**Note:** Large images and tables on this page may necessitate printing in landscape mode.



## PHARMACOGENETICS: INTRODUCTION

The genetic basis underlying variation in drug response among individuals has become evident with the introduction of modern analytical methods for the analysis of gene sequence and expression. *Pharmacogenetics* is the study of how genes affect the way people respond to drug therapy. The goal of pharmacogenetics is to individualize drug therapy to a person's unique genetic makeup. The environment, diet, age, lifestyle, and state of health can influence a person's response to medicine. An understanding of an individual's genetic makeup is thought to be the key to creating personalized drugs with greater efficacy and safety (; ). Pharmacogenetics is an established discipline that studies the genetic basis of interindividual variability in the response to drug therapy, and allows for individualization of drug therapy. In contrast, *pharmacokinetics* provides a means for estimating pharmacokinetic parameters of the drug in various population subgroups and then applying the information to drug therapy for the average patient.

A closely related, and considered by some to be an equivalent or overlapping field, is pharmacogenomics. *Pharmacogenomics* involves study of the role of genes and their genetic variations (DNA, RNA level) in the molecular basis of disease, and therefore, the resulting pharmacologic impact of drugs on that disease. Pharmacogenetics and pharmacogenomics are both important disciplines involved in the study of genes that code for drug-metabolizing enzymes (), drug receptors (), drug transporters, and ion channels or efflux systems (). Many of the above are new factors involved in determining how genetic variation contributes to variation in the response to drugs, including the ultimate fate of the drug and its ability to exert a therapeutic response without undue side effects.

Application of pharmacogenetics to pharmacokinetics and pharmacodynamics helps the development of models that predict an individual's risk to an adverse drug event and therapeutic response. With some drugs, pharmacogenetics allows the recognition of subgroups with different genetic makeup that results in alterations in drug receptors and the pharmacodynamic response to drugs. Understanding the genetic and molecular differences in disease etiology and drug mechanism produce insight on how a patient will respond to a given drug. For example, the monoclonal antibody Herceptin was designed to treat a subset of breast cancer patients who overexpress the HER-2 (human epidermal growth factor receptor-2) gene. Patients who lack HER-2 overexpression are considered to be nonresponders to Herceptin therapy. In the past, such differences would be apparent only after a trial-and-error period. This genetic knowledge improves our ability to select or design the proper drug for individuals suffering from a disease with a varying range of molecular defects.

Pharmacogenetics can provide justification for individualized dosing for many drugs known to be highly variable in their effects. The outcome of disease, resistance to treatment, and adverse reactions are increasingly recognized as an interaction of the individual's genes and the environment. Dominic Kwiatkowski, of the Wellcome Trust Center for Human Genetics, recently reviewed the role of genes in human susceptibility to infection (). He predicted that recent advances in genetics and high-throughput genotyping technology will make it feasible within the next decade to screen the whole genome for genetic factors that determine susceptibility to HIV and AIDS, malaria, and tuberculosis. Kwiatkowski listed many genes and their encoded proteins that play roles in immunity and disease fighting. Examples include genes with alleles that affect susceptibility to hepatitis B, HIV, and other known infections. Besides host factors, progress has been even faster for genes of microbes that play roles in efflux of drugs out of the system, a principal factor for antibiotic resistance in many pathogens. The subject of drug pumps in microbes has been reviewed by .

Pharmacogenetics (PGt), or pharmacogenomics (PGx), a more modern term preferred by some researchers, has been the subject of discussion by industry and regulatory agencies. These groups tried to generate a consensus on how and to what extent should PGx or PGt information be applied to improve drug therapy and safety of both old and new drugs ().

This chapter will focus on variations in drug response due to pharmacogenetics. However, variation in drug response is also in large part due to nongenetic factors, as listed on .

**Table 12.1 Pharmacogenetic and Nongenetic Influences on Variations in Disease and Drug Therapy[a]**

| | Variant Type | Example |
|---|---|---|
| Genetic influences | (PK) | Drug metabolism—Polymorphism in many cytochrome P-450 family enzymes (CYPs) and others in |
| | (PK) | P-glycoprotein or other drug transporter (difference in genetic expression) |
| | Drug receptor (PD) | Variation in receptor number, affinity, or response to drug |
| | Indirect drug response (PD) | Inherited differences in coagulation may predispose women to deep vein thrombosis when taking oral contraceptives |
| | | Variations in SCNA receptor predisposes patients to drug-induced arrhythmia () |

| | | |
|---|---|---|
| Nongenetic[a] influences | Environmental (PK, PD, or disease prognosis) | Cigarette smoking—enzyme induction<br><br>Exposure to mutagenic agents and occupational or environmental hazards |
| | | Geographic differences |
| | | • Climate (ultraviolet light on skin tumor) |
| | | • Diet[b] (effect of diet, including grapefruit, and influence on GI enzymes and drug absorption) |
| | | • Drinking water (dissolved minerals and effect on health) |
| | | • Nutrients or supplements[c] |
| Mixed covariates[d] | Gender, age, body weight/surface (PK/PD) | Male, female |
| | | Infant, young adult, or geriatric patient |
| | Pathophysiology (PK/PD) | Renal, hepatic, cardiovascular, or other disease |
| | Drug–drug interactions (PK/PD) | Metabolic, binding, or PD interactions |

[a]Environmental factors may switch on genes, and the nongenetic category may not be absolute. The two primary independent variables in life processes are genetics and environment.

[b]Examples of diet: Atkins diet, vegetarian, diabetic, and other hospital diets, etc. Foods rich in carbohydrates/protein may have an effect on urinary pH and affect renal tubular drug reabsorption. Diet can also affect PK drug absorption; certain groups lack or have abundant GI enzymes, lactase, etc (even though it might be genetic rather than adaptive, arguably).

[c]Examples of nutrients include vitamins, antioxidants, fortified fruit drinks, and supplements taken regularly, other than meals.

[d]Some genetic/environmental outcomes may become a covariate for a new pharmacogenetic response.

Some examples partially adapted from .

## EXAMPLE OF POLYMORPHISMS

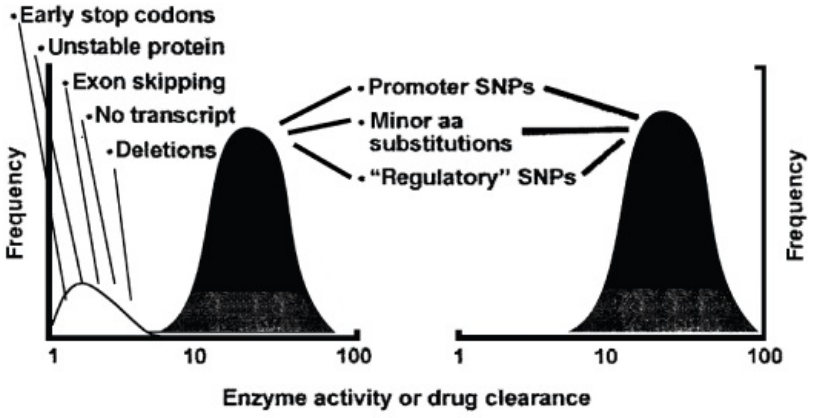
Interindividual differences in response to drug therapy due to differences in acetylation of drugs is a well-studied example of genetic polymorphism. Patients' ability to metabolize certain drugs such as hydralazine, procainamide, and isoniazid can be categorized as either "fast acetylators," "normal acetylators," or "slow acetylators." Acetylation status is dependent on the patient's genetic composition, which determines the activity of the acetylation enzyme N-acetyltransferase. Acetylation status determines whether a patient is dosed with a correspondingly higher or lower dose compared to "normal acetylators."

Although acetylation status is well known in the medical community, other metabolic or pharmacologic variations have been poorly understood until recently. Genetic variations are well known in bacteria and other microorganisms because the rapid changes in these organisms are easily observed. In humans, mutations and related changes occur to different degrees in thousands of proteins and other macromolecules.

*Polymorphisms* or genetic variations with a frequency of greater than 1% of the population, or *mutations,* in less than 1% of the population, in genetic sequences can affect patient therapeutic response or metabolism of a given drug (). However, many alleles encoding different drug receptors are being discovered and studied with increasing frequency. Pharmacokinetic parameters now known to be influenced by genetic differences include drug bioavailability, distribution, metabolism and tissue binding. Our understanding of the impact of these genetic differences on clinical pharmacokinetics and pharmacodynamics are in their infancy.

Polymorphism in cytochrome isozymes is well known in drug metabolism, and the corresponding allele genes involved have been widely studied and are fairly well understood clinically at this time. Genetic tests are available to screen polymorphisms for cytochrome P-450 drug-metabolizing enzymes in an individual. Prior knowledge of an individual's metabolic capability can reduce the risk of adverse drug reactions because dose regimens may be adjusted according to an individual patient's metabolic capability. The types of genetic mutations affecting metabolic enzymes are illustrated in .

**Figure 12-1.**




Source: Shargel S, Wu-Pong S, Yu ABC: *Applied Biopharmaceutics & Pharmacokinetics*, 5th Edition: http://www.accesspharmacy.com



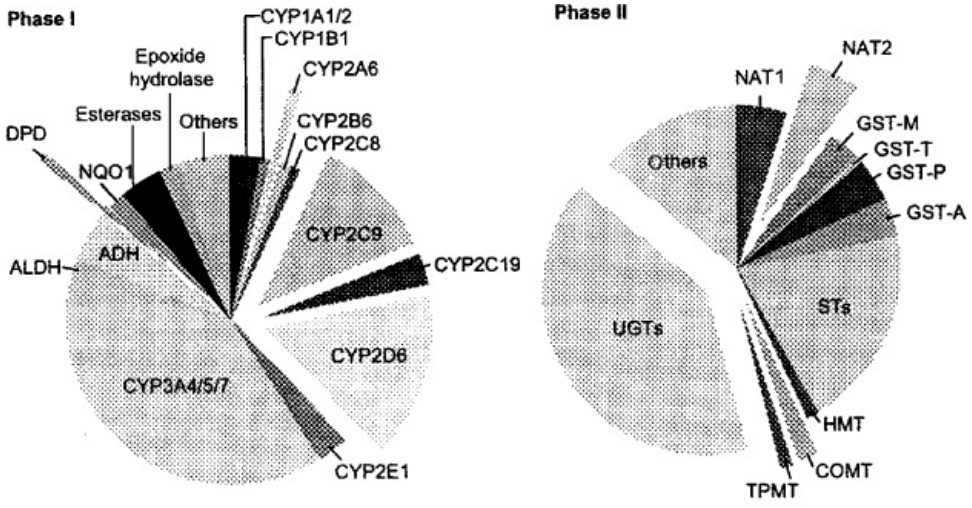
Plot of frequency of all drug metabolism phenotypes found in population versus enzyme activity or drug clearance. The left panel indicates mutations leading to almost complete loss of enzyme activity (note serious causes such as gene deletion or transcription failure); the right panel depicts mutations leading to altered but not complete loss of enzyme activity. (*Note:* SNP promoters may "switch on/off" and are responsible for individual variations in drug metabolism. The width of the bell-shaped curve shows the spread of enzymatic activity that corresponds to drug clearance.)

()

Genetic polymorphism within a specific genotype may occur with different frequencies depending on racial or population factors, which evolved from selective geographic, regional, and ethnic factors. The probability of carrying a specific allele is therefore different in different subjects depending on whether the dominating factor is geographic or ethnic. In practice, genetic polymorphisms with higher frequencies are more important because they are likely to affect more people. However, some rare mutations are important because they cause extreme medical consequences or may be fatal for the individual.

Using genetic polymorphism considerations, drugs may be developed that have less intersubject variation in pharmacodynamic response and less risk of an adverse event. In addition, doses for patients can be based on their metabolic capacity by using the frequency of genotypes of "poor metabolizers" or "ultrarapid metabolizers." Molecular studies in pharmacogenetics began with cloning of CYP2D6 and now have been extended to more than two dozen drug-metabolizing enzymes and several drug transport systems (). The most important isozymes with genetic polymorphism involved in drug metabolism are shown in .

**Figure 12-2.**




Source: Shargel S, Wu-Pong S, Yu ABC: *Applied Biopharmaceutics & Pharmacokinetics*, 5th Edition: http://www.accesspharmacy.com



Most drug-metabolizing enzymes exhibit clinically relevant genetic polymorphisms. Essentially all of the major human enzymes responsible for modification of functional groups [classified as phase I reactions (**left**)] or conjugation with endogenous substituents [classified as phase II reactions (**right**)] exhibit common polymorphisms at the genomic level; those enzyme polymorphisms that have already been associated with changes in drug effects are separated from the corresponding pie charts. The percentage of phase I and phase II metabolism of drugs that each enzyme contributes is estimated by the relative size of each section of the corresponding chart. ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CYP, cytochrome P-450; DPD, dihydropyrimidine dehydrogenase; NQO1, NADPH: quinone oxidoreductase or DT diaphorase; COMT, catechol *O*-methyltransferase; GST, glutathione *S*-transferase; HMT, histamine methyltransferase; NAT, *N*-acetyltransferase; STs, sulfotransferases; TPMT, thiopurine methyltransferase; UGTs, uridine 5'-triphosphate glucuronosyltransferases.

()

Recognition of the genotypes associated with drug disposition and metabolism and the ability to obtain a "specific pharmacogenetic profile" for the patient will individualize drug therapy and reduce drug interactions (; , ). To realize this lofty objective, pharmacogenomics research aims to elucidate these *polygenic* (multiple-gene) determinants of drug effects. The interplay of genetic polymorphism with interindividual differences in pharmacokinetics and pharmacodynamics is well reviewed (; ). The ultimate goal is to provide new strategies for optimizing drug efficacy and toxicity based on each patient's genetic determinants.

## PHARMACOGENOMICS

Pharmacogenomics developed rapidly as a result of advances in molecular genetics and genomics. High-throughput tests such as microarray technology allow many human genes and their sequences to be probed or detected rapidly. The previously held notion of the *monogenic* nature of disease (one gene causing one disorder) is yielding to the concept of *polygenic* disorders, by which several, or even dozens, of genes may be differentially expressed compared to normal, healthy tissue (). As such new information arises, new challenges and opportunities also emerge for novel drug development.

The coordinated goal to sequence the 30,000 or more human genes via the Human Genome Project has also fueled progress in pharmacogenomics. Many new genes or gene products are emerging from the Human Genome Project as new potential drug targets. These new targets may be receptors, membrane proteins, enzymes, or ion channels that may be directly or indirectly involved in disease pathogenesis. While these newly discovered receptors or target enzymes can be exploited as new drug targets, polymorphic variations of those genes must be considered when developing new drugs that target these proteins.

Specific genetic codes are known for thousands of proteins and endogenous substances that support normal cellular functions. The genetic information is coded in the two helical strands of DNA. DNA consists of four basic nitrogenous substance or bases (C, cytosine; A, adenine; T, thymine; and G, guanine), which combine with deoxyribose and phosphate to form the respective nucleotides. The four nucleotides are combined in unique sequences for each gene. Genes are coded in a special region or *locus* in the DNA.

A change or mutation in gene sequence may or may not result in chronically reduced or increased level or activity of a protein or an essential enzyme. In some cases, such changes result in an exaggerated or reduced therapeutic response to a drug. The cell is *homozygous* if the genetic sequences occupying the locus are the same on the maternal and paternal chromosome; if they are different, the cell is *heterozygous*. When more than one alternative forms of a gene exists, they are referred to as *alleles* of the gene. The identity of the alleles carried by an individual at a given gene locus is referred to as the *genotype*. Alleles that vary by a single nucleotide change can now be characterized rapidly at the DNA level by *single-nucleotide polymorphism* (SNP). The physical effect observed as a result of genotype difference is referred to as *phenotype*. Genes are considered functionally *polymorphic* when allelic variants exist stably in the population and their gene products exhibit altered activity in relation to the *wild type* ("normal"). Ideally, variation in drug response can be predicted by monitoring changes in phenotype or genotype for a single patient or a group of patients.

To determine whether a patient is a rapid or slow metabolizer, the patient is given a known substrate for that enzyme and the patient's intrinsic clearance () is measured. Traditionally, intersubject variation in metabolism has been investigated by this method followed by *in-vitro* verification of enzyme level. Alternatively, it is possible to determine metabolic-status genotype directly from subjects' DNA. The latter approach is more definitive and offers much insight during drug development into how genes affect the metabolism of drugs. Many drugs have been elucidated with both approaches. In practice, fragments of DNA samples are compared based on SNPs. If the SNP and its functional activity are known, the probable individual drug response can be predicted.

In the future, rapid sequencing and SNPs will play a major role in detecting unusual variations in heritable clinical phenotypes of drug response. SNPs occur in about one of every 100–1500 base pairs (bp) between two unrelated individuals. Any two individuals may differ by 0.1% of their more than 3 billion base pairs. Common or informative SNPs are those that occur at frequencies of greater than 1% (). Once a large number of these SNPs and their frequencies in different populations are known, they can be used to correlate a patient's genetic "fingerprint" and the patient's probable individual drug response.

SNPs in coding regions of genes (about 30,000–100,000 per genome) cause variations in amino acid and protein function. SNPs in gene regulatory regions can cause differences in protein expression that may affect drug response. SNP profiles of individuals may be analyzed to determine disease susceptibility as well as predisposition to pharmacogenetic considerations in drug efficacy or toxicity.

## ADVERSE DRUG REACTIONS ATTRIBUTED TO GENETIC DIFFERENCES

Variations in drug pharmacokinetics and pharmacodynamics are due largely to genetic polymorphism in genes involved in drug metabolism, absorption, disposition, and disease pathogenesis. As early as the 1950s, researchers realized that some adverse drug reactions were caused by genetically determined variations in enzyme activity. More recently, a review of the pharmacogenetic literature showed that a sizable portion of ADRs (~30%) involved in drug therapy implicated genetic polymorphism of drug metabolism by CYP2D6 (). For example, prolonged muscle relaxation in some subjects after receiving a cholinergic drug was explained by an inherited deficiency of a plasma cholinesterase. Hemolysis caused by antimalarial drugs is recognized as being caused by inherited variants of glucose 6-phosphate dehydrogenase. Slow metabolism of isoniazid in some patients (acetylation of isoniazid) has been found to be the cause of peripheral neuropathy caused by this drug.

More recently, adverse drug reactions of debrisoquin have led to the discovery of the genetic polymorphism of the drug-metabolizing enzyme, debrisoquin hydroxylase CYP2D6. The same isozyme deficiency causes more nausea, diplopia, and blurred vision after dosing of the antiarrhythmic drug sparteine in deficient patients. It is important to determine whether the variation in

ADR is truly genetic or due to other factors. A method used to distinguish hereditary and environmental components of variability is the comparison of monozygotic and dizygotic twins, or pharmacokinetically by repeated drug administration and comparison of the variability of the responses within and between individuals. Many examples of genetic polymorphism affecting drug response/side effect are listed in .

**Table 12.2 Clinically Important Genetic Polymorphisms of Drug Metabolism that Influence Drug Response**

| Enzyme/Receptor | Frequency of Polymorphism | Drug | Drug Effect/Side Effect |
|---|---|---|---|
| CYP2C9 | 14–28% (heterozygotes)<br>0.2–1% (homozygotes) | Warfarin<br>Tolbutamide | Hemorrhage<br>Hypoglycemia |
| | | Phenytoin | Phenytoin toxicity |
| | | Glipizide | Hypoglycemia |
| | | Losartan | Decreased antihypertensive effect |
| CYP2D6 | 5–10% (poor metabolizers) | Antiarrhythmics | Proarrhythmic and other toxic effects |
| | | | Toxicity in poor metabolizers |
| | 1–10% (ultrarapid metabolizers) | Antidepressants | Inefficacy in ultrarapid metabolizers |
| | | Antipsychotics<br>Opioids | Tardive dyskinesia<br>Inefficacy of codeine as analgesic, narcotic side effects, dependence |
| | | Beta-adrenoceptor antagonists | Increased—blockade |
| CYP2C19 | 3–6% (whites) | Omeprazole | Higher cure rates when given with clarithromycin |
| | 8–23% (Asians) | | |
| | | Diazepam | Prolonged sedation |
| Dihydropyrimidine dehydrogenase | 0.1% | Fluorouracil | Myelotoxicity, Neurotoxicity |
| Plasma pseudo-cholinesterase | 1.5% | Succinylcholine | Prolonged apnea |
| N-acetyltransferase | 40–70% (whites) | Sulphonamides | Hypersensitivity |
| | 10–20% (Asians) | Amonafide | Mylelotoxicity (rapid acetylators) |
| Thiopurine Methyltransferase | 0.3% | Procainamide, hydralazine, isoniazid<br>Mercaptopurine, thioguanine, azothioprine | Drug-induced lupus erythematosus<br>Myelotoxicity |
| UDP-glucuronosyl-transferase | 10–15% | Irinotecan | Diarrhea, myelosuppression |
| ACE | | Enalapril, lisinapril captopril | Renoprotective effect, cardiac indexes, blood pressure |
| Potassium channels | | Quinidine | Drug-induced QT syndrome |
| HERG | | Cisapride | Drug-induced torsade de pointes |
| KvLQT1 | | Terfenadine | Drug-induced long-QT syndrome |
| | | Disopyramide | |
| HKCNE2 | | Meflaquine<br>Clarithromycin | Drug-induced arrhythmia |

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## GENETIC POLYMORPHISM IN DRUG METABOLISM: CYTOCHROME P-450 ISOZYMES CYP2D6

CYP2D6 () is a large isozyme family that affects metabolism of many drugs. CYP2D6 is highly polymorphic. More than 70 variant alleles of the CYP2D6 locus have been reported. The metabolism of the tricyclic antidepressants amitriptyline, clomipramine, desipramine, imipramine, nortriptyline, and the tetracyclic compounds maprotiline and mianserin is influenced by the CYP2D6 polymorphism to various degrees. Genetic polymorphism of CYP2D6 was first investigated with debrisoquine (). Poor

metabolizers often carry two nonfunctional alleles of this gene, resulting in reduced drug clearance.

Since about 10% of the population are poor CYP2D6 metabolizers, CYP2D6 drug candidates are often dropped from further development by researchers in favor of others (). Poor metabolizers have increased plasma concentrations of tricyclic antidepressants when given recommended doses of the drug. Adverse effects may occur more frequently in poor metabolizers and may be misinterpreted as symptoms of depression and may further lead to erroneous increases in the dose. When determining CYP2D6 metabolic status (slow versus fast metabolizers) in patients on tricyclic antidepressants, co-administration of other CYP2D6 substrates such as serotonin-selective reuptake inhibitors may result in erroneously concluding poor CYP2D6 metabolic status.

In contrast, ultrarapid metabolizers have relatively fast drug metabolism due to the presence of more enzyme or increased enzyme activity. Patients in this group are prone to therapeutic failure due to the resulting subtherapeutic drug concentrations when "normal" doses are given. Pharmacogenomic studies have revealed that some fast metabolizers of CYP2D6 are the result of gene duplication among different racial groups. Depending on the population studied, 5–20% of patients can be classified as either rapid or poor metabolizers.

Polymorphic drug metabolism is found in a large number of drugs used in psychiatric patients. Retrospective analysis of psychiatric patients treated with substrates of CYP2D6 strongly indicate that genotyping can improve the likelihood of preventing adverse drug reactions, and decrease the costs of therapy.

The polymorphic O-demethylation of codeine is of clinical importance when this drug is given as an analgesic. About 10% of codeine is O-demethylated by CYP2D6 to morphine, and this conversion is deficient in poor metabolizers. Poor metabolizers therefore experience no analgestic effects of codeine.

### CYP1A2

Another isozyme, CYP1A2, which metabolizes 5% of randomly selected drugs, may also be considered during development, since up to 15% of a patient population can be classified as poor metabolizers, according to . Fluvoxamine is a substrate and potent inhibitor of CYP1A2, causing important interactions with drugs such as amitriptyline, clomipramine, imipramine, clozapine, and theophylline that are partly metabolized by this cytochrome P-450 enzyme.

### CYP2C9

Still another example of a clinically important drug metabolism polymorphism is the association of variant alleles of CYP2C9 with the requirement for lower warfarin dose. In a retrospective study of a population from an anticoagulant clinic, the CYP2C9 alleles associated with decreased enzyme activity (*2 and *3) were found to be overrepresented in patients stabilized on low doses of warfarin. These patients had an increase incidence of major and minor hemorrhage.

### CYP2C19

The 4'-hydroxylation of the (S)-enantiomer of mephenytoin is catalyzed by CYP2C19. The polymorphic enzyme has a poor metabolizer (PM) frequency of about 3% in Caucasians, 15–25% among Asians, and 4–7% among Black Africans (). The major defective allele responsible for the PM phenotype is CYP2C19***2,** which is found among 13% and 32% of Caucasians and Asians, respectively. A second allele, CYP2C19***3,** is found mostly among Asians and rarely in Caucasians.

Interestingly, few polymorphisms are reported for the isozyme subfamily CYP3A. This isozyme is involved in the metabolism of endogenous steroid and testosterone. Mutations of this vital enzyme may not be compatible with life.

Not all therapeutic variations and side effects result from genetic differences in the receptor or drug metabolism. Drug response (including therapeutic and unintended side effects) is influenced by many direct and indirect factors, including modifying effects from environmental factors on the disease process and drug disposition. As a result, some researchers are unsure whether prescribing drugs based on a pharmacogenetic profile will significantly reduce side effects for most drugs, since many side effects and therapeutic failures may be the result of incorrect diagnosis or failure to account for other influencing variables such as the nature and severity of the disease, the individual's age and race, organ function, concomitant therapy, drug interactions, and concomitant illnesses.

## GENETIC POLYMORPHISM IN DRUG TRANSPORT: P-GLYCOPROTEIN AND MULTIDRUG RESISTANCE

*Transporterpharmacogenetics* is a rapidly developing field that is concerned with drug uptake and efflux into or through tissues. Significant problems in the clinical application of drugs result from poor or variable oral drug bioavailability, and high intra- and interindividual variation in pharmacokinetics. Several membrane transporter proteins are involved in the absorption of drugs from the intestinal tract into the body, into nonintestinal tissues, or into specific target sites of action ().

*Drug efflux* is an important cause of drug resistance in certain types of cells. In cytotoxic chemotherapy for several human cancerous diseases, drugs are generally very effective, but in the case of *intrinsic* or *acquired multidrug resistance*, usually highly effective antineoplastic compounds, eg, vincristine, vinblastine, daunorubicin, or doxorubicin, fail to produce cures. One of the major causes of such multidrug resistance is the appearance of special integral membrane proteins, the *P-glycoprotein multidrug transporter*, or MDR1 (), which is one of the major causes of low drug level in targeted cells. P-glycoprotein is discussed in .

The multidrug resistance-associated proteins (MRPs) are members of the ATP-binding cassette (ABC) superfamily with six members currently, of which MRP1, MRP2, and MRP3 are commonly known to affect drug disposition. MRP1 is ubiquitous in the body. Substrates for MRP1 include glutathione, glucuronide, and sulfate. MRP1 is expressed basolaterally in the intestine, although its role in extruding drugs out of the enterocytes is still uncertain. There is some substrate overlap between MRP1 and apically located P-glycoprotein. The amino acid homology between MDR1 and P-glycoprotein was reported to be 15% in some cell

lines (see ).

## GENETIC POLYMORPHISM IN DRUG TARGETS

In the future, proteins involved in disease will become identified as important biomarkers for pharmacodynamic studies. Genomics has led to the development of proteonomics, which involves the study of biologically interesting proteins and their variants. Proteins can be used as probes for drug discovery or as biomarkers for drug safety, such as cell surface proteins (eg, COX-2, D-2R), intracellular proteins (eg, troponin I), and secreted proteins (eg, MCP-l).

The physiologic response of the body to a drug is generally the result of interaction of the drug at a specific target site in the body. It is estimated that about 50% of drugs act on membrane receptors, about 30% act on enyzmes, and about 5% act on ion channels (). Many of the genes encoding these target proteins exhibit polymorphisms that may alter drug response. Clinically relevant examples of polymorphism leading to variable responses are listed in . For example, the β-2-adrenergic receptor, and its common mutation of Arg→Gly at amino acid 16, greatly reduces the bronchodilator response of albuterol (). In addition, mutations in the angiotensin-converting enzyme (ACE) gene have been proposed to account for variations in the response to ACE inhibitors. Another study has shown that a combination of two mutations in the gene encoding a high-affinity sulphonylurea receptor leads to a 40% reduction in the insulin response to tolbutamide (). The response to clozapine in patients with schizophrenia appears to involve genetic polymorphisms in the 5-hydroxytryptamine (serotonin) receptor, HTR2A. Finally, mutations in five genes involved in the cardiac ion channels affect the risk of drug-induced long-QT syndrome (), a potential cause of sudden cardiac death in young individuals without structural heart disease. The prevalence of long-QT syndrome is about 1 in 10,000. All five genes code for membrane ion channels affecting sodium or potassium transport and are influenced by antiarrhythmics and other drugs ().

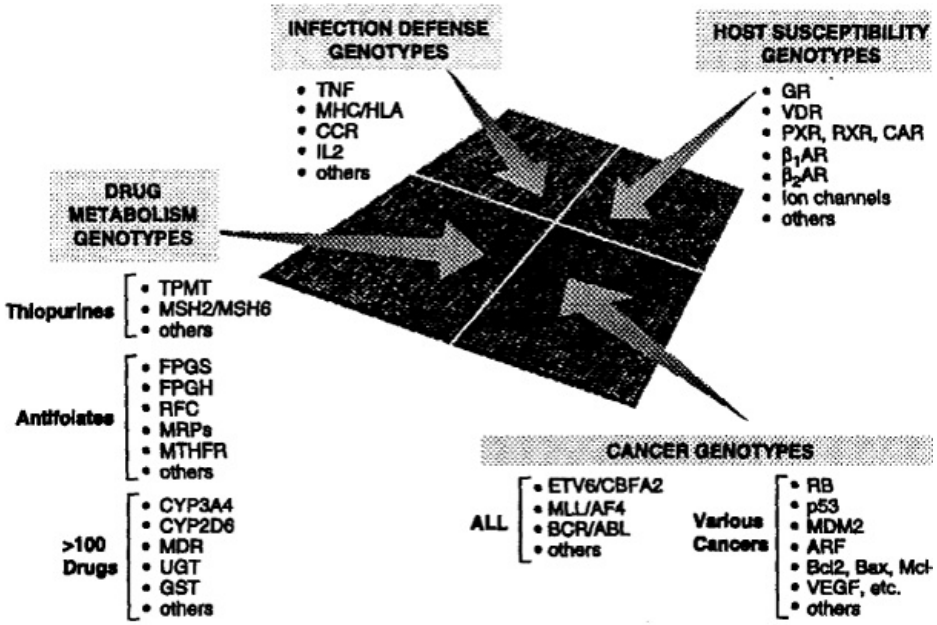
**Table 12.3 Clinically Important Genetic Polymorphisms of Drug Targets and Drug Transporters**

| Gene | Frequency | Drug | Drug Effect |
|---|---|---|---|
| Multidrug resistance gene (*MDR1*) | 24% | Digoxin | Increased concentrations of digoxin in plasma |
| Beta-2 adrenergic receptor gene (*2AR*) | 37% | Albuterol | Decreased response to Beta-2 adrenergic agonists |
| Sulphonylurea receptor gene (*SUR1*) | 2–3% | Tolbutamide | Decreased insulin response |
| Five genes coding for cardiac ion channels | 1–2% | Antiarrhythmics, terfenadine, many other drugs | Sudden cardiac death due to long-QT syndrome |

From , with permission.

The systematic identification and functional analysis of human genes is changing the study of disease processes and drug development. Pharmacogenetics enable clinicians to make reliable assessments of an individual's risk of acquiring a particular disease, be more specific in targeting drugs, and account for individual variation of therapeutic response and toxicity of drugs. Mutant alleles at a single gene locus are the best studied individual risk factors for adverse drug reactions, including the genes for N-acetyltransferase, thiopurine methyltransferase, dihydropyrimidine dehydrogenase, and the cytochrome P-450 isozymes. Genotyping can predict phenotype extremes in these situations and identify metabolic status in individual patients. Genomics is providing the information and technology needed to analyze the complex multifactorial situations involved in drug therapy (). Awareness of inherited variations of drug response can lead to dose adjustment on the basis of the patient's genetic makeup and provides a promising approach to reducing adverse drug reactions (; ). has reviewed the issues involving pharmacogenomics and the prescribing of antipsychotic drugs.

**Figure 12-3.**




Source: Shargel S, Wu-Pong S, Yu ABC: *Applied Biopharmaceutics & Pharmacokinetics*, 5th Edition: http://www.accesspharmacy.com



Molecular diagnostics of pharmacogenomic traits. DNA arrays are being made for automated, high-throughput detection of functionally important mutations in genes that are important determinants of drug effects, such as drug-metabolizing enzymes, drug targets (receptors), disease pathogenesis, and other polymorphic genes that influence an individual's susceptibility to drug toxicities or environmental exposures (such as pathogens, carcinogens, and others). This figure exemplifies components of a potential diagnostic DNA array for genes that could influence a patient's response to chemotherapy for acute lymphoblastic leukemia, including genes that determine drug metabolism, disease sensitivity, and the risk of adverse effects of treatment (cardiovascular or endocrine toxicities, infections, etc).

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## PHARMACOKINETICS/PHARMACODYNAMICS (PK/PD) CONSIDERATIONS AND PHARMACOGENETICS/PHARMACOGENOMICS (PGT/PGX)

The study of drug interactions and PGt/PGx has revealed that many unexpected pharmacodynamic responses and pharmacokinetic variations among individuals during drug therapy involve genetic factors. These genetic factors contribute to variation at many levels, including drug transport, metabolism, and interaction at the receptor site.

Applying genetics to study interindividual variations in drug response may greatly improve drug therapy. Understanding and monitoring the underlying pharmacogenetic factors will allow physicians to optimize efficacy and minimize side effects. The labeling of many new drugs now contains more information on drug interactions and metabolism based on our understanding of PGt/PGx. PGt/PGx is influencing the study of pharmacokinetics and pharmacodynamics. Population pharmacokinetics has already assimilated some of these changes by enriching what are often simple or even empirical PK/PD models to simulate the quantitative aspect of drug distribution and drug action.

Models that will predict dosing and an individual's drug clearance more accurately will require more details concerning the individual patient's genetic profile, demographic and pathologic information, as well as the drug's PK profile. If one of the patient's parents is homozygous for a specific isozyme that metabolizes the drug, how would that information be linked to a clearance prediction for the patient? Recognizing the allele that predisposes the patient to a severe adverse reaction or toxic plasma concentration may allow the dose to be reduced or the drug avoided entirely. Similarly, if a patient is known to be a nonresponder due to genetic variation, that information can be used to select an alternate drug *a priori.*

In , a mixed-effects model is described that takes into account the effect of enzyme induction due to concomitant administration of a drug that induces enzyme. Should new models be developed, or will an extension of some of the mixed-effect models suffice? Advances in PGt will no doubt stimulate developments in PK and PD.

The successful application of genetic screening tests to identify patients with specific risks in drug response or drug toxicity depends on many factors. Large amounts of relevant genetic information must be monitored. Robust, high-throughput, high-positive and low-negative predictive tests must be developed and implemented. Such an endeavor will also involve considerable training, adaptation, and acceptance of the new technology by physicians and other health care personnel. With genetic diagnostic tests becoming more common and affordable, it is expected that individual drug dosing will become more accurate and ultimately result in vast improvements in therapeutic response and better drug tolerance. Researchers have high expectations that the use of diagnostic DNA microarrays or gene chips will simplify and expand testing and have clinical applications in diagnosis, disease prevention, drug selection, and dose calculation. The challenge to pharmacokinetics is to integrate all the relevant information into a model that is accurate and simple enough for practical application.

## FREQUENTLY ASKED QUESTIONS

**1.** In genetic polymorphism of CY2C9, the frequency of polymorphism is much higher in heterocytes (14–28 %) than in

homocytes (0.2–1%). Why?

**2.** How do drug efflux transporters affect the rate and extent of drug absorption and the bioavailability of a drug?

**3.** Which human ABC-transport protein may influence the activation of transcription factors such as PXR in the liver, and which nuclear hormone receptors regulate drug detoxification genes?

**4.** How is biliary excretion affected if genetic expression of a transport protein is enhanced?

**5.** Without pharmacogenetic profiling of subjects, what are the chances of a subject with an unusually high expression of P-glycoprotein to be interpreted as a "statistical outliner" in a bioavailability study due to more rapid efflux that results in lower drug levels compared with other subjects? Is deviation from the mean a rational valid reason for data exclusion?

**6.** Should exclusion criteria based on pharmacogenomics be used in designing protocol for pharmacokinetics or clinical studies? In other words, minimize variation (prevent monkey wrench) and increase power in bioequivalence studies?

**7.** Often, the most likely functions are employed to minimize deviations from the data when pharmacokinetic parameters are estimated by iteration within a group. These parameters are estimated for an average individual such that most subjects resemble the mean within a group. However, for a subject with the unusual allele, the underlying genetic factors contribute parameter differences that are the result of genetic expression. How useful are the parameters obtained from a classical pharmacokinetic model that does not incorporate parameters accounting for genomic determinants? (*Note:* An attempt to force data fitting the mean would seriously err for a few individuals, ie, fit most subjects well, but err badly for the few, on the basis of minimization of the sum of squared deviations.)

**8.** What are the differences between pharmacogenetics and pharmacogenomics? There are many but no uniformly accepted definition. The two terms are regarded as similar by some and different by others. The latter term is preferred by more recent investigators. It is regarded by some to be more focused on the deployment of high-throughput technologies, besides its applications in drug therapy and drug discovery.

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