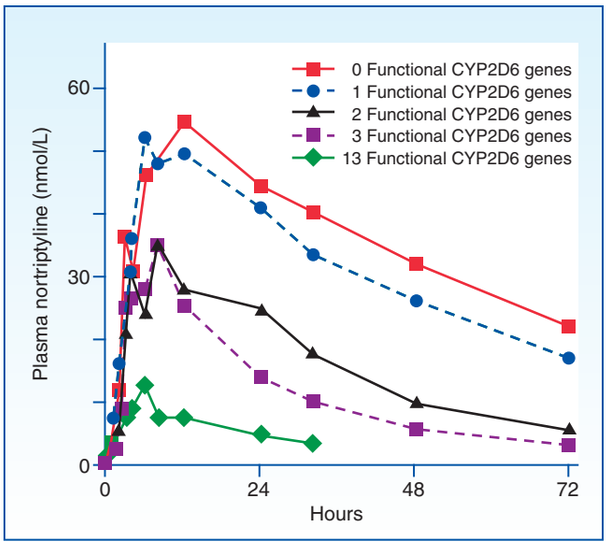

Figure 8–4. European population and the *CYP2D6* substrate nortriptyline.

Table 8–3 *CYP2D6*

Substrate	Inhibitors	Inducers
Codeine	Amiodarone	Carbamazepine
Dextromethorphan	Fluoxetine	Phenytoin
Metoprolol	Labetalol	Phenobarbital
Paroxetine	Paroxetine	Rifampin
Haloperidol	Propafenone	
Propranolol	Quinidine	
Risperidone	Sertraline	
Timolol	Cimetidine	
Amitriptyline		
Nortriptyline		
Clozapine		
Morphine		
Methadone		

CYP2D6 and Opioid Analgesics (Codeine)

Opioid analgesics such as codeine rely on CYP2D6 enzymes to convert them to their active form, morphine (Belle & Singh, 2008). Genetic polymorphisms of the CYP2D6 enzyme can greatly alter the effect that codeine has on patients who are PM or UM types. UM types may not experience the analgesic effects of the drug at normal therapeutic doses, and PMs may not be able to convert codeine to its active metabolite morphine, thus experiencing little or no clinical benefit. Other narcotics that are active when administered to patients may produce the effects of excess drug at even the lower end of therapeutic dosing. See Table 8-3.

A recent area where CYP2D6 polymorphism has generated interest is the effects of its pharmacogenomic variation on infants who are breastfed milk by UM mothers on codeine. In UMs, excessive codeine activation to morphine may cause fatal respiratory depression. Since the active metabolite, morphine, is lipophilic and may be found in breast milk, severe respiratory depression could result in infants who have not yet been weaned. However, a study published in 2012 demonstrated that the main concern in this scenario is CNS depression in UM mothers on codeine, which was found to be a significant risk factor when compared with CNS depression as measured by sleepiness and lethargy in the infants being fed breast milk by the same mothers (Sistonen et al, 2012).

In addition, fatalities have been observed in some pediatric patients following the administration of codeine for postoperative pain management after tonsillectomy and/or adenoidectomy procedures. In August 2012, the Food and Drug Administration (FDA) acknowledged that it was considering the lethal effects of codeine in some pediatric patients and estimated that "the number of "ultrarapid metabolizers" is generally 1 to 7 per 100 people, but may be as high as 28 per 100 people in some ethnic groups." Consequently, as recently as February 2013, the FDA issued a Black-Box Warning for the cautious use of codeine in children, particularly for pain management following surgery.

Genetic Testing for CYP2D6 Polymorphisms

There are commercially available tests that can provide immensely helpful information. One such test called the Tag-It system is described at the end of the chapter.

CYP2C9

CYP2C9 is the primary route of metabolism for about a hundred different drugs in humans. While some CYP2C9 substrates are the more common drugs, such as phenytoin, glipizide, and losartan, other drug substrates include those that evince a narrow therapeutic index, such as the coumarin-related anticoagulant agents warfarin and acenocoumarol.

CYP2C9 and Warfarin

Warfarin is one of the most effective, cheapest, and widely prescribed anticoagulant drugs that act by inhibiting the enzyme vitamin K epoxide reductase, which prevents the formation of functional vitamin K. This action in turn inhibits the activation of clotting factors in the liver, causing the anticoagulant effect. Warfarin is available as a racemic mixture, of which the *S*-enantiomer, which is the more bioactive form, is metabolized by CYP2C9. The presence of CYP2C9 mutations is associated with a reduction in the metabolism of *S*-warfarin. Clinically, warfarin maintenance dosing requirements are lower in patients with CYP2C9*2 polymorphisms and further reduced in patients with CYP2C9*3 variants (Gulseth, Grice, & Dager, 2009), making these two the most common "reduced function variants" for the CYP gene in terms of its effect on warfarin. The CYP2C9*2 variant evinces a 30% and the CYP2C9*3 variant a 90% reduction in warfarin clearance (Rettie,

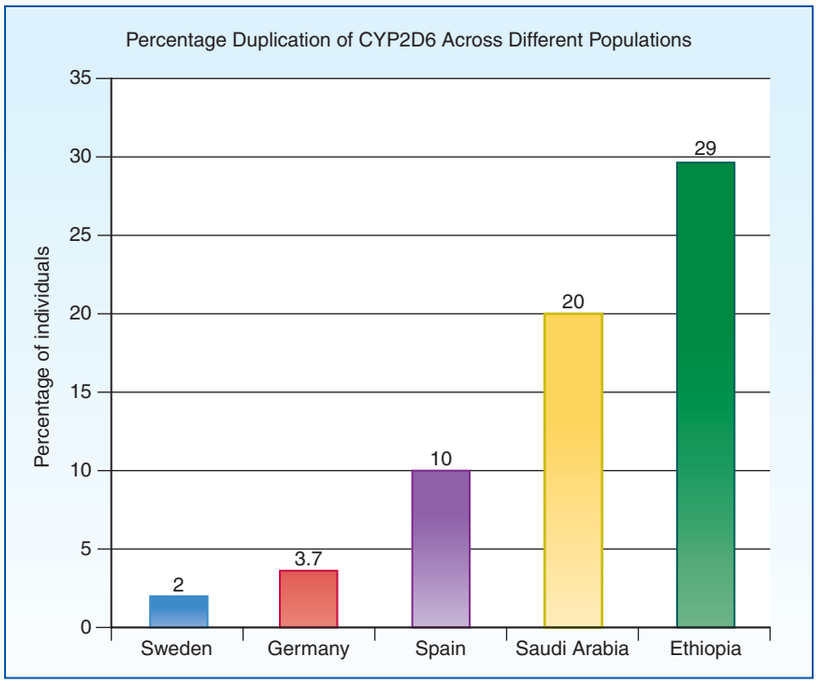

Figure 8–5. Percentage distribution of individuals across countries showing a duplication of an allele of CYP2D6. The figure explains the exaggerated metabolism of some drugs in the specified percentage of individuals belonging to certain ethnic backgrounds (generously assuming ethnic homogeneity in some countries) due to increased 2D6 activity.

Haining, Bajpai, & Levy, 1999), resulting in adjusted dose requirements in these patients compared with noncarriers.

In addition, patients with homozygous presentation of a CYP2C9 mutation appear to have a greater reduction in dosing requirement than do heterozygotes. Approximately one-third of the population carries at least one allele for the slow-metabolizing form of CYP2C9 (U.S. Food and Drug Administration, 2009). The clinical implications of altered warfarin metabolism can be significant; the clinical implications of pharmacogenomic variants are found later in this chapter. See Table 8-4.

In spite of the information outlined above, controversy exists in the field of pharmacogenetic testing over initiation or maintenance of anticoagulant therapy following an adverse cardiovascular event, in part due to some conflicting reports in the literature about the clinical effectiveness and relevance of pharmacogenomic variability in warfarin drug metabolism. However, recent studies on the pharmacogenomics of warfarin have included an analysis of the effects of polymorphism of CYP2C9 and vitamin K epoxide reductase (VKORC1) across populations in Europe, Asia, and elsewhere. Polymorphisms in these two genes account for nearly 40% of the differences in warfarin therapy across populations (Yip & Pirmohamed, 2013). Taken together, the data from these pharmacogenomic studies indicate a strong connection between the variant effects of CYP2C9 polymorphism and the metabolism and therapeutic efficacy of warfarin and acenocoumaral. CYP2C9 variation was reported to be important for the maintenance therapy of warfarin in a genome-wide association analysis in the Swedish and Japanese population (Cha et al, 2010).

Data from disparate research resources need to be organized to obtain clinically relevant information that assists in guiding the therapeutic rationale for the use of warfarin in special populations. One such approach would be to analyze studies on the frequency of allelic variation of the three drug-metabolizing genes for warfarin in selected populations. Thus, for the CYP2C9 gene, the frequency of allelic variation for CYP2C9*2 is about 10% in Caucasians, compared to less than 1% in Africans and Asians, while the frequency of allelic variation for CYP2C9*3 is about 6% in Caucasians, 4% in Asians, and less than 1% in Africans (Fig. 8-6). The frequency of allelic variation of the VKORC1 gene is substantially higher in all the three ethnicities compared with the mutational frequency of CYP2C9. For the specific VKORC1 (-1639) mutation, allelic frequency is 98% for Africans, 60% for Caucasians, and 2% for Asians; see Figure 8-7 (Voora & Ginsburg, 2012).

Table 8–4 *CYP2C* (*9* and *19*)

Substrate	Inhibitors	Inducers
S-warfarin	Amiodarone	Carbamazepine
Losartan	Cimetidine	Phenytoin
Diazepam	Chloramphenicol	Rifampin
Imipramine	Fluconazole	
Amitriptyline	Isoniazid	
Phenytoin	Ketoconazole	
Rosiglitazone	Zafirlukast	
	Fluoxetine	
	Fluvoxamine	
	Sertraline	
	Rosiglitazone	

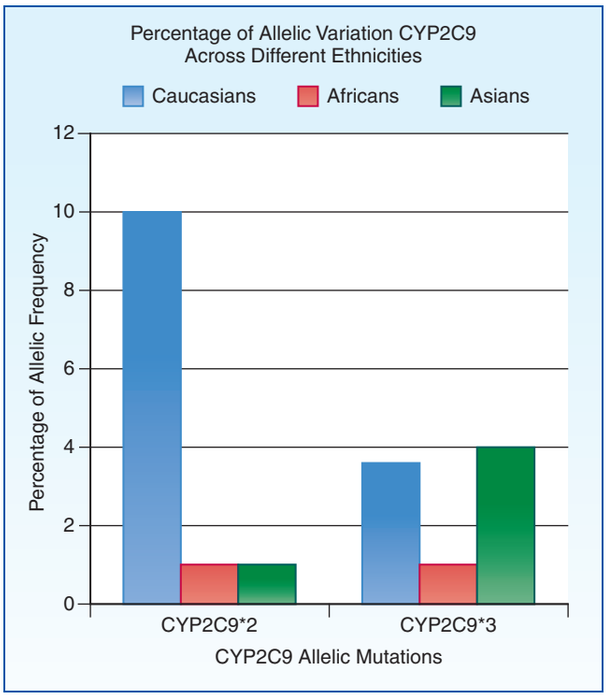

Figure 8–6. Percentage distribution of individuals across ethnicities exhibiting polymorphism in *CYP2C9*.

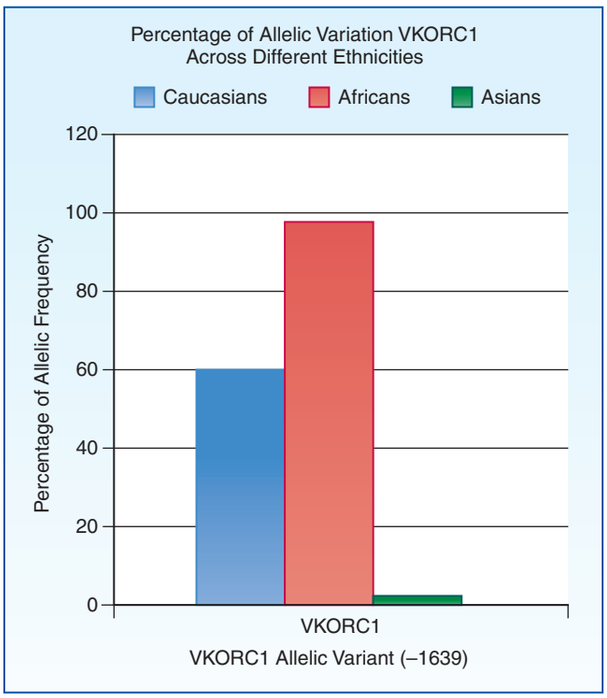

Figure 8–7. Percentage distribution of individuals across ethnicities showing variation in *VKORC1*.

The wealth of scientific data documenting evidence of *CYP2C9*2, CYP2C9*3*, and VKORC1 (-1639 G > A) polymorphisms affecting dose response to warfarin in different populations resulted in the FDA updating the label of the drug in 2007 and then again in 2010 (Finkelman et al, 2011). A number of algorithms have been published to help physicians in making a decision about warfarin dosing after pharmacogenetic testing, of which two prominent examples are the Gage algorithm and the International Warfarin Pharmacogenetics Consortium (IWPC) algorithm.

Interestingly, the nonsteroidal anti-inflammatory drugs (NSAIDs) celecoxib and flubiprofen have received "use with caution" in PM label warnings from the FDA owing to CYP2C9 polymorphism.

CYP3A4

The CYP3A group of isoenzymes is responsible for up to 50% of drug metabolism (Howe, 2009). CYP3A4 isoenzyme is responsible for metabolism of several important classes of drugs that are commonly used in primary care (see Table 8-1). Examples of these classes include azole antifungals, calcium channel blockers, antihistamines, anticonvulsants, antimicrobials, and corticosteroids. Both drug-related induction or inhibition of CYP450 3A4 isoenzyme may complicate drug therapy in patients (Howe, 2009). Predicting the onset and offset of these effects is very difficult. The time to onset and offset of drug–drug interactions is closely related to each drug's half-life and the half-life of enzyme production. Clinically significant drug interactions in this setting may increase the risk of toxicity. For example, amiodarone has a half-life of close to 60 days and requires months to reach steady state and inhibit the CYP450 enzyme system effectively (Table 8-5). Conversely,

Table 8–5 ***CYP3A4***

Substrate	Inhibitors	Inducers
Cyclosporine, FK 506	Erythromycin	Carbamazepine
Corticosteroids	Clarithromycin	Phenobarbital
Erythromycin	Diltiazem	Rifampin
Felodipine, isradipine	Ketoconazole	Rifabutin
Nifedipine	Fluconazole	Phenytoin
Nisoldipine	Itraconazole	Corticosteroids
Nitrendipine	Quinidine	INH
Digoxin, quinidine	Grapefruit juice	St. John's wort
Verapamil	Cimetidine	
Warfarin	Indinavir	
Sildenafil	Fluoxetine	
Astemizole	Zileuton, zafirlukast	
Terfenadine	Verapamil	
Pioglitazone	Amiodarone	
R-warfarin	Corticosteroids	
	Fluvoxamine	

it takes less than 2 days for rifampin, which is a nonspecific CYP450 inducer with a shorter half-life, to decrease blood concentrations of many drugs to a subtherapeutic level and significantly increase the risk of therapeutic failure. Close monitoring is required when prescribing drugs that induce or inhibit CYP3A4 enzymes. See Table 8-5 for further information.

P-GLYCOPROTEIN

P-glycoprotein (Pgp) is a membrane-bound, ATP-dependent transport system responsible for the efflux of a variety of xenobiotics from cells to the extracellular fluid. This includes the ejection of drugs from cells, usually against their concentration gradients. Pgp, also known as multidrug resistance (MDR1) protein, is the product of the *ABCB1* and *ABCB4* genes and is a member of adenosine triphosphate (ATP)-binding family of proteins. Differential expression of Pgp may explain tissue-specific and temporal variations in efflux efficiency in different cells. In fact, chemoresistance in cancer therapeutics has been strongly linked with Pgp expression—the more Pgp protein expressed by the cell, the greater the efflux potential of xenobiotics such as anticancer drugs. In addition to differences in protein expression, polymorphic variation of the Pgp genes may also dynamically affect intracellular and plasma drug concentration. Over 50 SNPs within the *ABCB1* gene have been identified, which may lead to variability in drug responses (Reed & Parissenti, 2011).

Pharmaceutically relevant examples of this include the variation in drug response to agents such as antiepileptic drugs, select cardiovascular agents, and so on. Interestingly, P-glycoprotein at the site of the gastrointestinal (GI) tract effluxes hydrophilic drugs out of the cell and inhibits drug absorption through the GI tract (Howe, 2009). As drugs passively diffuse through the GI tract, Pgp pumps move drugs from cytoplasmic areas to extracellular fluid. Some examples of substrates of P-glycoprotein include carvedilol, diltiazem, and digoxin (Howe, 2009). Several antiepileptic drugs such as phenytoin, carbamezapine, lamotrizine, phenobarbital, valproic acid, and gabapentin are substrates or inhibitors of Pgp, but there is considerable controversy in the literature regarding these. In the case of digoxin, Pgp affects the level of digoxin available for absorption and elimination (Howe, 2009). P-glycoprotein inhibitors include verapamil, quinidine, cyclosporine, and ketoconazole (Howe, 2009). If an inhibitor of P-glycoprotein is administered, then blood levels of substrates will rise, as seen if quinidine is administered with digoxin.

Drugs can be categorized as reversible or suicidal inhibitors or P-glycoproteins. For example, calcium channel blockers and high-dose steroids are considered as reversible inhibitors of both P-glycoproteins and CYP450. However, grapefruit and ritonavir are suicidal agents for both P-glycoprotein and CYP450, meaning the effect of grapefruit juice will be prolonged, perhaps up to 24 hours. See Figure 8-8.

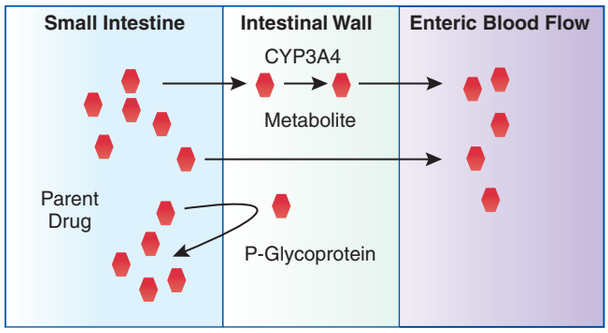

Figure 8–8. Drug–metabolism interactions.

CLINICAL IMPLICATIONS OF PHARMACOGENOMICS

Adverse Drug Reactions

One benefit of understanding pharmacogenomics is the possibility of a decrease in the number of ADRs. The CYPP450 enzymes in families 1 to 3 mediate 78% to 80% of all phase I–dependent metabolism of clinically used drugs (Spatzenegger & Jaeger, 1995). The polymorphic forms of CYPP450s are responsible for the development of idiosyncratic ADRs (Kalgutkar, Obach, & Maurer, 2007). According to Phillips and Van Bebber (2005), 56% of drugs cited in ADR studies are metabolized by polymorphic phase I enzymes, of which 86% are P450s.

Warfarin

In 2008, the package insert for warfarin was updated by the U.S. Food and Drug Administration to include application of pharmacogenomics to the dosing of warfarin. Previous work had identified variable metabolism by CYP2C9 as a major contributor to the variable response to the drug. In 2004, coding-region mutations in VCORC1, encoding a subunit of the vitamin K epoxide reductase complex (the pharmacological target for the drug), were found to cause a rare syndrome of warfarin resistance. Subsequently, the variants in VCORC1 have been found to account for a much greater fraction of variability in warfarin response (21%) than do variations in CYP2C9 (6%) (Gulseth et al, 2009). Although genetic testing prior to prescribing has not yet been required by the FDA, numerous warfarin dosing calculators exist on the Web where a clinician can insert clinical information about the patient, including genetic test results and indications, and a dosing regimen will be calculated or "individualized" for that patient (http://www.warfarindosing.com; http://www.globalrph .com/warfarin.htm).

Pharmacogenetic Testing Prior to Prescribing

The FDA now requires additional pharmacogenomic information on several drug package inserts (Table 8-6). Within the anticoagulant drug class, warfarin is a drug with

Table 8–6 U.S. Food and Drug Administration Positions on Necessity of Pharmacogenetic Testing as Indicated on Drug Labeling

Pharmacogenetic Biomarker	Drug
Test Required	
EGFR expression	Cetuximab
HER2/NEU overexpression	Trastuzumab
CCR-5-tropic HIV-1	Maraviroc
Presence of Philadelphia	Dasatinibchromosome
Test Recommended	
HLA-B*1502	Carbamazepine
HLA-B*5701	Abacavir
CYP2C9 variants	Warfarin
VKORC1 variants	Warfarin
Protein C deficiency	Warfarin
TPMT variants	Azathioprine, mercaptopurine, thioguanine
UGT1A1 variants	Irinotecan
G6PD deficiency	Rasburicase
Urea cycle disorders	Valproic acid
Information Only	
c-KIT expression	Imatinib
CYP2C19 variants	Voriconazole
CYP2C9 variants	Celecoxib
CYP2D6 variants	Atomoxetine, tamoxifen, fluoxetine
DPD deficiency	Capecitabine, fluorouracil
EGFR expression	Erlotinib
G6PD deficiency	Rasburicase, primaquine
NAT variants	Isoniazid, rifampin
Philadelphia chromosome	Busulfandeficiency
PML/RAR gene expression	Tretinoin

EGFR = epidermal growth factor receptor; *HER2/NEU* = v-erb-b2 erythroblastic leukemia viral oncogene homolog 2; *CCR-5* = chemokine C-C motif receptor; HLA = human leukocyte antigen; *CYP2C9* = cytochrome P-450 isoenzyme 2C9; *VKORC1* = vitamin K epoxide reductase complex subunit 1; TPMT = thiopurine *S*-methyltransferase; *UGT1A1* = uridine diphosphate-glucuronosyltransferase 1A1; *c-KIT* = v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog; *CYP2C19* = cytochrome P-450 2C19; *CYP2D6* = cytochrome P-450 isoenzyme 2D6; DPD deficiency = dihydropyrimidine dehydrogenase; G6PD = glucose-6-phosphate dehydrogenase; *NAT* = *N*-acetyltransferase; *PML/RAR* = promyelocytic leukemia/retinoic acid receptor.cytochrome P-450 isoenzyme 2D6; DPD deficiency = dihydropyrimidine dehydrogenase; G6PD = glucose-6-phosphate dehydrogenase.
Source: Derived from U.S. Food and Drug Administration. (2010b). Table of valid genomic biomarkers in the context of approved drug labels. Retrieved from http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm

a narrow therapeutic index. In 2007, the FDA recommended an important label update suggesting genetic testing to prevent possibly fatal bleeding in patients with polymorphic variants of CYP2C9, the metabolizing enzyme, and VKORC1, the target enzyme of warfarin. Patients with CYP2C9 variations require more time to achieve the International Normalized Ratio, or INR, and are at an increased risk for bleeding (Sconce, 2005); they may also require lower doses of warfarin to achieve and maintain therapeutic INR (Limdi, 2007). Thus, if there are indications of inherited differences in these genes, the patient should be genotyped. However, monitoring INR is still as much of a requirement while dosing warfarin as before. While the FDA did not explicitly require genetic testing in patients prior to prescribing warfarin, the package labeling did show changes in dosage amounts. There is an FDA-approved genetic testing kit available, but others may also be used. Generally, cell samples are collected from the mouth or from blood. However, it should be emphasized that genetic testing is not the sole consideration, since patient-related factors such as age, sex, body weight, and some other parameters may need to be considered with the genetic results. A variety of online algorithms can aid physicians and hospital staff in making these dosage adjustments, as presented, for example, at www.warfarindosing.org.

The pharmacogenetic tests mentioned on drug labels can be classified as "test required," "test recommended," and "information only." Currently, four drugs are required to have pharmacogenetic testing performed before they are prescribed: cetuximab, trastuzumab, maraviroc, and dasatinib. Cetuximab treatment needs a confirmation of epidermal growth factor receptor *(EGFR)* expression. Trastuzumab therapy requires testing for HER2/NEU overexpression. Infection with *CCR-5*-tropic HIV-1 should be confirmed before initiation of therapy with maraviroc (an antiretroviral). Dasatinib is used for the treatment of patients with Philadelphia chromosome-positive acute lymphoblastic leukemia resistant to or intolerant of prior therapy (U.S. Food and Drug Administration, 2010b).

In December 2007, the FDA added a Black-Box Warning on the carbamazepine label, recommending testing for the HLA-B*1502 allele in patients with Asian ancestry before initiating carbamazepine therapy because these patients are at high risk of developing carbamazepine-induced Stevens–Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN). Interestingly, although Asians or patients with Asian ancestry have been reported to have a strikingly high frequency (10 times higher than whites) of carbamazepine-induced SJS or TEN if they carry an HLA-B*1502 allele, other races carrying the allele do not seem to have the increased risk (U.S. Food and Drug Administration, 2007).

The anticancer agent irinotecan is a prodrug used for the treatment of colorectal cancer, small-cell lung cancer, and other solid tumors. The active metabolite of irinotecan is SN-38, a topoisomerase I inhibitor, and uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) plays a critical role in inactivating SN-38 (McLeod & Hoskins, 2007).

The low activity of the UGT1A1 enzyme may increase the risk for adverse events associated with irinotecan therapy (e.g., neutropenia) by increasing serum concentrations of the active metabolite. A polymorphism in the promoter region of the UGT1A1 gene determines patient exposure to SN-38 (McLeod & Hoskins, 2007). Patients homozygous for the polymorphism (UGT1A1*28) are at a 5-fold greater risk of irinotecan-related toxicity compared with patients with one or two normal alleles. Additionally, the FDA has approved a test for detection of the UGT1A1*28 genotype for irinotecan dosing. Additional genotype tests approved by the FDA and their implications are summarized in Table 8-7.

SUMMARY

We live in remarkable times, in which multiple therapeutic options are available for most common diseases. However, the selection of the optimal medication for an individual patient is still problematic. Practitioners still pick the "right" initial medication only half the time and ADRs are still unpredictable. In addition, the expense of new biological agents is such that even wealthy countries like the United States cannot afford to treat all patients.

The completion of the Human Genome Project has enabled the development of clinical tools for patient evaluation. Pharmacogenomics may allow identification of patients most likely to benefit from a given therapy and those patients for whom the cost and risk outweigh the benefits. Both the safety and efficacy of drug therapy may improve. In the future, genotyping may be used to personalize drug treatment for vast numbers of patients, decreasing the cost of drug treatment and increasing the efficacy of drugs and health in general.

Table 8–7 FDA-Approved Diagnostic Test Commercially Available for Commonly Prescribed Pharmacologic Therapies

Genetic Test	Drug	Benefit of Genetic Test
CYP2C9/ VCORC1	Warfarin	Reduce time to target INR; possibly decrease bleeding episodes
CYP2D6	Tamoxifen	Reduce therapeutic failure
	Codeine	Reduce GI toxicities/ improve pain control
	Oxycodone	Reduce GI toxicities/ improve pain control
	Tricyclic antidepressants	Reduce therapeutic failure
TMPT	Azathiaprine	Reduce myelosuppression
	6-mecaptopurine	Reduce myelosuppression
UGT1A1	Irinotecan	Reduce neutropenia

Source: Derived from McLeod, H. L., & Hoskins. J. M. (2007). Personalized drug therapy: The era of pharmacoeconomics. Retrieved from http://www.ipit.unc.edu/files/1233076125-one.pdf

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