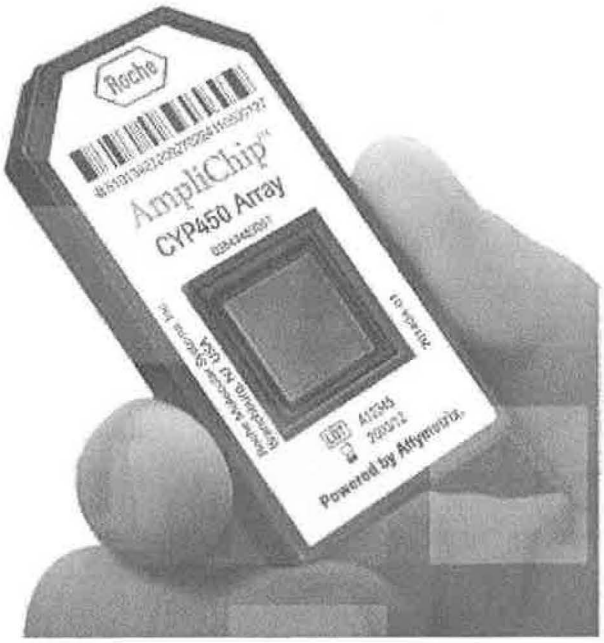
Pharmacogenetics: Is it ready to be a prime time player?

Keith L. Parker



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GENOMIC MEDICINE

NEWS

Preventing Toxicity With a Gene Test

To test or not to test? That is the question clinicians are asking about screening for genes that affect how the body metabolizes drugs

U.S. Food and Drug Administration (FDA) seems unlikely to recommend one.

[Internal Medicine Grand Rounds]
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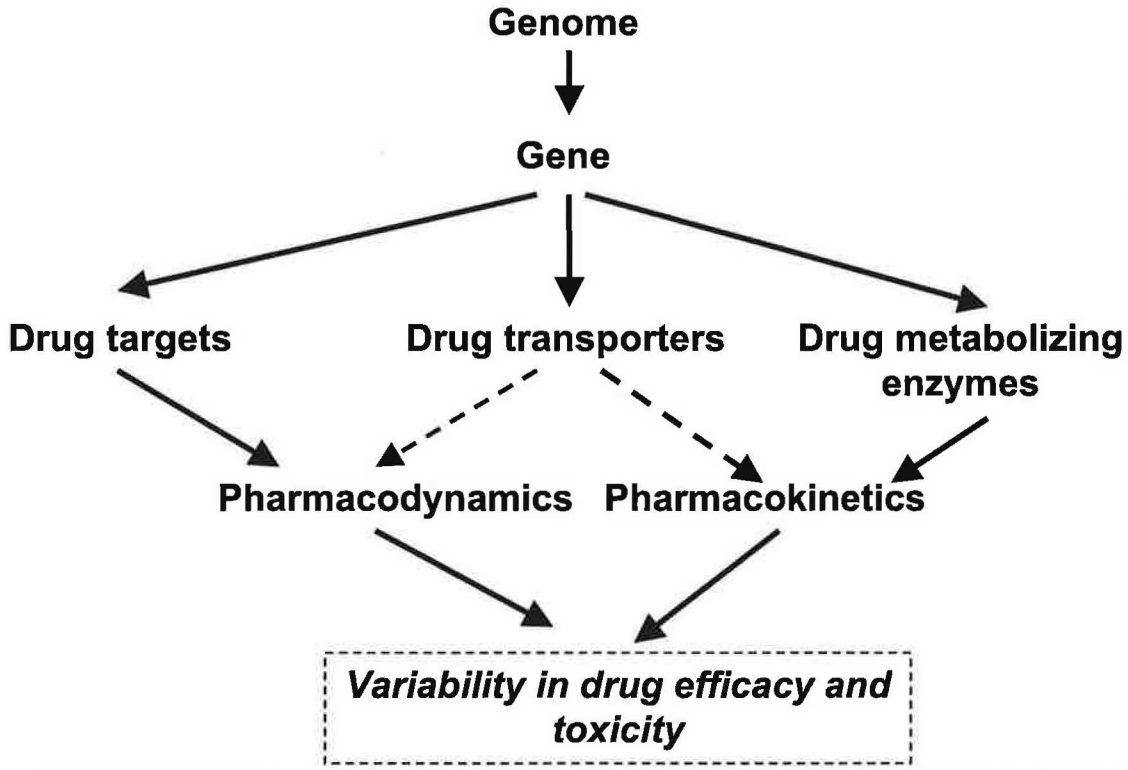
Introduction

Adverse drug reactions in the U.S. are estimated to cause 2 million hospitalizations annually, resulting in 100, 000 deaths (Lazarou *et al.*, 1998) and costing between 30 and 150 billion dollars/year. The underlying causes are complex, including medication errors (Allard *et al.*, 2002), drug allergies (Solensky 2006), and environmental factors; increasingly, it has been recognized that genetically determined, individual differences in drug absorption, metabolism, or action play important roles in determining drug efficacy and adverse reactions (Wilkinson, 2005).



An overview of the many levels that can influence the response to a drug is shown above. Important terms to keep in mind are "Pharmacodynamics", or the biological response elicited by a drug, and "Pharmacokinetics", or the rate of elimination of a drug from the body (which usually is related to drug metabolism). The field of Pharmacogenetics seeks to associate characteristic drug response endpoints (*e.g.*, efficacy, drug interactions, and adverse effects)--termed the phenotype--with genetic markers (polymorphisms). Although most phenotypic traits for drugs are complex, the goal is to identify specific genetic polymorphisms that are linked to phenotypes for individual drugs, thereby providing personalized pharmacotherapy that maximizes efficacy for an individual patient while minimizing the likelihood of adverse effects (Meyer, 2004; Eichelbaum *et al.*, 2006). Retrospective analyses indicate that 56% of drugs that frequently cause adverse drug reactions are substrates for polymorphic drug-metabolizing enzymes; only 20% of drugs that are metabolized by non-polymorphic enzymes

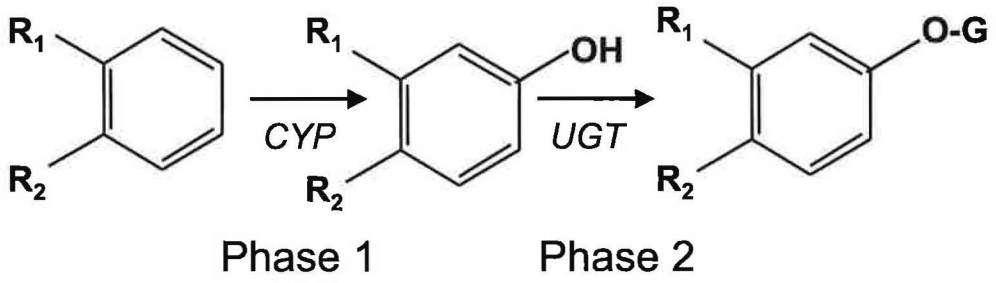
are frequent offenders (Phillips *et al.*, 2001). Prospective genotyping of these enzymes may allow us to avoid many adverse drug reactions and optimize drug efficacy.

Although pharmacogenetics has focused on genetic polymorphisms in drug metabolizing enzymes, it is apparent that polymorphisms in drug transporters and receptors also can be important determinants of drug efficacy and adverse effects (*see* below).

Today, we will briefly review mechanisms of drug handling, focusing especially on the Cytochrome P450 enzymes (CYPs), consider classic examples in which drug phenotypes have been linked to specific genes, discuss the current status for application of pharmacogenetics to pharmacotherapy with specific drugs, and speculate on how new advances such as pharmacogenomics may enhance our ability to treat individual patients safely and effectively with specific drugs.

Drug Transport and Metabolism

Xenobiotic metabolism involves phase 1 reactions (oxidation, reduction, or hydrolytic reactions) and phase 2 reactions, in which enzymes form a conjugate of the phase 1 product. Phase 1 enzymes (left side) introduce functional groups (*e.g.*, –OH, -COOH, -SH, -O- or NH_2) into the parent compound; these moieties usually lead to drug inactivation but can also result in bioactivation of a prodrug (*e.g.*, the hydrolysis of an ester or amide linkage). Phase 2 enzymes (right side) facilitate the elimination of drugs and the inactivation of electrophilic and potentially toxic metabolites produced by oxidation (in this case, glucuronidation).

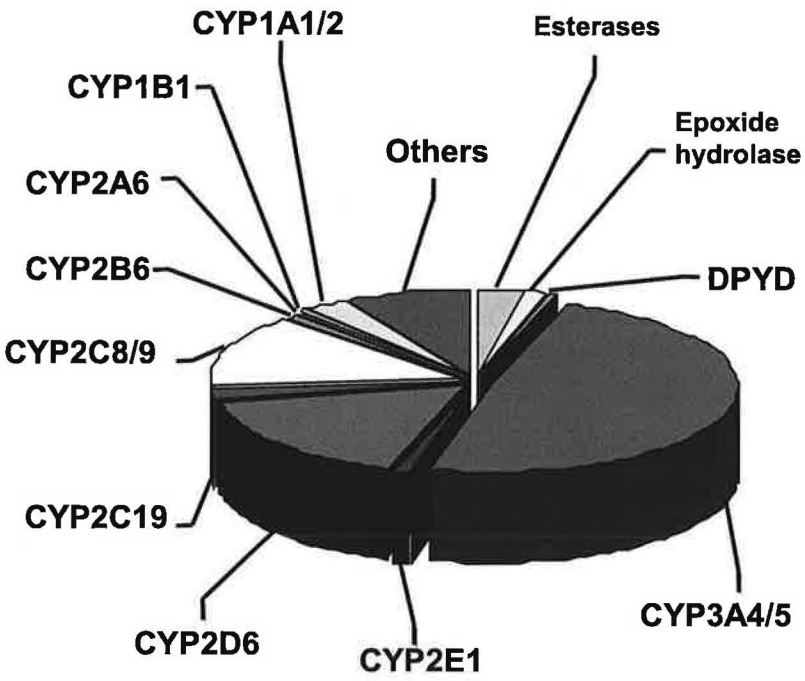


Phase 1 oxidation reactions are catalyzed by the superfamilies of CYPs, flavin-containing monooxygenases, and epoxide hydrolases. The CYPs are especially important in drug metabolism and also provide striking examples of polymorphic variation in drug phenotype associated with genetic variants (*see* below). Shown below is a breakdown of the roles of the different phase 1 enzymes in the metabolism of drugs in clinical use. Note the key role in the metabolism of multiple drugs by CYP3A4, which in part accounts of its preeminent involvement in drug interactions. Polymorphic variations in its activity, however, are not widespread, and CYP3A4 does not play a major role in personalized assessment of risk of adverse drug effects.

The phase 2 enzymes include several superfamilies of conjugating enzymes (transferases), such as the glutathione-S-transferases (GSTs), UDP-glucuronosyltransferases (UGTs), sulfotransferases (SULTs), *N*-acetyltransferases (NATs), and methyltransferases (MTs). These conjugation reactions usually require that the substrate have O (hydroxyl or epoxide groups), N, or S atoms that serve as acceptor sites for a hydrophilic moiety (*e.g.*, glutathione, glucuronic acid, sulfate, or an acetyl group) that is covalently conjugated to an acceptor site on the molecule. Phase 1 oxidation generally either adds or exposes a functional group, permitting the products to then serve as substrates for phase 2 enzymes.

Cytochrome P450 Enzymes

The human genome contains 57 functional CYP genes, which are divided into families and subfamilies. These genes all carry the generic "CYP" label, followed by a number designating the family, a letter denoting the subfamily, and a second number designating the specific isoform. In addition to their roles in the metabolism of xenobiotics, including drugs, other CYPs play key roles in the conversion of cholesterol into steroid hormones and bile acids.



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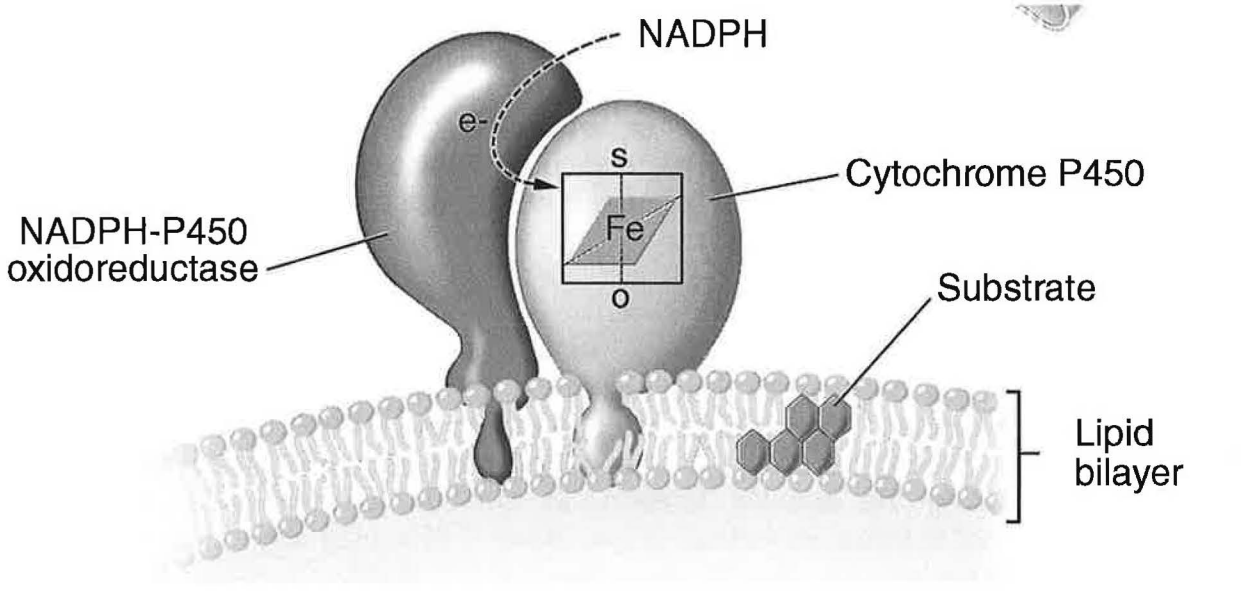
The CYP proteins contain heme; the heme iron binds oxygen in the CYP active site. CYPs use molecular oxygen to carry out the oxidation of substrates and

obtain electrons from NADPH-cytochrome P450 oxidoreductase and its cofactor, NADPH. The CYPs that play major roles in drug metabolism generally reside in the endoplasmic reticulum.



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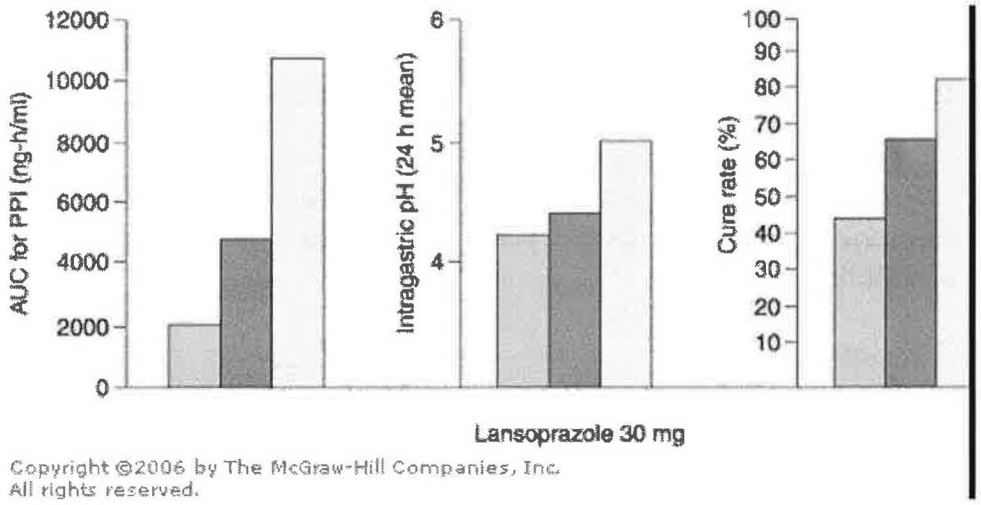
Among the diverse reactions carried out by mammalian CYPs are *N*-dealkylation, *O*-dealkylation, aromatic hydroxylation, *N*-oxidation, *S*-oxidation, deamination, and dehalogenation. The CYPs that carry out xenobiotic metabolism have the capacity to metabolize a large number of structurally diverse chemicals. This is due both to multiple forms of CYPs and to the capacity of a single CYP to metabolize structurally dissimilar chemicals. A single compound can be metabolized by multiple CYPs and CYPs can metabolize a single compound at multiple positions. This promiscuity of CYPs, which reflects their large and fluid substrate-binding pocket, comes at the price of relatively slow catalytic rates. Eukaryotic CYPs metabolize substrates at a fraction of the rate of more typical enzymes involved in intermediary metabolism and mitochondrial electron transfer. As a result, drugs generally have half-lives in the range of hours, while endogenous compounds (*e.g.*, steroid hormones) have half-lives of minutes.

The liver has the highest levels of xenobiotic-metabolizing CYPs, which also are found throughout the GI tract, and, in lower amounts, in lung, kidney, and the CNS. The most important CYPs for drug metabolism are those in the CYP2C, CYP2D, and CYP3A subfamilies. CYP3A4—the most abundantly expressed—is involved in the metabolism of ~50% of clinically used drugs (*see* Figure) and thus is susceptible to multiple drug interactions of clinical significance. However, although allelic variants of CYP3A4 that affect activity have been found, they are very rare and apparently do not play a major role in interindividual levels of enzyme expression. In contrast, several human CYP genes exhibit

polymorphisms, including *CYP2A6*, *CYP2C9*, *CYP2C19,* and *CYP2D6*, that play key pharmacogenetic roles.

CYP2C19

CYP2C19, originally designated mephenytoin hydroxylase, is highly polymorphic drug metabolizing enzyme. The deficient phenotype (poor metabolizers) is much more common in Chinese and Japanese populations. Several proton pump inhibitors, (*e.g.*, omeprazole and lansoprazole*),* are inactivated by CYP2C19. Thus, CYP2C19-deficient patients have higher exposure to active parent drug, a greater pharmacodynamic effect (higher gastric pH), and a higher probability of ulcer cure than individuals who are heterozygous or homozygous for the wild-type allele (Furota *et al.* 2004).



CYP2C9

Inactivating polymorphisms in *CYP2C9* are common—with 2% to 10% of most populations being homozygous for low-activity variants—and play important roles in determining therapeutic response and adverse effects of warfarin (*see* below).

CYP2D6

Approximately 15% to 25% of all drugs in clinical use are substrates for or inhibitors of CYP2D6, including β blockers, tricyclic antidepressants, antiarrhythmics, antipsychotics, selective-serotonin reuptake inhibitors, and opioids. Studies of multiple ethnic groups have defined seven variant alleles that account for >90% of "poor metabolizer", low-activity CYP2D6 alleles; the frequency of these variant alleles depends on geographic origin of a given individual. One especially common variant allele, CYP2D6*4, results from defective splicing and is found with an allele frequency of 12-21% in Caucasians. In contrast, a small percentage of individuals carry stable duplications of CYP2D6 (up to 13 copies of the active gene), resulting in an "ultra-rapid"

metabolizer phenotype. Phenotypic consequences of the deficient CYP2D6 phenotype include increased risk of toxicity of antidepressants or antipsychotics that are catabolized by CYP2D6 and lack of analgesic effect of codeine, which is activated by the enzyme. Conversely, the ultra-rapid metabolizers exhibit extremely rapid clearance and decreased efficacy of SSRIs used for depression.

Drug Transporters

Transporters are membrane proteins that control the influx of essential nutrients and ions and the efflux of cellular waste, environmental toxins, and other xenobiotics. Approximately 6% of genes in the human genome encode transporters or transporter-related proteins. Drug-transporting proteins contribute to both therapeutic and adverse effects of drugs. Moreover, polymorphisms in membrane transporters that play a role in drug response are yielding new insights in pharmacogenetics.

The two major classes of transporters that are relevant to pharmacology are the ATP binding cassette (ABC) superfamily and the solute carrier (SLC) transporters. Prominent members of the ABC family in pharmacology include P-glycoprotein, while prominent SLC members include SLC6A4, which encodes the serotonin transporter (SERT)—the target for the selective serotonin reuptake inhibitors.

P-glycoprotein

The most widely studied drug transporter is the P-glycoprotein, which is an ATP-binding cassette protein encoded by *ABCB1*. Polymorphisms in *ABCB1* are associated with responses to anticancer drugs (hence, its other name as multiple drug resistance-1, MDR1), antiviral agents, immunosuppressants, antihistamines, cardiac glycosides, and anticonvulsants. SNPs in *ABCB1* also have been associated with tacrolimus and nortriptyline neurotoxicity and susceptibility for developing ulcerative colitis, renal cell carcinoma, and Parkinson's disease. Although analysis of genetic variants of P-glycoprotein is not widely used, it is possible that developing approaches in cancer chemotherapy will make this an important gene for genetic analysis.

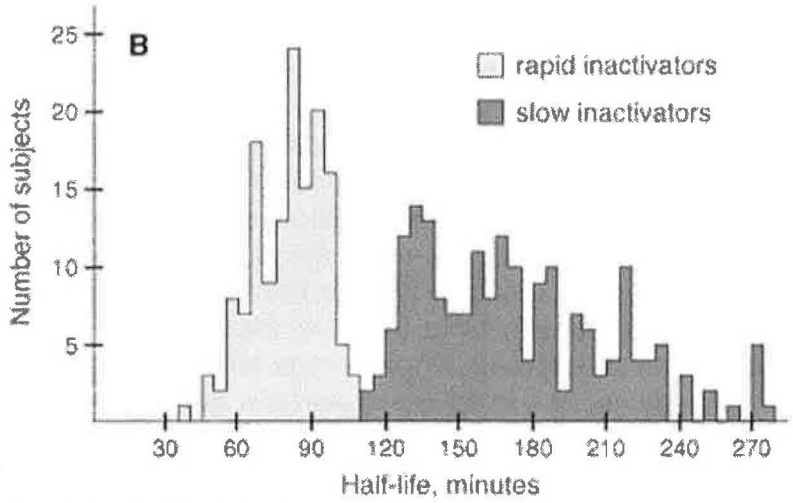
Pharmacogenetic Vignettes

Discussed below are several clinical vignettes that illustrate the potential benefits and limitations of pharmacogenetic testing in drug therapy. This discussion is by no means complete but rather is focused on a few examples.

Isoniazid

After isoniazid was introduced for the treatment of tuberculosis, toxicities were noted in 5-15% of patients. Patients with toxic effects were found to excrete large

amounts of unchanged drug and low amounts of acetylated isoniazid. Subsequent studies led to the classification of "rapid" and "slow" acetylators (Tiitinen, 1969).



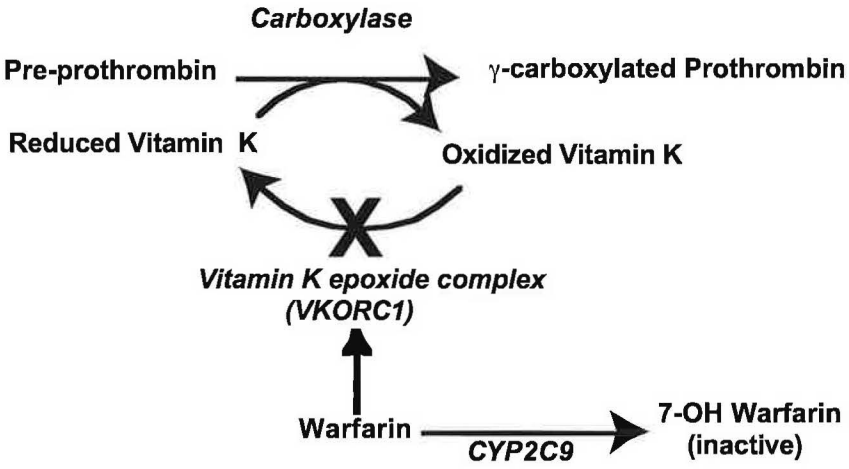
Molecular analysis of the cytosolic N-acetyltransferase 2 (*NAT2)* gene revealed polymorphisms that correspond to the "slow" and "fast" acetylator phenotypes. Polymorphisms in the *NAT2* gene and their association with the slow acetylation of isoniazid provided the first link between a pharmacogenetic phenotype and a genetic polymorphism in a Phase 2 enzyme.

Subsequent studies defined a family of enzymes—the N acetyl transferases (NATs)--that metabolize drugs and other xenobiotics containing an aromatic amine or hydrazine group. The addition of the acetyl group from the cofactor acetyl-coenzyme A often leads to a metabolite that is less water soluble because the ionizable amine is neutralized by covalent addition of an acetyl group. NATs are among the most polymorphic of all human xenobiotic drug-metabolizing enzymes. There are two functional NAT genes in humans, *NAT1* and *NAT2*. Over 25 allelic variants of *NAT1* and *NAT2* have been characterized, and homozygous genotypes for at least two variant alleles are required to predispose to lowered drug metabolism. Slow acetylation patterns are attributed mostly to *NAT2* polymorphisms, as seen with isoniazid and hydralazine, and may be associated with an increased risk of adverse effects (more convincing evidence is available for hydralazine than isoniazid, Gardiner and Begg, 2006).

Acetylator status appears to relate more directly to adverse effects (*e.g.*, drug-induced lupus) than to efficacy, but genetic testing for acetylation status has not and likely will not achieve widespread clinical use (Gardiner and Begg, 2006).

Warfarin

The oral anticoagulant warfarin is commonly employed for the chronic prevention of thromboembolic events; in the U. S., 21 million prescriptions were written for warfarin in 2003 (Marketos 2004). It inhibits an enzyme complex (vitamin K epoxide reductase) that recycles vitamin K and thereby interferes with clotting factors II, VII, IX, and X. Warfarin use is complicated by its narrow therapeutic window, considerable individual variation in drug response to a given dose, and potentially life-threatening consequences of either over- or undertreatment. Maintenance doses of warfarin must be adjusted to compensate for changes in diet or for concomitant use of many other medications (*e.g.*, many drugs affect protein binding, antibiotics can affect vitamin K metabolism by intestinal bacteria). Thus, the maintenance dose of warfarin ranges from 0.5 mg/day to >10 mg/day. These factors have prompted efforts to improve the warfarin dosage selection and thereby improve clinical outcome. Pharmacogenetic studies have focused on polymorphisms in two genes, one involved in warfarin metabolism (*CYP2D9*) and one representing the molecular target for warfarin action (*VKORC1*).



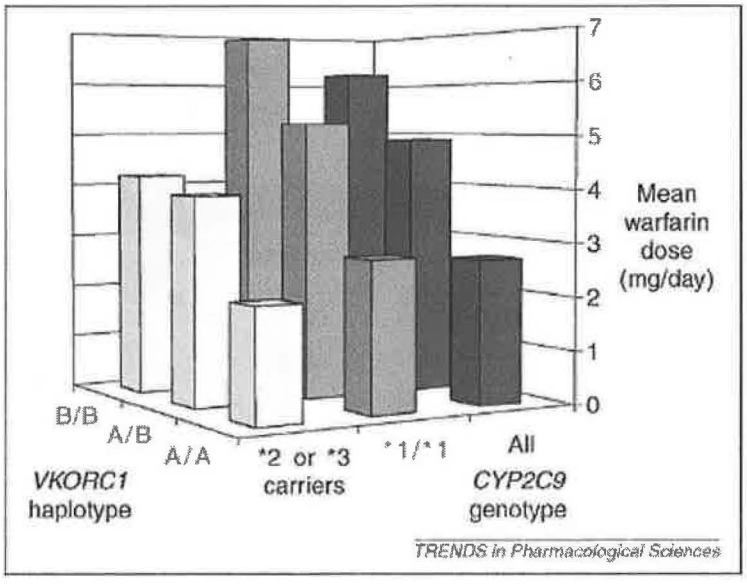
CYP2D9

One critical genetic factor in warfarin dosing is polymorphic variation in CYP2C9. In the liver, *S*-warfarin (the more active form of the racemic mixture given clinically) is metabolized by CYP2C9 to 7-hydroxywarfarin. Approximately 200 different SNPs in CYP2C9 have been described, some of which cause coding region variants. One common inactivating mutation is termed CYP2C9*3 (Ile359Leu), which is widely distributed among ethnic groups. A second inactivating mutation, CYP2C9*2 (Arg144Cys) is relatively common in Caucasians but absent in Asians. Thus, ~35% of Caucasians and 3% of African-Americans and Asians carry > 1 variant allele. There is a graded increase in warfarin sensitivity with increasing numbers of deficient alleles, such that individuals carrying 2 copies of the CYP2C9*3 allele need a warfarin dose only 20% of that typically used. In addition, studies have correlated CYP2C9

genotype with bleeding complications in patients who were initiated on the standard warfarin dose (Aithal *et al.*, 1999). Finally, in a rarity in pharmacogenetic studies, two small prospective trials have documented the utility of CYP2D9 genotype analysis in improving the prediction of warfarin dose at drug initiation (Hillman *et al.*, 2005; Voora *et al.*, 2005).

VKORC1

A second important factor in warfarin pharmacogenetics emerged from the recent discovery of the warfarin target gene, which encodes vitamin K epoxide reductase complex 1 (*VKORC1*). This complex recycles reduced vitamin K, which is essential for the post-translational γ-carboxylation of vitamin K–dependent clotting factors (II, VII, IX, and X). Several mutations in the VKORC1 protein have been discovered in rare patients with warfarin-resistance, but these mutations are not prevalent in the general population. However, common SNPs in VKORC1 are strongly associated with warfarin maintenance dose (Rieder *et al.*, 2005). For genotyping, these are arranged into haplotypes, or groups of SNPs that are in linkage disequilibrium and therefore cluster together on a given chromosome. These haplotypes have been divided into A and B subgroups, with the A haplotype correlating with increased warfarin sensitivity. If anything, the VKORC1 haplotypes are more predictive of warfarin dose than are the polymorphisms in CYP2D9, but combined analysis for both loci has greater predictive value than either locus alone (Beitelshees and McLeod, 2006).

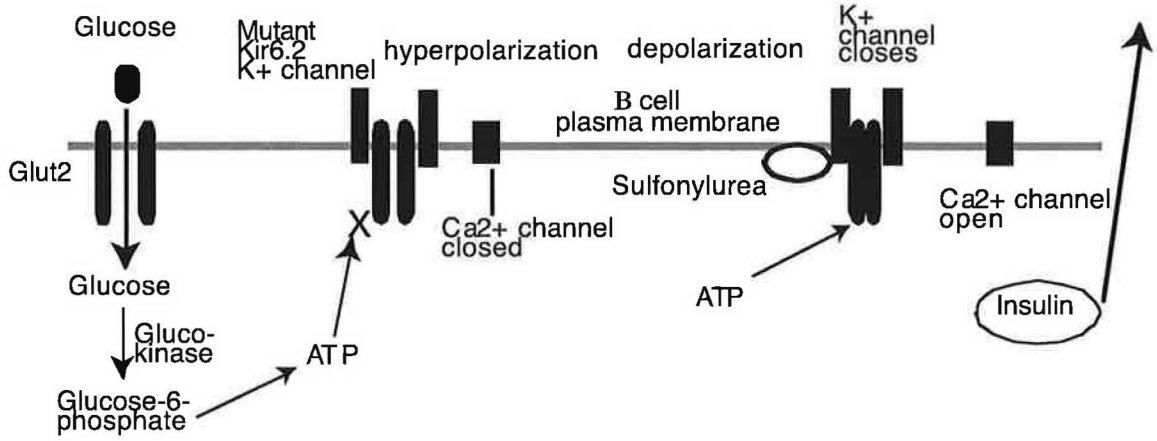


The ability to predict the appropriate dose of warfarin based on genetic analysis of the CYP2D9 and VKORC1 loci has led to recommendations that pharmacogenetic analysis should routinely be used in dosing patients with warfarin (*see* Haga *et al.*, 2006). Several companies provide genetic analysis and recommendation for dosing following ascertainment of genotype using leukocyte DNA in individual patients. Given that the value of such

pharmacogenetic analysis in improving efficacy, decreasing bleeding complications, or decreasing the expense of outpatient monitoring of therapeutic response has not been demonstrated in a prospective trial, a consensus has not yet been achieved. Nonetheless, this is one application of pharmacogenetics that likely will achieve widespread use in clinical practice (albeit not currently at UT Southwestern or Parkland Memorial Hospital).

Sulfonylurea Receptor and Type 1 Diabetes Mellitus

Although type 1 diabetes mellitus typically presents in childhood, some patients present as neonates with diabetic ketoacidosis or severe hyperglycemia. Approximately half of these patients have heterozygous activating mutations in *KCNJ11*--a component of the sulfonylurea-sensitive potassium channel--that inhibit the closing of the β-cell K_{ATP} channel in response to increased intracellular ATP (Gloyn *et al.* 2004; Sagen *et al.* 2004). Unlike typical patients with type 1 diabetes mellitus, these patients respond favorably to treatment with sulfonylureas and do not require insulin (Pearson *et al.* 2006). It has been proposed that this clinical response at least partly involves sulfonylurea-induced responsiveness of the β cell to incretins such as GLP-1. Because patients with other causes of neonatal diabetes mellitus (*e.g.,* homozygous mutations in glucokinase, mutations in IPX1 associated with pancreatic agenesis) and because a trial of sulfonylureas could lead to ketoacidosis in non-responding patients, it has been proposed that genetic analysis of KCNJ11 be routine part of the diagnosis and treatment of neonatal diabetes mellitus.

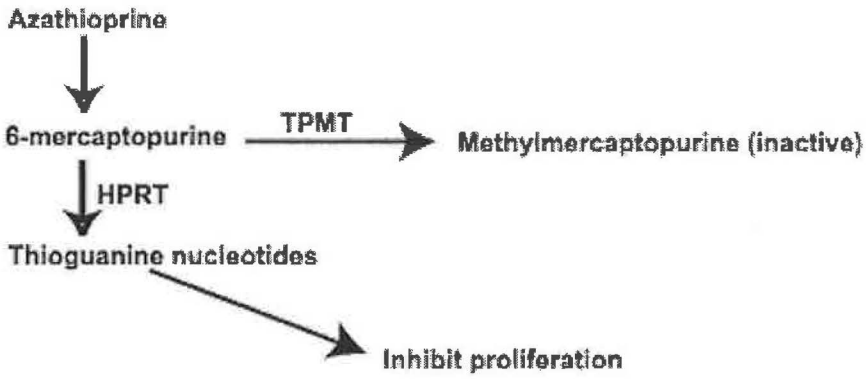


Thiopurines

The phase 2 methytransferases methylate both exogenous and endogenous compounds on O-, N-, and S-moieties. One member of this family, thiopurine methyltransferase (TPMT), catalyzes the S-methylation of the thiopurine drugs azathioprine and mercaptopurine. Azathioprine and mercaptopurine are used for

immunosuppression in autoimmune diseases (*e.g.*, inflammatory bowel disease, systemic lupus erythematosus, rheumatoid arthritis) or following transplantation. Mercaptopurine also is used for childhood acute lymphoblastic leukemia.

Azathioprine and mercaptopurine are prodrugs. Azathioprine is converted to mercaptopurine, which then has three potential fates. First, it can be metabolized to 6-thioguanine nucleotides that are the presumed active agents in impairing purine biosynthesis and inhibiting cell proliferation, a reaction catalyzed by HPRT. Alternatively, mercaptopurine can be metabolized by xanthine oxidase to the inactive thiourate. Finally, it can be methylated by TPMT to 6-methyl-mercaptopurine, which also is inactive.



TPMT is a polymorphic enzyme and three SNPs account for >90% of the inactivating alleles. In the U. S., one in 300 individuals is homozygous deficient, 10% are heterozygotes, and about 90% are homozygous for wild-type *TPMT* alleles. Because methylation of mercaptopurine by TPMT competes with activation of the drug to thioguanine nucleotides, the concentration of the active (but toxic) thioguanine nucleotides is inversely related to TPMT activity and directly related to the probability of pharmacologic effects (both efficacy and adverse effects). Dosage reduction from that appropriate for the "average" individual may be required to avoid toxic levels resulting in myelosuppression in 100% of homozygous deficient patients, 35% of heterozygotes, and only 7% to 8% of those with homozygous wild-type activity.

Because these drugs have a narrow therapeutic index, dosing by trial and error can place patients at higher risk of toxicity; thus, prospective adjustment of thiopurine dose based on *TPMT* genotype has been proposed, both for leukemia and for immunosuppression. Enzymatic assays for TPMT activity are already used clinically to identify individuals who are predisposed to thiopurine toxicity and should be given a lower dose, but are not informative in patients with recent blood transfusions and may give inaccurate results. As DNA-based analyses gain greater use clinically (the test is a Clinical Laboratory Improvement Amendments-certified molecular diagnostic), it is likely that this pharmacogenetic testing will become a standard part of the use of these drugs. One caveat is that

one retrospective analysis concluded that ~80% of adverse drug reactions were linked to factors other than the TPMT genotype (van Aken *et al.*, 2003).

Cancer Therapy

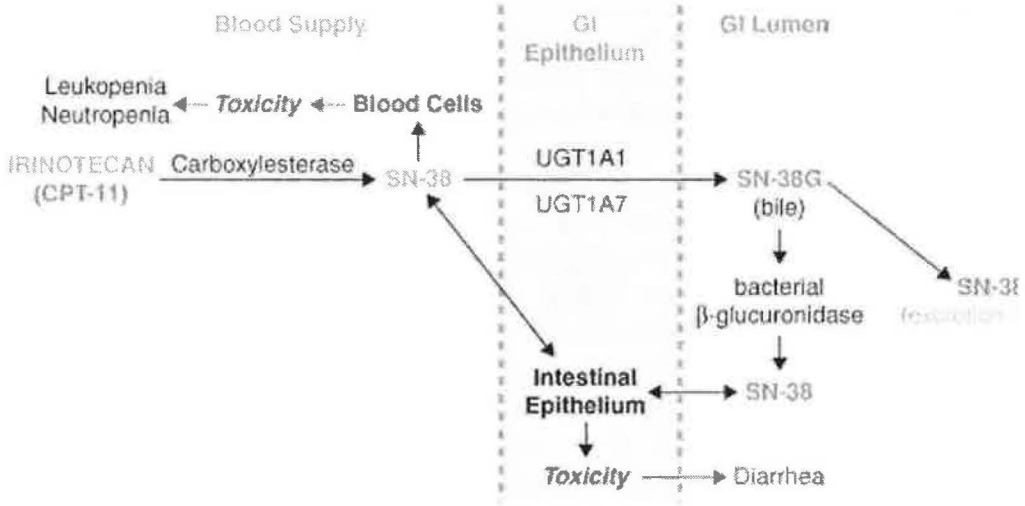
Pharmacogenetics rapidly is finding clinical utility in the chemotherapy of neoplastic disease. This relates partly to the highly complex regimens of different drugs used in cancer chemotherapy but also reflects the fact that we can consider cancer pharmacogenetics at two different levels: i) the polymorphisms in the patient in factors such as drug metabolizing enzymes, and transporters; ii) the varying genetic compositions that are seen in individual tumors due to somatic mutations.

Pharmacogenetics of Metabolism of Drugs Used for Cancer Chemotherapy

The thiopurines and TPMT are discussed above as an example in which genetic polymorphisms in drug metabolism enzymes alter cancer chemotherapy. Other situations in which pharmacogenetic analysis likely will be widely applied in the near future are described here.

Irinotecan

Irinotecan is a topoisomerase inhibitor that is used for solid tumors such as metastatic colorectal, ovarian, and non-small cell lung cancer. Its use is limited by a relatively high frequency (~25%) of severe diarrhea and neutropenia. Irinotecan is a prodrug that is metabolized to the active species SN-38 by serum and hepatic carboxylesterases. SN-38 is catabolized by the phase 2 enzyme uridine diphosphate-glucuronosyltransferase 1A1 (UGT1A1) and excreted into the bile. In the intestine, SN-38 glucuronide is cleaved by bacterial β-glucuronidase to liberate SN-38, which then is reabsorbed. Elevated levels of SN-38 in intestine lead to diarrhea while increases in blood lead to leukopenia.



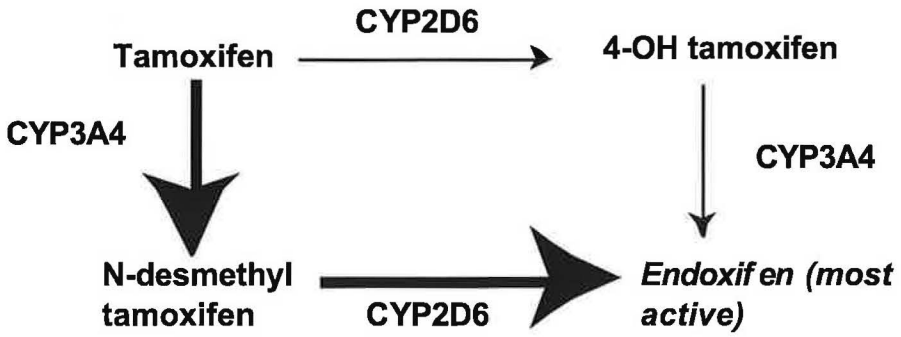
Loss-of-function mutations of *UGT1A1* cause Crigler-Najjar Syndrome. More commonly, the relatively mild Gilbert's syndrome results from differences in the *UGT1A1* gene promoter (extra TA dinucleotide repeats) that reduce the expression of UGT1A1. One variant, termed *UGTA1*28*, has 7 TA repeats in the 5'-flanking region. Subjects with the *UGT1A1*28* allele are predisposed to developing higher circulating and tissue levels of SN-38, increasing considerably the likelihood of severe neutropenia and diarrhea (reviewed by Maitland *et al.*, 2006). In one prospective European trial in which all patients received the standard dose of 350 mg/m^2, all patients with severe neutropenia had at least one copy of UGT1A1*28 and the incidence was 50% in subjects with 2 copies of this allele (Innocenti *et al.*, 2004).

The FDA has approved a genetic test—the Invader UGT1A1 Molecular Assay, Third Wave Technologies—and has revised the safely labeling for irinotcan to recommend that the dose be reduced in patients who are homozygous for the *UGT1A1*28* allele (Anonymous, 2006). The test costs ~250-500 dollars.

Tamoxifen

In breast cancer, tumor phenotyping based on expression of estrogen receptor and progesterone receptor has long served as a predictor of the likelihood of response to hormonal therapy (*e.g.*, tamoxifen, aromatase inhibitors). More recently, it has been recognized that polymorphism in a metabolic enzyme also plays a key role in determining tamoxifen efficacy.

Although tamoxifen has affinity for ER, its metabolites bind ER with roughly 100-fold higher affinity. Thus, the level of the endoxifen metabolite apparently is crucial for anti-tumor effect. Recent studies suggest that up to 7-10% of women with breast cancer may have CYP2D6 alleles (especially CYP2D6*4) that decrease tamoxifen efficacy by impaired conversion to the more active metabolite (Goetz *et al.*, 2005). In October, 2006, a FDA panel agreed that CYP2D6 polymorphisms are a predictor of drug efficacy and recommended revision of the product labeling to note that a genetic test is available for CYP2D6 that may impact dosing.



Given the complexity of combination chemotherapy regimens, pharmacogenetics undoubtedly will play an increasing role in adjusting drug dosing to maximize efficacy while diminishing adverse effects. An example of the way that pharmacogenetics can be incorporated into therapeutic regimens for cancer is provided below. Without pharmacogenetics, 3 different combination regimens are used, depending on tumor stage. The incorporation of a single polymorphism in a major drug-metabolizing enzyme for each of three drugs (thymidylate synthetase for methotrexaste, MDR-1 for paclitaxel, and glutathione transferase for cyclophosphamide) can identify 8 different genotypes that will then be treated with one of 11 different therapeutic regimens (thanks to Mary Relling, St. Jude's).

A

Cancer X

Stage I	Stage II	Stage III	Stage IV
Regimens:			
Surgery Only	LD MTX LD Paclitaxel	HD MTX HD Paclitaxel	HD MTX HD Paclitaxel HD Cyclophosphamide

3 drug regimens

B

Each Gene Has 2 Major Alleles
Each Drug Affected by 1 Polymorphism

Multilocus Genotype	*TYMS* Low	*TYMS* High	*MDR1* Low	*MDR1* High	*GSTM1* Low	*GSTM1* High
A	X		X		X	
B	X			X	X	
C	X		X			X
D	X			X		X
E		X	X		X	
F		X		X	X	
G		X	X			X
H		X		X		X

C

Use Genotypes to Individualize Drug Regimens for Cancer X

Multilocus Genotype	Stage II MTX	Stage II Paclitaxel	Stage II Cyclo	Stage III MTX	Stage III Paclitaxel	Stage III Cyclo	Stage IV MTX	Stage IV Paclitaxel	Stage IV Cyclo
A	Low	Low	0	Low	Low	0	Low	Low	Low
B	Low	High	0	Low	High	0	Low	High	Low
C	Low	Low	0	Low	Low	0	Low	Low	High
D	Low	High	0	Low	High	0	Low	High	High
E	High	Low	0	High	Low	Low	High	High	Low
F	High	High	0	High	High	Low	High	High	High
G	High	Low	0	High	Low	Low	High	High	Low
H	High	High	0	High	High	Low	High	High	High

3 drug regimens expand to 11 regimens

Pharmacotherapy of Cancer Based on Tumor Genetics

In addition to personalized cancer chemotherapy based on the genetics of the individual patient (*e.g.*, irinotecan, mercaptopurine, tamoxifen), the identification of somatic mutations in specific genes in cancer cells also provides the potential for personalized chemotherapy. As noted above, expression analyses of ER and PR are a standard part of therapeutic decisions, but increased application of

microarray analyses to other tumors is expanding the apparent utility of tumor pharmacogenetics.

Tyrosine Kinase Inhibitors

Specific somatic mutations in tumors can play a role in increased tumor cell proliferation or avoidance of apoptosis. The availability of drugs that specifically inhibit these tyrosine kinases has revolutionized the therapy of a subset of these tumors and has led to much greater emphasis on identifying genetic profiles that will identify those tumors that are most likely to respond to specific drugs. The data that have provided proof-of-principle are discussed below, followed by a discussion of new directions to extend such efforts.

The major breakthrough in the concept that cancer could be controlled by impinging on signal transduction pathways driving tumor proliferation came with imatinib (GLEEVEC). Imatinib inhibits activated forms of the ABL, KIT, or PDGFR receptors, which play dominant roles in driving tumor growth, either due to fusion with another protein or point mutations. Thus, imatinib shows remarkable therapeutic benefits in patients with chronic myelongenous leukemia (BCR-ABL), gastrointestinal stromal tumors (KIT mutation positive), chronic myelomonocytic leukemia (EVT6-PDGFR), hypereosinophilic syndrome (FIP1L1-PDGFR), and dermatofibrosarcoma protuberans (constitutive production of the ligand for PDGFR). The situation in GIST is particularly instructive, as patients with an exon 11 mutation of KIT have a significantly higher partial response rate (72%) than those with no detectable KIT mutations (9%). Thus, KIT mutational status predicts response and some centers are now routinely looking for somatic mutations in KIT to identify patients who are more likely to respond (Sequist *et al.* 2006).

EGF receptor and lung cancer

Some patients with non-small cell lung cancer–typically non-smoking women with adenocarcinoma—have somatic mutations that result in constitutive activation of the EGF receptor (Paez *et al.*, 2004; Lynch *et al.*, 2004). As recently reviewed (Rossell *et al.*, 2006). two mutations account for >90% of these mutations. Most common are in-frame deletions of nucleotides in exon 19 that affect amino acids in the tyrosine kinase domain. Alternatively, a Leu858Arg missense mutation is found in exon 21. These patients have demonstrated dramatic responses to the EGF receptor kinase inhibitors gefitinib (IRESSA) and erlotinib (TARCEVA), and a commercial assay is now available to identify patients with such mutations who are more likely to respond to these drugs (Couzin, 2004). At UT Southwestern, testing for EGFR mutations is limited to non-smokers, Asian women, and younger patients with light smoking histories, groups in which mutations in the EGFR are most frequently found (Shigematsu *et al.*, 2005).

HER2/neu and breast cancer

The monoclonal antibody trastuzumab (HERCEPTIN) targets the HER2/neu (ErbB2) member of the EGF family of protein tyrosine kinase receptors. Activation of HER2/neu enhances metastatic potential and inhibits apoptosis. HER2/neu is over-expressed in up to 30% of breast cancers and is associated with clinical resistance to cytotoxic and hormonal therapies.

Trastuzumab is approved for HER2/neu over-expressing metastatic breast cancer, either as initial treatment in combination with paclitaxel or as monotherapy following chemotherapy relapse. Thus, analysis of HER2 status is used routinely in making therapeutic decisions for breast cancer. Although immunohistochemical assays have traditionally been used, a FISH assay that looks for HER2 amplification is becoming the more favored assay to assess HER2/neu status (including at UT Southwestern).

In November, 2006, the FDA expanded the use of trastuzumab, approving its use in combination with other cancer drugs, for the treatment of HER2 positive breast cancer after surgery (lumpectomy or mastectomy). The approval was based on data from 2 NCI-sponsored trials in which the addition of trastuzumb significantly decreased breast cancer relapses in this setting.

Global Gene Profiling in Cancer Therapy

In addition to pharmacogenetic testing for specific somatic mutations, techniques are now available to examine the complete cohort of genes that are expressed by human tumors, and several studies have attempted to correlate tumor behavior with patterns of gene expression. For breast cancer, an important issue has been differentiating between tumors that are ER positive, as about 40% of these individuals do not respond to hormonal therapy and have a more aggressive tumor progression. A number of groups have tried to use microarray analyses to identify genes whose expression pattern correlated with tumor behavior. These studies have been hampered by the retrospective nature of most of the studies and by differing results obtained by different groups studying distinct groups of patients (Lo *et al.*, 2006). In one encouraging finding, a retrospective use of 5 different tumor progression models showed an excellent correspondence between four of the five models in predicting tumor prognosis (Fan *et al.*, 2006), even though these programs contained almost no shared genes. To the extent that this finding is confirmed, it suggests that microarray analyses will add new insights into tumor prognosis and appropriate therapy that extend beyond the current parameters of tumor stage and grade, ER and PR positivity, and HER2 amplification (Bild *et al.*, 2006).

Non-medical Issues

One important issue to consider is the cost of genotyping. For example, severe neurotoxicity with 5-fluorouracil has been associated with mutations in dihydropyrimidine dehydrogenase (DPD) that cause reduced enzyme activity (Tuchman *et al.* 1985). In the general population, however, DPD alleles

associated with impaired activity are rare (<1%), and it therefore may not be cost-effective to use genetic testing for DPD alleles in the general population. This is one example of a genetic test that is routinely employed at UT Southwestern.

Even when the tests are directed at polymorphisms that play important roles in the general population, cost benefit issues may become important. Using DNA obtained from cheek swabs, one company offers genetic testing for the most important polymorphic enzymes CYP2D6, CYP2C9, and CYP2C19 ($250 each or $600 for all three). Also available is a combination test for warfarin dosing that includes CYP2C9 and VKOR1C ($550), as well as tests for CYP1A2 and NAT2 ($1000 total). In none of these cases has it been shown that the use of genetic testing is cost effective in terms of lowering the costs of adverse effects,

In addition to questions about clinical benefit of routine genotyping for the known pharmacogenetic markers and cost-benefit issues, important "non-medical" issues also must be considered. For example, the increased information provided by genotyping may also become relevant to issues such as disease susceptibility, long-term prognosis, and other factors. Mechanisms to protect this information from employers and health insurance companies are an important issue for both pharmacogenetics and for genetic testing in general.

Future Directions

One lesson for the future is that relatively rare pharmacogenetic variations associated with adverse drug reactions may also have important ramifications for the rest of the population. For example, the role of *TPMT* polymorphisms in adverse reactions to thiopurines is described above. A wider utility beyond avoiding these relatively rare events emerged with the finding that ALL patients who are homozygous for the fully active *TPMT* allele had a lower initial response rate to standard chemotherapy with mercaptopurine than did heterozygous subjects (Stanulla *et al.* 2005). These data imply that the subset of patients with more active metabolism of mercaptopurine to inactive compounds had lower antitumor efficacy.

Despite striking successes in identifying specific genetic polymorphisms associated with drug phenotypes, it also has become apparent that the responses to many drugs are highly complex and cannot be explained by single genes in a manner that readily translates to important endpoints. Ideally potentially interacting genes for a given drug should be analyzed as integrated sets (*e.g.*, the genes encoding a receptor complex, other genes that influence the pathophysiology of a given disease, and drug-metabolizing enzymes). For example, the effect of specific genes on drug response in congestive heart failure might include genes that influence drug action (*e.g.*, β–adrenergic receptor), metabolism (*e.g*, cytochrome P450 enzymes, drug transporters), and various components of the renin-angiotensin system (*e.g.*, renin, angiotensinogen, the angiotensin receptor). By considering these functionally related genes as a

cluster, each of which may only have a minor quantitative effect on its own, it may be possible to define discrete subsets of patients that are most effectively treated with one agent versus other available drugs.

Pharmacogenomics is one strategy that has been proposed to expand our repertoire of genetic polymorphisms that influence drug response (Weinshilboum and Wang, 2004). The human genome is highly variable, such that single nucleotide polymorphisms (SNPs) and insertions/ deletions are distributed throughout the genome. These often are not randomly distributed, such that haplotypes can be characterized by single SNPs that will also predict polymorphisms at linked regions. Increasingly robust technologies are being developed to interrogate sets of single-nucleotide polymorphisms (SNPs) across the entire genome, and such whole genome association approaches have been applied to identify new disease susceptibility genes and pathways. A similar genome-wide approach may identify novel genes that determine the drug response phenotype.

To facilitate the application of pharmacogenetics and pharmacogenomics, the NIH initiated the Pharmacogenetics Research Network to investigate the relationship of genetic variation to variable drug response, to bring together investigators working in this field; and to provide a publicly available database linking drug phenotypes and genotypes. This database is found at www.pharmgkb.org.

Drug companies also are incorporating pharmacogenetics into the drug development process. Based on the appreciation of the role of CYP2D6 polymorphisms in drug metabolism, most pharmaceutical companies routinely use cell based assays to identify drugs that are metabolized predominantly by this pathway; such drugs generally are disfavored for further development (Weinshilboum and Wang, 2004). In an approach called efficacy pharmacogenetics, subjects in phase IIA studies are subgrouped by pharmacogenetic approaches in an attempt to identify subgroups that would be predicted to be responders versus non-responders. By selecting out these patients, phase III trials to document efficacy can then include fewer subjects, making them faster and less expensive to conduct (Roses, 2004).

Surveillance for Adverse Drug Reactions

Following testing in the 3 phases of clinical trials, a new drug may gain FDA approval for use in the U. S. Although this process typically takes from 2-10 years, only a few thousand selected patients will receive the drug during phase III trials, often for durations of only 3 to 6 months. It is inevitable that adverse effects that are rare (*e.g.*, < 1 in 1000 patients) or delayed will not be apparent prior to marketing. Similarly, adverse effects that are associated with drug interactions or limited to selected populations such as children or the elderly will often be missed. For these reasons, the FDA relies on postmarketing surveillance by

clinicians to provide warning that such adverse effects may be associated with a given drug. Such efforts are particularly important for newly approved drugs, whose adverse effects may have been missed in clinical trials for the reasons noted above. This reporting system—called MEDWATCH--is voluntary in the U. S. (legally mandated systems exist in Canada, the United Kingdom, and other European countries). However, many clinicians in the U. S. are unaware of this reporting system for postmarketing surveillance of adverse drug effects. Forms to report adverse effects can be obtained 24 hours a day from the FDA by calling (800)-FDA-1088, or can be reported at www.fda.gov/medwatch. Even with ongoing improvements in our understanding of pharmacogenetics and adverse drug reactions, this will continue to be an important role for the astute clinician.

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