Basics of Pharmacogenomics for the Compounding Pharmacist

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People respond in different ways to similar doses of a drug. A drug that works well in one person may have a different effect in another individual. Pharmacogenomics is the study of interindividual genetic variations in drug disposition and the applications of these variations in selecting an optimal drug therapy and dosage for each individual. Most drug effects are determined by the interaction of several gene products such as drug targets, drug-metabolizing enzymes, and transporters that influence the pharmacokinetics and pharmacodynamics of medications.[1]

The field of pharmacogenomics evolved from its predecessor, pharmacogenetics. The two terms are used interchangeably, although a slight difference exists. The main difference between the two is that pharmacogenetics studies the association between genotype and drug metabolism, whereas pharmacogenomics is the application of genomic technologies to drugs in clinical development.[2] Pharmacogenetics has been around for hundreds of years and dates back to Pythagoras and his observation that the ingestion of fava beans triggered a potentially fatal hemolytic anemia in some but not all individuals. The associations between adverse drug reactions and inherited variations were first recognized only in the 1950s, when peripheral neuropathy occurred as an adverse effect of treatment with isoniazid.[3] Over the past 50 years, pharmacogenomics has evolved with the advent of new technology and applications.

Gene Terminology

Genes are made of DNA, which comprises four units called nucleotides: adenine (A), guanine (G), cytosine (C), and thymine (T). These units form base pairs, A-T and G-C, and each gene is made of thousands of these A-T and G-C pairs in specific sequences. A gene has many variants, which differ slightly in sequence; these variants are called alleles. Individuals inherit two alleles of each gene, one from each parent, and the alleles inherited determine traits (such as eye color). A person is said to be homozygous for a specific trait if he/she has two identical alleles of that gene; heterozygous indicates that the alleles are not identical. A single base-pair variation in a genetic sequence is known as a single nucleotide polymorphism (SNP) (Figure 1). Over 1.4 million SNPs have been identified in the human genome. The phenotype is the external manifestation of a trait, whereas the genotype is the internal genetic code of an organism that determines the trait. Interindividual differences in phenotype are often caused by genetic polymorphisms.

Figure 1. Example of a Single Nucleotide Polymorphism.

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ACGTGTTT    ACGTG G TT
TGCACAAA    TGCAC C AA
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Drug Metabolism

The majority of drugs get metabolized in the liver to a more water-soluble form, which gets excreted (Figure 2). Polymorphisms of drug-metabolizing enzymes can lead to interindividual variations in protein expression or metabolic activity, and this in turn could result in unique drug-metabolism phenotypes. There are more than 30 families of drug-metabolizing enzymes, and essentially all of them have genetic variants. Metabolism in the liver is mainly carried out by two groups of enzymes: phase I and phase II enzymes.

Phase I Enzymes

The cytochrome P450 (CYP450) enzymes are the most important phase I enzymes, and they act by modifying functional groups on drugs. The CYP450 enzyme system is responsible for the metabolism of a majority of drugs and has been a major focus of pharmacogenetic research.[4] Table 1 presents a list of selected CYP enzymes and their substrates. The CYP2D6 enzyme was the first human drug-metabolizing enzyme to be cloned and characterized at the molecular level.[5] CYP2D6 metabolizes 25% to 30% of all drugs, and it is the most polymorphic CYP enzyme.[6]

Figure 2. Metabolic Process.

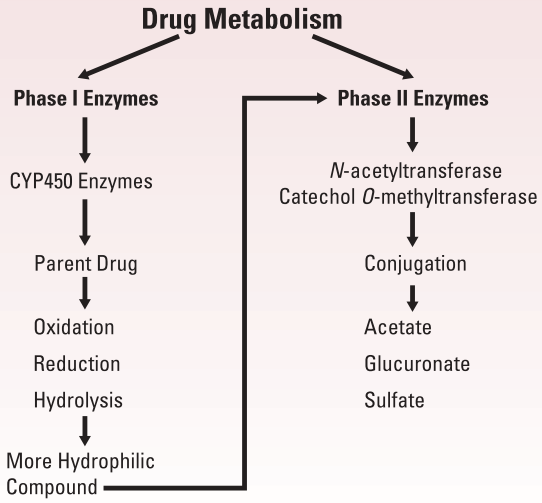

Table 1. Selected List of CYP Enzymes and Substrates.

Enzyme	*Drugs Metabolized*
CYP1A2	Amitriptyline, clomipramine, clozapine, erythromycin, fluvoxamine, haloperidol, imipramine, naproxen, paracetamol, propranolol, theophylline, verapamil, warfarin
CYP2C9	Dextromethorphan, diclofenac, fluoxetine, ibuprofen, naproxen, phenytoin, S-warfarin, tolbutamide
CYP2C19	Aminopyrine, citalopram, clomipramine, diazepam, imipramine, mephenytoin, omeprazole, phenytoin, sertraline
CYP2D6	Beta-blockers, chlorpromazine, debrisoquine, dextromethorphan, fentanyl, flecainide, haloperidol, meperidine, mexiletine, paroxetine, procainamide, propafenone, risperidone, selective serotonin reuptake inhibitors (eg, fluoxetine), thioridazine, trazodone, tricyclic antidepressants Prodrug activation of tramadol, venlafaxine, and oxycodone
CYP2E1	Acetaminophen, chlorzoxazone, ethanol, halothane, isoflurane, pentobarbitone, propranolol, rifampicin, tolbutamide
CYP3A4	Alprazolam, calcium channel blockers, carbamazepine, codeine, cyclosporin A, dexamethasone, erythromycin, fentanyl, haloperidol, HIV protease inhibitors, ketoconazole, lidocaine, methadone, midazolam, ropivacaine, sufentanil, terfenadine, testosterone, triazolam

On the basis of genetic polymorphisms of CYP2D6, three metabolizer types, or profiles, are recognized.[7,8]

- Ultraextensive metabolizers
- Extensive metabolizers
- Poor metabolizers

Clinically, these drug metabolism profiles could translate to a variety of responses in different patients, from therapeutic inefficacy to toxicity for the same dosage of a drug. Identification of a patient's genotype and subsequent dose adjustment is likely to improve response. The ability to identify these profiles could provide opportunities for the compounding pharmacist to compound specific dosages based on the individual's likely response. The enzyme CYP2C9, for example, is known to metabolize warfarin, phenytoin, and a number of nonsteroidal anti-inflammatory drugs.[4,9-11] Slight differences in dose requirements for warfarin and phenytoin may be clinically relevant because of their narrow therapeutic range.[4,12] Genotyping for CYP2C9 alleles before initiation of anticoagulant therapy can provide valuable information on whether anticoagulant treatment is appropriate, the most suitable induction regimen, and the degree of clinical monitoring required.[13]

The CYP3A enzymes are considered one of the most important enzyme groups involved in drug metabolism.[14,15] CYP3A4 metabolizes the largest number of drugs, including antilipid drugs, benzodiazepines, HIV protease inhibitors, and macrolide antibiotics.

Phase II Enzymes

Phase II enzymes enhance drug excretion through conjugation of polar groups, such as acetate and glucuronate (Figure 2), onto the drug molecules. An example of a phase II enzyme is thiopurine *S*-methyltransferase (TPMT), an enzyme that metabolizes the anticancer drugs mercaptopurine and azathioprine. Administration of standard thiopurine drug dosages to individuals homozygous for the gene that codes for low TPMT levels can lead to adverse effects due to buildup of toxic metabolites of these drugs. To avoid a toxic response, individuals with this genotype must be treated with considerably lower doses of these drugs, about 5% to 10% of the standard dose.[16] Tests for the TPMT phenotype and genotype were among the first pharmacogenetic diagnostic tests.[17] Other phase II enzymes include glutathione-*S*-transferase, *N*-acetyltransferase, and sulfotransferases.

Transport Proteins

Transport proteins mediate the absorption of drugs across physiological barriers such as the blood-brain barrier. Polymorphisms affecting either levels or function of the transporter proteins are likely to be important determinants of both overall drug levels and drug levels within cells. The multidrug resistant protein (MDR1) is a good example of a polymorphic transport protein. An MDR1 is a member of the p-glycoprotein (P-gp) family, and MDR1 is expressed on the inner surface of barrier organs, such as the small intestine and the brain. Interindividual variability in the disposition of certain drugs has been attributed to genetic variations in P-gp expression.[4,18] The MDR1 protein is associated with the transport of digoxin, HIV protease inhibitors, and some natural product anticancer drugs. Genetic elucidation of P-gp function may have predictive value in determining the risk for certain diseases and predicting therapeutic outcomes of treatment with P-gp substrates.[4] Other substrates of P-gp include morphine, methadone, fentanyl, and sufentanil (Table 2).[19] An increased understanding of transporter pharmacogenetics may prove beneficial in the future.

Drug Targets

Most drugs exert their pharmacologic effect by interaction with specific targets, ie, receptors, enzymes, or proteins. Examples of these targets include the β_2– adrenoceptors, insulin receptors, and the angiotensin-converting enzyme. Polymorphisms in genes encoding these targets may influence sensitivity to selected drugs.[20] These polymorphisms may cause an indirect effect on drug response, which is unrelated to drug metabolism or transport.

An example is the anticancer drug 5-fluorouracil (5-FU), which acts by inhibiting its target, the enzyme thymidylate synthase (TS). Thymidylate synthase is a critical element in DNA synthesis and

Table 2. Selected Drugs that Are Substrates of P-gp.[4]

Anticancer Agents	*Cardiac Drugs*	*Antibiotics*	*Protease Inhibitors*	*Miscellaneous*
Actinomycin D	Atorvastatin	Erythromycin	Indinavir	Dexamethasone
Irinotecan	Digitoxin	Levofloxacin	Ritonavir	Morphine
Mitomycin C	Diltiazem	Sparfloxacin	Saquinavir	Phenytoin
Vincristine	Quinidine			Ranitidine
	Verapamil			Rifampin

repair, and clinical resistance to 5-FU and other folate-based antimetabolites has been linked to overexpression of TS. Level of expression of TS levels appears to be regulated by the number of polymorphic tandem repeats in the TS enhancer region, and TS activity has been observed to increase with an increased number of tandem repeats.[21]

Drug-Metabolism Profile

The drug-metabolism type may be identified through phenotyping or genotyping. Phenotyping is used to evaluate the level of activity of an enzyme that is purported to metabolize a group of drugs.[22] The test drug or substrate used is typically one whose metabolism is solely dependent on a specific enzyme.[7] For example, omeprazole and mephenytoin have been used as substrates to check for CYP2C19 activity,[14,23] and debrisoquine and sparteine are used for phenotyping CYP2D6 activity.[8] On the basis of enzyme activity, an individual is assigned a drug-metabolism profile, which would help predict likely response to other drugs/substrates metabolized by the same enzyme. Genotyping involves the identification of genetic variants or mutations that produce the specific drug-metabolism phenotype.[7] Such applications of genotyping are becoming increasingly popular in molecular diagnostics.

Ethnic Variations in Drug Disposition

An individual's drug-metabolism phenotype is determined partly by the inheritance of alleles that encode enzymes, transporters, and targets. These allele frequencies have exhibited variations among different ethnic groups. Isoniazid, an antitubercular drug, exhibits a metabolic phenotype of rapid and slow metabolizers whose distribution varies in different ethnic or geographic populations. The slow metabolizer phenotype exhibits a frequency of 5% among Canadian Eskimos, 80% among Egyptians, and 90% among Moroccans. The variable acetylation phenotype was correlated with at least 20 variations of the *N*-acetyltransferase 2 (*NAT2*) gene.[4]

Scope of Pharmacogenomics

Applications of pharmacogenomics are being explored and identified in a vast variety of disciplines. At present, individuals with a particular condition are treated the same way, without taking into account any genetic variations that may influence clinical response. This philosophy may in part account for annual hospital expenditures of as much as $5.6 billion due to adverse drug reactions, which are also one of the leading causes of death.[24] As already noted, predetermination of certain genetic polymorphisms could help predict the clinical response to a certain drug. From a fiscal perspective, a study on CYP2D6 indicated that as much as $4000 to $6000 more is spent per year for patients who have the poor or ultrarapid genotype. The high cost of genotyping delays its potential economic benefits.[11]

Clinical medicine has benefited from SNP-based applications, especially as a tool of molecular diagnostics.[3] The identification of drug targets and subpopulation-specific drug development has aided the discovery and development of new drugs.[2] Traditionally, most new drugs have not reached the clinic because of intolerable toxicity profiles and insufficient efficacy. Even after clinical trials involving hundreds of patients, toxicity is still a possibility, as in the cases of the drugs fenfluramine, terfenadine, and mibefradil.[25]

Predictive genomic technologies are being embraced more frequently in the biotechnology and pharmaceutical industries, to identify targets for drug discovery. A genotype-based, population-specific clinical trial leading to the discovery of a new drug could potentially save time, money, and patient distress by reducing the incidence of adverse effects. Clinical trials would definitely benefit from the incorporation of pharmacogenomic data, by identifying individuals with increased risk of adverse effects. Furthermore, recognition of a genetic subtype would allow a drug to be marketed exclusively to individuals expressing a "positive response" genotype, thus increasing drug efficacy.[26] Chemotherapeutic efficacy of drug combinations and predetermination of drug efficacy and toxicity are more recent extensions of pharmacogenomics. Identification of genetic polymorphisms that predispose individuals to drug toxicity could help pre-empt unnecessary expenditure on drugs and could "kill" or allow modification of drugs that are potentially harmful to a small subset of the population.[2] These medications could be prescribed selectively to the genomically determined population of patients who would most likely benefit.

Applications of Pharmacogenomics to Compounding Pharmacy

Presently, most drugs are available at prescription doses. Because "poor metabolizers" have lower levels of drug-metabolizing enzymes, administration of regular prescription drug dosages in such individuals might lead to cumulative toxicity and adverse effects owing to their relatively slow metabolic rate. In "ultrarapid metabolizers," the opposite effect occurs: drug metabolism is accelerated, and a normal dose would be therapeutically ineffective. Compounding pharmacies could have a crucial role in individualized therapeutics for these patients.

Narcotic analgesics and drugs with a low therapeutic index often exhibit variable responses in individuals. In the foreseeable future, molecular diagnostics could become a valuable tool for clinicians to individualize medications and drug doses, and pharmacogenomics could serve as a useful adjunct to empirical prescription guidelines. The sequence of events in treatment would begin with molecular diagnostics, which allows interpretation of the disease/condition, and progress to formulation and delivery of individualized therapeutic preparations. Genomically determined treatment programs will mean more frequent prescription of atypical dosages of drugs, in formulations tailor-made for each person. Issues of drug selection and dosage would be under the purview of the compounding pharmacist. An in-house genomic diagnostic laboratory would allow the compounding pharmacist to decide whether the prescribed drug is safe, and if so, the optimal dose to be compounded.

An individual's genotype needs to be determined only once, because with the rare exception of somatic mutations, the genotype is constant.[1] This has significant financial implications, and could obviate the need for a plethora of expensive laboratory tests and prolonged drug monitoring. With newer, higher throughput methods, a small quantity of DNA can be screened for thousands of SNPs, thus also elucidating the polygenic effects of drugs. In the future we can anticipate a paradigm shift from a "one size fits all" approach to the advent of genomically individualized medicine.

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