0031-6997/11/6302-437–459$25.00
PHARMACOLOGICAL REVIEWS
U.S. Government work not protected by U.S. copyright
Pharmacol Rev 63:437–459, 2011

Vol. 63, No. 2
3533/3680085
Printed in U.S.A.

ASSOCIATE EDITOR: DAVID R. SIBLEY

Pharmacogenetics, Pharmacogenomics, and Individualized Medicine

Qiang Ma and Anthony Y. H. Lu

Receptor Biology Laboratory, Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, West Virginia (Q.M.); and Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers University, Piscataway, New Jersey (A.Y.H.L.)

***Abstract*——Individual variability in drug efficacy and drug safety is a major challenge in current clinical practice, drug development, and drug regulation. For more than 5 decades, studies of pharmacogenetics have provided ample examples of causal relations between genotypes and drug response to account for phenotypic variations of clinical importance in drug therapy. The convergence of pharmacogenetics and human genomics in recent years has dramatically accelerated the discovery of new genetic variations that potentially underlie variability in drug response, giving birth to pharmacogenomics. In addition to the rapid accumulation of knowledge on genome-disease and genome-drug interactions, there arises the hope of individualized medicine. Here we review recent progress in the understanding of genetic contributions to major individual variability in drug therapy with focus on genetic variations of drug target, drug metabolism, drug transport, disease susceptibility, and drug safety. Challenges to future pharmacogenomics and its translation into individualized medicine, drug development, and regulation are discussed. For example, knowledge on genetic determinants of disease pathogenesis and drug action, especially those of complex disease and drug response, is not always available. Relating the many gene variations from genomic sequencing to clinical phenotypes may not be straightforward. It is often very challenging to conduct large scale, prospective studies to establish causal associations between genetic variations and drug response or to evaluate the utility and cost-effectiveness of genomic medicine. Overcoming the obstacles holds promise for achieving the ultimate goal of effective and safe medication to targeted patients with appropriate genotypes.**

Address correspondence to: Q. Ma, Receptor Biology Laboratory, Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, 1095 Willowdale Rd., Morgantown, WV 26505. E-mail: qam1@cdc.gov

This article is available online at http://pharmrev.aspetjournals.org.
doi:10.1124/pr.110.003533.

I. Introduction

In a large patient population, a medication that is proven efficacious in many patients often fails to work in some other patients. Furthermore, when it does work, it may cause serious side effects, even death, in a small number of patients. Although large individual variability in drug efficacy and safety has been known to exist since the beginning of human medicine, understanding the origin of individual variation in drug response has proven difficult. On the other hand, the demand to overcome such variation has received more attention now than ever before. It is well documented that large variability of drug efficacy and adverse drug reactions in patients is a major determinant of the clinical use, regulation, and withdrawal-from-market of clinical drugs and a bottleneck in the development of new therapeutic agents.

Factors that cause variations in drug response are multifold and complex, some of which involve fundamental aspects of human biology, because a drug response directly affects well being and survival (Table 1). Genetic variation in humans was recognized as an important determinant of individual variability of drug response from clinical observations in late 1950s (Kalow and Staron, 1957; Kalow and Gunn, 1959; Evans et al., 1960). In these cases, patients with very high or low plasma or urinary drug concentrations that correspond to a specific phenotype of a drug response were identified, and the biochemical traits leading to the variation of drug concentrations were found to be inherited. The observation that individual variation of a drug response is often larger among members in a population (population variability) than within the same person at different times (intrapatient variability) further supports inheritance as a major determinant of drug response (Vesell, 1989; Kalow et al., 1998). These clinical and population-based findings fostered the formation of pharmacogenetics to specifically address genetic contribution to individual variability in drug therapy.

TABLE 1
Major factors affecting individual drug response

Factors	Effects
Genetic Factors	Major variables; stable and inherited
Therapeutic targets	Drug efficacy (pharmacodynamics)
Drug-metabolizing enzymes	Drug metabolism (pharmacokinetics)
Drug transporters	Drug disposition (pharmacokinetics)
Targets of adverse drug reactions	Drug toxicity (pharmacodynamics and pharmacokinetics)
Factors with indirect effects	Drug efficacy, pharmacokinetics, and toxicity
Other Factors	Mostly transient
Environmental factors	Drug efficacy, pharmacokinetics, and toxicity
Environmental chemicals, coadministered drugs, tobacco smoking, alcohol drinking, and dietary constituents	
Physiological factors	Drug efficacy, pharmacokinetics, and toxicity
Age, sex, disease state, pregnancy, exercise, circadian rhythm, and starvation	

The human genome sequence provides a special record of human evolution that varies among populations and individuals. Sequence variations in drug target proteins, drug-metabolizing enzymes, and drug transporters can alter drug efficacy, drug side effects, or both to cause variable drug responses in individual patients (Lu, 1998; Meyer, 2000; Evans and McLeod, 2003; Weinshilboum, 2003a; Evans and Relling, 2004; Eichelbaum et al., 2006; Lin, 2007; Lu and Ma, 2010). From this prospect, the availability of the complete human genome sequence has made it possible to analyze the impact of variations of the human genome sequence on the pathogenesis of important diseases and the response to drug therapy at an accelerating rate in recent years. The rapid accumulation of knowledge on genome-disease and genome-drug interactions has also impelled the transformation of pharmacogenetics into a new entity of human genetics—pharmacogenomics—and, at the same time, provided a rationale for the hope that individualized medicine can be achieved in the near future.

It is evident that both pharmacogenomics and individualized drug therapy are increasingly influencing medicine and biomedical research in many areas, including clinical medicine, drug development, drug regulation, pharmacology, and toxicology, a thematic reflection of the postgenomic era of today's medicine. This article is intended to provide a comprehensive review of recent progress in the understanding of the basis of individual variability of drug efficacy and adverse drug reactions with focus on genetic polymorphisms of drug targets, drug-metabolizing enzymes, drug transporters, and targets of drug toxicity. Translation of pharmacogenetics and pharmacogenomics into clinical practice and drug

[1]Abbreviations: ABC, ATP-binding cassette; ACE, angiotensin I-converting enzyme; AD, Alzheimer's disease; apo, apolipoprotein; BCR-ABL, breakpoint cluster region-Abelson kinase fusion protein; BCRP, breast cancer resistance protein; Calu/CALU, calumenin; CBZ, carbamazepine; CCR, C-C chemokine receptor; CML, chronic myeloid leukemia; D, deletion; DHR, drug hypersensitivity reaction; DILI, drug-induced liver injury; DMPK, drug metabolism and pharmacokinetics; EM, extensive metabolizer; ER, estrogen receptor; FDA, U.S. Food and Drug Administration; GGCX, γ-glutamyl carboxylase; GIST, gastrointestinal stromal tumor; HLA, human leukocyte antigen; Hsp70, 70-kDa heat-shock protein; I, insertion; INR, international normalized ratio; KCNE, potassium voltage-gated channel, Isk-related family, member 3; LDL, low-density lipoprotein; LQTS, long QT syndrome; MHC, major histocompatibility complex; NAT, *N*-acetyltransferase; OATP, organic anion-transporting polypeptide; P450, cytochrome P450; PDGFR, platelet-derived growth factor receptor; Pgp, P-glycoprotein; PM, poor metabolizer; SCFR, mast/stem cell growth factor receptor; SJS, Stevens-Johnson syndrome; SN-38, 7-ethyl-10-hydroxycamptothecin; SNP, single-nucleotide polymorphism; TEN, toxic epidermal necrosis; TNF, tumor necrosis factor; TPMT, thiopurine *S*-methyltransferase; UGT, UDP-glucuronosyltransferase; UM, ultrarapid metabolizer; VKOR, vitamin K epoxide reductase; VKORC1, VKOR complex subunit 1.

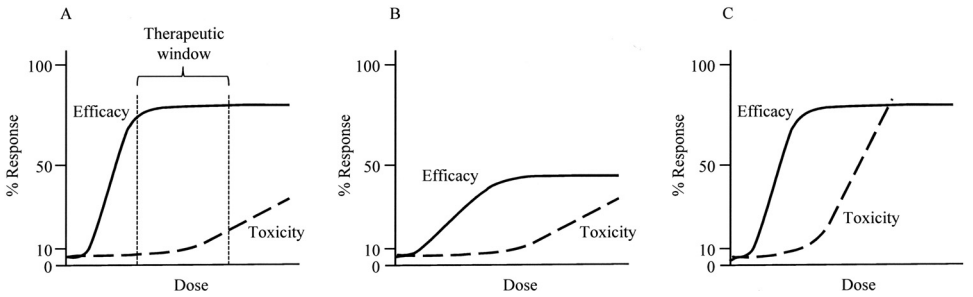

FIG. 1. Illustration of dose curves of drug response. A, dose-dependent increase in drug therapeutic effect and toxicity. A therapeutic window defines the dosage between a therapeutic effect and an apparent adverse reaction. Factors may alter the dose curve of drug therapeutic effect (B) or toxicity (C) to affect a drug response in individual patients.

development and the challenges of reaching the goal of individualized medicine are also discussed.

II. Drug Response and Inheritance

A. Individual Variability in Drug Therapy

Drug efficacy and adverse drug reactions dose-dependently determine the clinical outcome of a medication (Fig. 1). A higher dose boosts drug therapeutic effect but simultaneously increases the propensity for new or greater undesirable side effects. The drug dosage, between its therapeutic effect and apparent adverse reaction, defines the therapeutic window. For many drugs, the optimum dose required for effective and safe therapy varies significantly from patient to patient. A drug dose within the therapeutic window for the majority of a patient population can be too low or too high for a small number of patients who have an atypical dose-response curve for a drug therapeutic effect, toxicity, or both, resulting in unexpected, undesirable outcomes in the patients. Individual variability generally has a larger impact on drugs that have a narrow therapeutic window than those with a wide one. For example, warfarin is a mainstay anticoagulant for the treatment of thromboembolic diseases but has a very narrow therapeutic dose range and potentially life-threatening side effects. Moreover, the required daily dose of warfarin for inhibition of thrombosis and embolism in many disease conditions varies up to 20- to 30-fold from patient to patient. Therefore, frequent blood coagulation testing in patients who receive warfarin therapy is mandated to achieve effective and safe anticoagulation (Rettie and Tai, 2006).

The clinical use of simvastatin, a member of the statin class of HMG-CoA reductase inhibitors, to lower blood cholesterol level provides another example that demonstrates large and dose-dependent individual variations in drug efficacy and drug safety. In a cohort of 156 subjects with low-density lipoprotein (LDL[1]) cholesterol levels of >160 mg/dl, 6 weeks of simvastatin at doses of 40, 80, and 160 mg/day resulted in median reduction of LDL cholesterol by 41, 47, and 53%, respectively, demonstrating that simvastatin is highly effective in reducing LDL cholesterol in the majority of the patients (Table 2) (Davidson et al., 1997). However, the range of reduction at all doses is quite large because approximately 5% of patients had little or no reduction in LDL cholesterol levels, even at the dose of 160 mg/day. Furthermore, as the dose increases, a few subjects exhibited elevated plasma alanine aminotransferase activities at doses of 80 and 160 mg/day (0.7 and 2.1%, respectively) indicating liver damage. One subject developed myopathy (0.7%) at 160 mg/day. Why simvastatin failed to lower LDL cholesterol but caused liver and muscle toxicities in a small number of patients is not clear. Recent studies suggest that genetic polymorphisms of HMG-CoA reductase and drug transporters, such as organic anion-transporting polypeptide (OATP) 1B1, OATP-C, and ATP-binding cassette transporter (ABC) G2, which regulate hepatic uptake or efflux of statins and statin metabolites, contribute to the variability in the efficacy and side effects of the cholesterol-lowering drugs (Chasman et al., 2004; Mwinyi et al., 2004; SEARCH Collaborative Group, 2008; Tomlinson et al., 2010).

TABLE 2
Dose-dependent efficacy and toxicity of 6-week simvastatin

Data from Davidson et al. (1997). Cholesterol change calculated from baseline LDL cholesterol of ~199 mg/dl (n = 147). Values in parentheses indicate S.D. (second column) and percentages (fourth and fifth columns).

Dose and No. of Patients	LDL Cholesterol Change		Patients with	
	Median	Range	Myopathy	ALT Elevation
	%		n (%)	
40 mg/day (n = 141)	−41 (−48 to −32)	−64–11	0	0
80 mg/day (n = 144)	−47 (−53 to −37)	−65–30	0	1 (0.7)
160 mg/day (n = 140)	−53 (−59 to −45)	−71–19	1 (0.7)	3 (2.1)

ALT, alanine aminotransferase.

B. Factors Affecting Individual Drug Response

Genetic and nongenetic factors affect individual variability of a drug response by modulating the dose-response curves of drug efficacy and drug toxicity of patients. Clinical outcome is altered if drug dose is not adjusted accordingly (Fig. 1). Genetic factors generally cause permanent changes in protein functions, whereas environmental and physiological factors and their impact on drug response are transient in most cases (Table 1). In the former case, individual variations are stable over the course of a lifetime and are inherited through germline transmission, but a drug response modulated by a nongenetic factor often returns to a normal state after the factor is corrected or removed.

Genetic polymorphisms of proteins involved in drug targeting (i.e., pharmacodynamics) and drug metabolism and transport (i.e., pharmacokinetics) are likely to be the most important sources of individual variability in drug efficacy (Table 1). Drug target responsible for an adverse drug reaction can be the same as, or different from, the therapeutic target of the drug, resulting in on-target or off-target side effects. Variations in drug pharmacokinetics alter the concentration of a toxic drug or metabolite in the target tissue to cause variable toxicity. Some genetic variations affect drug efficacy and drug safety indirectly by modulating the biological context in which a drug reaction occurs.

At the molecular level, genetic variations can change the structure of a target protein via mutations in the coding region of the gene or the amount of the protein expressed by modulating gene regulation, both of which ultimately alter the function of the protein or the rate and kinetic constants in the case of an enzyme. Mutations can also modulate gene expression by way of epigenetic regulation. Structural changes of receptors or enzymes may affect drug-receptor or drug-enzyme interaction and, consequently, drug response. Genetic polymorphisms of drug-metabolizing enzymes and transporters can affect the absorption, distribution, metabolism, and elimination of drugs and thereby modulate their plasma and target tissue concentrations. Defective DNA repair enzymes reduce the ability of cells to repair mutations induced by alkylating chemotherapeutic agents. Mutations that alter the structure or reduce the amount of the enzymes involved in the biosynthesis of glutathione are likely to reduce the intracellular content of glutathione, which is critical in protecting cells from oxidative stress and reactive intermediates commonly encountered in adverse drug reactions.

Environmental chemicals, coadministered drugs, dietary constituents, tobacco smoking, and alcohol use are all known to induce or inhibit P450s, other drug-metabolizing enzymes, and drug transporters; to alter drug efficacy; and to induce drug-drug and drug-chemical interactions and drug side effects. Large individual variability in the induction and inhibition of human P450 enzymes has been well documented (Lin and Lu, 2001; Ma and Lu, 2003). Environmental factors may also interact with drug targets to produce antagonism or synergy with drugs to alter drug therapeutic effects or toxicity. Physiological factors, including age, sex, disease states, pregnancy, exercise, starvation, and circadian rhythm, can also contribute significantly to individual variations of the pharmacokinetic and pharmacodynamic properties of administered drugs. Some physiological traits are also genetic—generally polygenic in nature, such as sex, body weight, and susceptibility to chronic diseases.

C. Human Genetics in Drug Response

Early clinical examples of genetic influence on drug response involved variations in single genes (i.e., monogenic inheritance) in which polymorphisms of a single gene encoding a drug-metabolizing enzyme responsible for the metabolism and disposition of a substrate drug caused aberrant response to the drug. The phenotypic variation can be dramatic, especially when no alternate pathway exists to perform the same function. Exemplary studies include the identification of an atypical form of butyrylcholinesterase (pseudocholinesterase) as the cause of prolonged muscle paralysis and apnea by the muscle relaxant succinylcholine (Kalow and Gunn, 1959); the phenotyping of thiopurine *S*-methyltransferase (TPMT) to identify patients with cancer with low activity in methylating toxic anticancer drugs, an inactivation pathway of the drugs (Evans and McLeod, 2003; Weinshilboum, 2003a); the identification of "slow acetylators" (*N*-acetyltransferase 2 or NAT2 variants) for isoniazid acetylation in the treatment of tuberculosis (Evans et al., 1960; Weber, 1987); and the phenotyping of "poor metabolizers" of CYP2D6 for debrisoquine hydroxylation (Eichelbaum et al., 2006).

In these examples, the so-called "traditional approach" was used to establish a genotype-phenotype connection in three steps: identifying individual phenotypes (normal or extensive metabolizers versus poor or slow metabolizers) by measuring drug levels in the urine or plasma before the genetic mechanism was known; establishing a correlation between drug pharmacokinetics and drug response (efficacy or toxicity); and finally, identifying the genetic defects that account for the low or lack of the enzyme activity years later. Although this process is generally slow and tedious, the outcome of the genetic polymorphism studies of drug-metabolizing enzymes is often clinically significant and meaningful. On the other hand, numerous variants of drug-metabolizing enzymes have been discovered since the completion of the human genome project. With few exceptions, however, the significance of these genetic variations in pharmacokinetics and clinical outcome of drug therapy has not been established. Therefore, even though the DNA sequence-based analysis of variants is much faster than the classic approach in identifying new variants, estab-

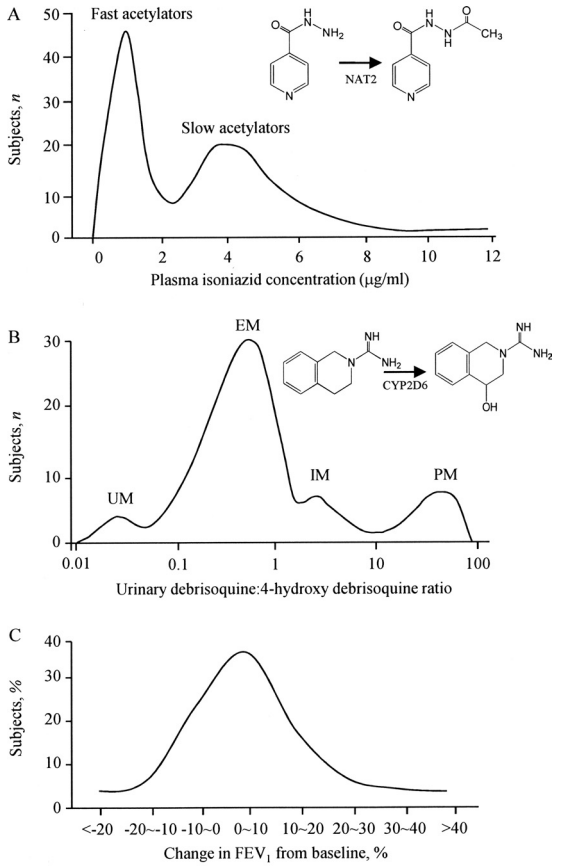

FIG. 2. Simulated distribution of drug response phenotypes. A, bimodal distribution of plasma isoniazid concentrations as a result of polymorphisms of *NAT2* in subjects receiving isoniazid. Simulation based on information from Evans et al. (1960). B, Multimodal distribution of urinary debrisoquine–to–4-hydroxydebrisoquine ratio as a result of polymorphisms of *CYP2D6* in subjects taking debrisoquine. Simulation based on information from Dahl et al. (1995). C, broad distribution of changes in forced expiratory volume in 1 s (FEV_1) in subjects given steroids for antiasthmatic therapy. Simulation based on information from Tantisira et al. (2004).

lishing the impact of the newly identified genetic variants from genomic studies on drug therapy remains a major challenge.

Monogenic inheritance may exhibit gene dosage effects that manifest in the phenotypic distribution of genetic polymorphisms in a population. Three types of genetic phenotype distributions associated with drug response have been recognized: bimodal, multimodal, or broad without an apparent antimode. The acetylation of isoniazid illustrates a monogenic, bimodal distribution in which individuals are separated into fast acetylator or slow acetylator subpopulations that correspond to genotypes of *NAT2* (Fig. 2A) (Evans et al., 1960; Weber, 1987). The 4-hydroxylation of debrisoquine by CYP2D6 follows a monogenic, multimodal distribution that includes poor metabolizers (PM) who have inactive CYP2D6, ultrarapid metabolizers (UM) who have multiple copies of *CYP2D6* and very high activities of 2D6, intermediate metabolizers who have reduced activities of CYP2D6, and extensive metabolizers (EM) who have a normal rate of metabolism (Fig. 2B) (Table 3) (Dahl et al., 1995; Eichelbaum et al., 2006). Some drug responses exhibit a broad distribution without a clear antimode. Although a near-normal distribution of variations generally suggests influences from multiple factors, this pattern of response can also include a prominent genetic component, as suggested by studies with twins (Fig. 2C) (Vesell, 1989; Tantisira et al., 2004). For drug responses that reflect combined effects of multiple genes (i.e., polygenic inheritance), the phenotypic distribution of polygenic traits and their impact on drug therapy can be complex and obscure. For instance, a CYP3A5 deficiency occurs in approximately 75% of white persons and 50% of black persons because of a single nucleotide polymorphism (*CYP3A5*3*, *6986A>G*) within intron 3 that introduces a premature stop codon and truncation of the protein (Kuehl et al., 2001). Because many drugs metabolized by CYP3A5 are also substrates of CYP3A4, the clinical effects of the *CYP3A5*3* polymorphism may well be obscured by the presence of functional CYP3A4, or vice versa.

Genetic variations can result from single-nucleotide polymorphism (SNP), insertion, deletion, or duplication of DNA sequences. SNP is probably the most common variation. More than 90% of human genes contain at least one SNP, and nearly every human gene is marked by a sequence variation. More than 14 million SNPs have been identified in the human genome. More than 60,000 SNPs are located in the coding regions of the genes (Sachidanandam et al., 2001). Most SNPs seem to have no apparent effect on gene function. Nonetheless, some SNPs do have profound impact on the function of associated genes, whether the SNPs occur in the coding regions or at a significant distance from the transcription starting site of the gene. Certain SNPs are known to be associated with significant changes in drug efficacy and drug disposition (Evans and Relling, 1999; McLeod and Evans, 2001; Eichelbaum et al., 2006; Roden et al., 2006). However, it is increasingly clear that identification of a single SNP may not be sufficient to relate the

TABLE 3
CYP2D6 Polymorphism and Characteristics

Phenotype	Characteristics	Clinical Consequence
PM	Major variants: CYP2D6*3, -*4, -*5, -*6	High plasma drug level
	Enzyme inactive	Risk of drug-related side effects
	5–10% White; 1–2% Chinese and Japanese	Use of reduced drug dose
IM	Major variants: CYP2D6*9, -*10, -*41	Lower dose for some patients
	Low residual enzyme activity	
EM	Not a uniform group	Standard dose for most patients
	Normal rate of metabolism	
UM	Multiple copies of CYP2D6	Very low plasma drug level
	Very high enzyme activity	Loss of drug efficacy
	1–2% Whites; 30% Ethiopians	Higher drug dose required

IM, intermediate metabolizer.

variation of a target protein to a disease or a drug response. A haplotype defines a set of genetic variants that are inherited together in linkage disequilibrium and thus is particularly useful in genome-phenotype analyses. For this reason, new techniques are being developed to integrate sets of SNPs across the entire genome to identify genetic loci that exist in linkage disequilibrium. This approach increases the likelihood of success in identifying polymorphisms of drug targets, drug-metabolizing enzymes, drug transporters, and other genes that influence drug response, as well as new disease susceptibility genes and pathways that are important in the etiology and pathogenesis of chronic diseases.

III. Genetic Polymorphisms of Drug Targets

Polymorphisms in genes encoding drug targets directly affect target protein function, drug-target interaction, or both to produce profound effects on drug response.

Warfarin is the most commonly used oral anticoagulant worldwide. The enzyme vitamin K epoxide reductase (VKOR) was recognized as warfarin's target more than 30 years ago (Bell, 1978). However, the direct target protein was identified as VKOR complex subunit 1 (VKORC1) only in 2004 (Li et al., 2004; Rost et al., 2004). VKOR catalyzes the conversion of vitamin K epoxide to reduced vitamin K, which is required for post-translational γ-carboxylation of the glutamic acid residues of coagulation factors II, VII, IX, and X and the anticoagulant proteins C, S, and Z by γ-glutamyl carboxylase (GGCX) (Fig. 3). Inhibition of VKORC1 by warfarin leads to depletion of reduced vitamin K and, consequently, production of hypofunctional coagulation factors resulting in anticoagulation. Several mutations in the coding region of *VKORC1* have been identified, including A41S, V45A, R58G, V66M, and L128R (Rost et al., 2004; Bodin et al., 2005; Rieder et al., 2005; Rettie and Tai, 2006; Au and Rettie, 2008). These mutations are generally rare in human populations (<0.1%), reflecting the highly conserved nature of *VKORC1*, but all mutations are associated with warfarin resistance that necessitates a daily dose greater than 15 mg/day (Table 4). A41S is associated with a daily dose of 16 mg; R58G, 32–36 mg; V66M, 27–35 mg; L128R, >45 mg; and V45A with target international normalized ratio (INR; a standardized prothrombin time) never reached. On the other hand, most *VKORC1* polymorphisms are within the regulatory or intron regions of the gene and influence warfarin dose across the normal dosing range. For instance, the C1173T polymorphism in the intron 1 of *VKORC1* has been consistently associated with interindividual variability in warfarin dose (Rieder et al., 2005; Wadelius et al., 2005). Overall, polymorphisms of *VKORC1* were estimated to account for ~25% of the variation in warfarin dose (Schwarz and Stein, 2006).

The angiotensin I-converting enzyme (ACE) encoded by the *ACE* gene catalyzes the conversion of decapeptide angiotensin I to octapeptide angiotensin II (a potent vasoconstrictor), and the degradation of bradykinin (a potent vasodilator). ACE is a primary target in the treatment of high blood pressure, heart failure, diabetic nephropathy, and type 2 diabetes. An insertion (I)/deletion (D) polymorphism of *ACE* has been shown to affect ACE

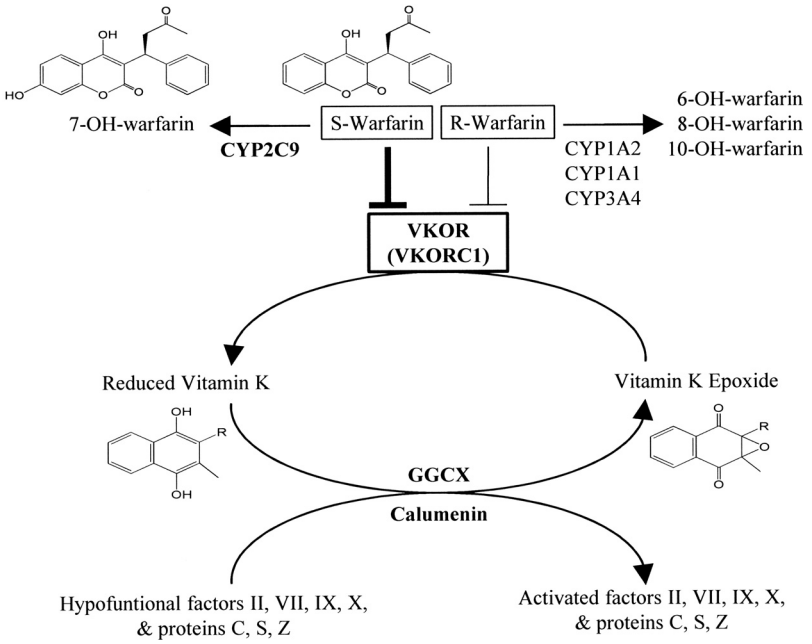

FIG. 3. Metabolism and therapeutic action of warfarin. (*S*)-warfarin is the active enantiomer that targets VKORC1 to inhibit vitamin K reduction, resulting in the depletion of reduced vitamin K and, consequently, decreased γ-carboxylation of coagulation factors II, VII, IX, and X by GGCX and prolonged blood coagulation. CYP2C9 catalyzes the 7-hydroxylation of (*S*)-warfarin to inactivate the drug, whereas, calumenin modulates GGCX function to affect blood clotting. The figure is based on information from Au and Rettie (2008) and Limdi and Veenstra (2008).

TABLE 4
VKORC1 polymorphisms and warfarin resistance
Data from Rost et al. (2004), Bodin et al. (2005), Rieder et al. (2005), and Rettie and Tai (2006).

Polymorphism	Daily Warfarin Dose	Resistance Phenotype
	mg	
Wild type	4–6	
A41S	16	Moderate
R58G	34	Major
V66M	31	Major
L28R	>45	Severe
V45A	Target INR never reached	Severe
Common SNPs in noncoding regions	1–15	Variations across the "normal" dosing range

inhibition therapy in a number of disease conditions. The antiproteinuric effect of ACE inhibition depends in part on the reduction of systemic blood pressure. Patients with hypertension and albuminuria with insulin-dependent diabetes mellitus were particularly susceptible to ACE inhibition treatment if they carried the homozygous I/I genotype. On the other hand, the deletion polymorphism of *ACE*, particularly D/D homozygote, is a risk factor for an accelerated loss of kidney function that reduces the long-term beneficial effect of ACE inhibition on progression of diabetic and nondiabetic kidney diseases (Jacobsen et al., 1998). In patients with essential hypertension and left ventricular hypertrophy who participated in a long-term ACE inhibitor therapy, the magnitudes of regression of septal wall thickness and left ventricular mass index during the therapy were less in the D/D group than in the I/I group, indicating that patients with hypertension who have the D/D genotype are less likely to have regression of left ventricular hypertrophy with ACE inhibitor therapy than those with other *ACE* genotypes (Kohno et al., 1999). In a recent study of 96 patients who have essential hypertension and received metoprolol monotherapy for 8 weeks, patients with the *ACE* I/I genotype showed greater reduction in 24-h average heart rate than those with I/D or D/D genotypes (Liu et al., 2010).

The *ADRB2* gene encodes the β_2-adrenoreceptor, the target receptor of β-agonists, such as albuterol, for bronchodilation. Two SNPs of *ADRB2* result in mutations of R16G and Q27E, respectively. Both SNPs are common with allele frequencies of 0.4 to 0.6. The mutations have significant functional impacts on drug therapy. For instance, β-agonist albuterol evokes a larger and more rapid bronchodilation response in Arg16/Arg16 homozygotes than in carriers of the Gly16 allele (Arg16/Gly16 and Gly16/Gly16). The maximal percentage increase in albuterol-evoked forced expiratory volume in 1 s is 18% for individuals carrying the Arg16/Arg16, but only 5% for those with the Gly16 allele after an oral dose of 8 mg of albuterol (Lima et al., 1999). In a separate study, infusion of β-agonist isoproterenol induced nearly complete desensitization after 90-min infusion in patients with homozygous Arg16/Arg16 genotype, but not in patients homozygous for Gly16. On the other hand, patients homozygous for the Glu allele at codon 27 had higher maximal vasodilation by isoproterenol than those with the Gln27 allele (Dishy et al., 2001).

The development of novel cancer therapies in which molecular targeted agents specifically interfere with key molecular events responsible for tumor phenotypes have revolutionized the treatment of a number of malignancies. Molecular targeted agents hold great promise to achieve a wider therapeutic window and more effective treatment compared with conventional cytotoxic therapies. A key to the success in targeted cancer therapy is to separate responders from nonresponders in clinical practice, so that the drug is targeted to patients who have a particular molecular abnormality. In targeted anticancer therapy against non–small-cell lung cancer, most patients have no response to gefitinib, a tyrosine kinase inhibitor that targets oncogenic epidermal growth factor receptor; however, approximately 10% of the patients have a rapid and often dramatic response. This high sensitivity to gefitinib seems to be due to somatic mutations clustered around the ATP-binding pocket of the tyrosine kinase domain of epidermal growth factor receptor that cause enhanced tyrosine kinase activity in response to epidermal growth factor and increased sensitivity to inhibition by gefitinib (Lynch et al., 2004). Screening for such mutations in lung cancers may identify patients who will have a response to gefitinib.

Molecular characterization of drug resistance that may arise against a targeted anticancer drug is also important in targeted therapy. The Abelson kinase (ABL) is constitutively activated in chronic myeloid leukemia (CML) by a chromosomal translocation that produces the breakpoint cluster region (BCR)-ABL fusion protein. Imatinib effectively inhibits oncogenic ABL kinase activity, upon which CML tumor cells critically depend for growth and tumorigenicity. In phase II studies, over 90% of the patients with CML who were resistant to interferon therapy had complete hematological responses to imatinib (Druker, 2008). A subset of the patients had relapse within the first few years as a result of the amplification of the BCR-ABL oncogene or mutations within the ABL kinase domain that rendered the kinase refractory to imatinib. Thus, identifying patients with CML who have imatinib-resistant mutant genotypes of BCR-ABL and developing second-generation inhibitors that inhibit most of these BCR-ABL resistant variants is critical in targeted therapy against CML.

HIV infection in humans involves C-C chemokine receptor type 5 (CCR5, CD195), a chemokine receptor used by HIV as a coreceptor for their entry into target cells, and CCR2 (CD192), a chemokine receptor for monocyte chemoattractant protein 1. In white and black persons, a polymorphism of CCR2 at codon 64 (V64I) is common with an allele frequency of 10%. Subjects who carry the Ile allele and are infected with HIV progress to AIDS 2

to 4 years later than those carrying the wild-type allele (Smith et al., 1997). Approximately 9% of white persons carry a 32-base pair deletion (Δ32) in the *CCR5* gene, but the deletion is generally not found in Africans. The deletion results in a frame shift in the coding region of CCR5, producing a nonfunctional receptor that does not support membrane fusion or infection by macrophage- and dual-tropic HIV-1 strains. The white blood cells from persons homozygous for the null allele are highly resistant to infection by HIV, whereas heterozygotes of Δ32 are partially protected from transmission of HIV (Samson et al., 1996). Thus, the *CCR2 V64I* allele has a significant impact on AIDS progression and *CCR5-Δ32* protects against HIV-1 transmission. Anti-HIV drugs called entry inhibitors, such as PRO 140, vicriviroc, and maraviroc, specifically interfere with the interaction between CCR5 and HIV. These examples demonstrate the utility of genomic studies in identifying important genes of disease pathogenesis and drug targeting.

Pravastatin and other statin drugs inhibit HMG-CoA reductase encoded by *HMGCR* to lower blood cholesterol levels. In a cohort of 1536 subjects treated with pravastatin at 40 mg/day, two common and tightly linked SNPs (SNP12 and SNP29) within the *HMGCR* gene were significantly associated with reduced success of pravastatin therapy (Chasman et al., 2004). Subjects with a single copy of the SNP allele had a 22% smaller reduction in total cholesterol, and 19% smaller reduction on LDL cholesterol compared with those homozygous for the major allele of one of the SNPs. The SNPs were not associated with changes in HDL cholesterol levels or baseline lipid levels. The mechanism by which these alleles affect pravastatin efficacy is unclear but may reflect the function of additional alleles of *HMGCR* that are linked to these SNPs. It remains possible that these SNPs also reduce cholesterol-lowering efficacy of other statin drugs.

IV. Genetic Polymorphisms of Drug-Metabolizing Enzymes

A. Cytochromes P450

Most, if not all, clinical drugs are metabolized by one or more microsomal cytochrome P450 enzymes. P450s catalyze the mono-oxygenation of lipophilic drugs to give rise to metabolites with altered activity and increased water solubility or metabolites more suitable to further metabolism by other enzymes (Ma and Lu, 2008). In many cases, P450 polymorphism is a major variable affecting drug plasma concentration, drug detoxification, and drug activation in the case of a prodrug.

CYP2D6 is responsible for the metabolism of approximately 20 to 25% of all marketed drugs (Williams et al., 2004). Many drug substrates of CYP2D6 have been identified, including β-adrenergic receptor blockers, antidepressants, antiarrhythmics, and antipsychotics. *CYP2D6* polymorphism is one of the best studied among P450s (Eichelbaum et al., 2006; Zhou et al., 2008). *CYP2D6* is highly polymorphic. Variant alleles of *CYP2D6* are classified on the basis of enzymatic activities. The frequency and genetic basis of major variants of *CYP2D6* are well documented (Table 3; Fig. 2B). In addition, methods for rapid, effective clinical testing of the variants are available. Therefore, if CYP2D6 is mainly responsible for the blood level of a drug in humans and the genetic polymorphism of drug target is not an issue, knowing the CYP2D6 phenotype of an individual patient would allow physicians to prescribe a safe and effective dose of the drug to the patient.

CYP2D6 is the rate-limiting enzyme in catalyzing the conversion of the prodrug tamoxifen into active metabolites 4-hydroxytamoxifen and endoxifen. Both metabolites have significantly higher affinity for the drug target [the estrogen receptor (ER)], and greater ability to inhibit cell proliferation in endocrine therapy for prevention and treatment of ER-positive breast cancer than the parent drug (Fig. 4) (Jin et al., 2005). Patients with

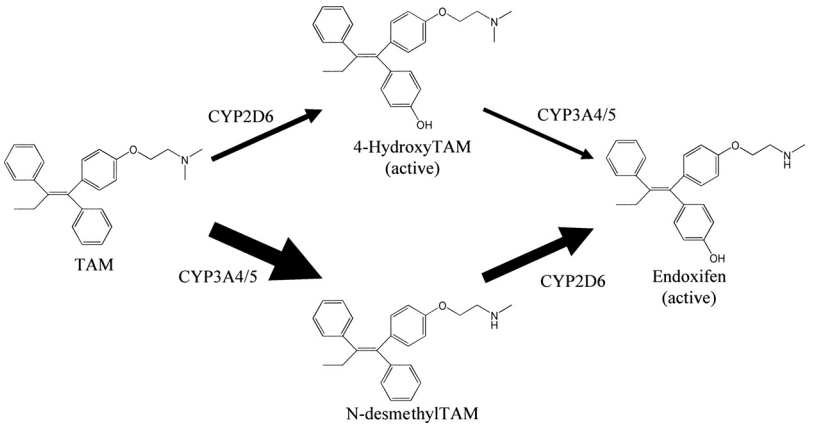

FIG. 4. Metabolic activation of tamoxifen. CYP2D6 is the rate-limiting enzyme in the activation of tamoxifen (TAM) to active metabolites 4-hydroxyTAM and endoxifen. Polymorphisms of CYP2D6 have a significant impact on the anticancer efficacy and side effects of TAM. Arrow thickness indicates relative contributions of the reactions. The figure is based on information from Jin et al. (2005).

multiple copies of functional alleles of *CYP2D6* (UM) have higher mean plasma concentrations of endoxifen than those without a UM allele as a result of high CYP2D6 activities in the UM patients (Borges et al., 2006). *CYP2D6*4* is the most common null allele contributing to the CYP2D6 PM phenotype in white persons. In a retrospective study of a prospective adjuvant tamoxifen trial in postmenopausal women with surgically resected ER-positive breast cancer, patients with the *CYP2D6*4/*4* genotype (PM) had shorter relapse-free time and worse disease-free survival compared with patients with either one or no *4 alleles (Goetz et al., 2005). On the other hand, higher incidence of moderate or severe hot flashes were found in patients with one or no *4 alleles (20%) compared with homozygotes of the *4 allele (0%). Differences in CYP2D6-mediated formation of active metabolites between PM and EM patients probably caused the differential therapeutic efficacy and side effects observed in these patients.

CYP2C19 catalyzes the metabolism of many commonly used drugs, including (*S*)-mephenytoin (anticonvulsant), omeprazole (antiulcerative), and diazepam (anxiolytic). CYP2C19 plays an important role in the proton-pump inhibitor therapy for peptic ulcer and gastroesophageal reflux diseases. More than 20 polymorphisms of *CYP2C19* have been reported (Zhou et al., 2008). Most "poor metabolization" of CYP2C19 is attributable to the *CYP2C19*2* and *-*3* genotypes, which are null alleles. Approximately 15 to 25% of the Chinese, Japanese, and Korean populations are PMs of (*S*)-mephenytoin, whereas the PM frequency in white persons is less than 5%. The effect of omeprazole on the intragastric pH value largely depends on the *CYP2C19* genotypes of the patients. At a single dose (20 mg) of omeprazole, the plasma area-under-the-curve of the drug is the highest among PM subjects, the lowest among EM subjects, and the medium in heterozygous EMs. Moreover, the pharmacokinetic data are in a good agreement with clinical outcomes in that the intragastric pH is 4.5 for the PM subjects, 3.3 for the heterozygous EM subjects, and 2.1 for the EM subjects (Furuta et al., 1999). In a separate example, the rate of eradication of *Helicobacter pylori* by a combination therapy with lansoprazole and antibiotics was shown to be highly dependent on *CYP2C19* in white patients who took a standard dose of lansoprazole (30 mg twice a day). Those with the EM phenotype of CYP2C19 had lower serum concentrations of lansoprazole and lower rates of *H. pylori* eradication (Schwab et al., 2004). These patients would benefit from a higher dose of the proton-pump inhibitor than patients with the PM phenotype.

CYP2C9 is involved in the metabolism of many clinically important drugs, including tolbutamide (hypoglycemic agent), glipizide (hypoglycemic agent), phenytoin (anticonvulsant), (*S*)-warfarin (anticoagulant), and flurbiprofen (anti-inflammatory agent). More than 30 variants of *CYP2C9* have been identified. *CYP2C9*2* and *CYP2C9*3*, two most common variant alleles, exhibit largely reduced enzymatic activities. Furthermore, the extent of such reduction is often substrate-dependent. Approximately 1% of white persons are *CYP2C9**2 homozygotes and 0.4% are *CYP2C9*3* homozygotes (Lee et al., 2002). In Chinese and Japanese populations, homozygous *CYP2C9*2*, homozygous *CYP2C9*3*, and heterozygous *CYP2C9*1/-*2* carriers are very rare, whereas heterozygous *CYP2C9*1/-*3* accounts for 4% of the populations. Ethnic distributions of the *CYP2C9* polymorphisms are clinically significant in anticoagulation therapy because CYP2C9 is the major P450 to inactivate the active *S*-enantiomer of warfarin (Fig. 3). In patients who receive warfarin therapy and carry the wild-type *CYP2C9*1* allele, (*S*)-warfarin is cleared from the body normally. Poor metabolizers who carry the *CYP2C9*2* and/or *CYP2C9*3* alleles have impaired capacity of metabolizing (*S*)-warfarin and thus require reduced daily doses of warfarin. These patients have 2- to 3-fold higher risks of having an adverse event than those with the wild-type allele in warfarin therapy.

CYP3A4 is the most abundant P450 enzyme in human liver and is responsible for the metabolism of more than 50% of clinical drugs. More than 20 *CYP3A4* variants have been identified. Many of the variants have altered enzyme activities, ranging from modest to significant loss in catalytic efficiency (Miyazaki et al., 2008; Zhou et al., 2008). There is also a large difference across ethnic groups in the frequency of *CYP3A4* variants. High frequencies of *CYP3A4*2* and *-*7* were found in white persons and high frequencies of *CYP3A4*16* and *-*18* in Asian populations (Sata et al., 2000; Lamba et al., 2002). The clinical significance of the *CYP3A4* variant alleles for many drugs metabolized by CYP3A4 remains uncertain or is minimal to moderate based on the current data. These coding region variants are unlikely to account for the >10-fold differences in CYP3A4 activities observed in vivo, because the alleles cause only small changes in the enzyme activity and many of the alleles exist in low frequencies (Lamba et al., 2002). One factor that may contribute to the complexity of the CYP3A4 puzzle is CYP3A5. Virtually all CYP3A4 substrates, with a few exceptions, are also metabolized by CYP3A5. Although CYP3A5 metabolizes these drugs at slower rates in most cases, some drugs can be metabolized by CYP3A5 as fast as or faster than the CYP3A4 enzyme. Therefore, the metabolic rates of CYP3A4 drugs measured in vivo are likely to reflect combined activities of CYP3A4 and CYP3A5. Because approximately 25% of whites and 50% of blacks express functional CYP3A5 (Kuehl et al., 2001), this dual pathway potentially obscures the clinical effects of *CYP3A4* variants in human studies. The effect of polymorphisms of the CYP3A4 gene on the activity of a variant enzyme is known to be often substrate-dependent. In addition, clinically meaningful

phenotyping of CYP3A4 by using probe drug substrates has not been successfully established.

The CYP3A5*3 allele (6986A>G) is the most frequently occurring allele of *CYP3A5* that results in a splicing defect and absence of enzyme activity. Persons with at least one allele of 6986A designated as *CYP3A5*1* are classified as CYP3A5 expressors. Tacrolimus is a calcineurin inhibitor and a more effective alternative to cyclosporine for immunosuppressive therapy after kidney transplantation. Tacrolimus has a low therapeutic index with a wide range of side effects and large interindividual variability in its pharmacokinetics, particularly in the dose required to reach target trough blood concentrations (C_0), thus necessitating routine therapeutic drug monitoring in clinical practice. The whole-blood tacrolimus C_0 and tacrolimus dose requirements are closely associated with *CYP3A5* polymorphisms. Carriers of at least one *CYP3A5*1* allele have functional CYP3A5 and require a higher dose of tacrolimus to reach the targeted whole-blood concentrations (Hesselink et al., 2003). In the first 2 weeks after transplantation, CYP3A5 expressors experience a delay in reaching target concentrations (MacPhee et al., 2004). In a randomized, controlled trial that compared the efficacy of tacrolimus dosing on the basis of individual *CYP3A5* genotypes with a standard tacrolimus dosing regimen that was based on body weight, pharmacogenetic adaptation of the daily dose of tacrolimus is found to be associated with improved achievement of the target C_0 (Thervet et al., 2010).

B. Other Drug-Metabolizing Enzymes

Many non-P450 drug-metabolizing enzymes also play critical roles in the metabolism of a variety of drugs. Polymorphisms of these enzymes influence the metabolism and therapeutic effect of the drugs, some of which are clinically significant.

Thiopurine methyltransferase catalyzes the S-methylation of 6-mercaptopurine, azathioprine, and thioguanine, to inactivate the thiopurine drugs, which are used for the treatment of leukemia and autoimmune diseases. More than 20 variant alleles of the *TPMT* gene have been identified, among which *TPMT*2*, *TPMT*3A*, and *TPMT*3C* are defective alleles that produce poor enzymatic activities (Zhou et al., 2008). Approximately 90% of white persons inherit high enzyme activity, 10% inherit intermediate activity (heterozygous), and 0.3% inherit low or no activity. Persons carrying defective *TPMT* alleles accumulate higher levels of cytotoxic thiopurine nucleotides than those with the wild-type alleles after receiving a standard dose of the drugs, leading to severe hematological toxicity by the parent drugs. In these scenarios, a reduced drug dose should be prescribed.

The serum butyrylcholinesterase hydrolyzes the muscle relaxant succinylcholine and thereby determines the serum concentration of the drug and the duration of muscle relaxation. A variant allele of the gene encodes an atypical form of the enzyme that is not active in hydrolyzing succinylcholine. Approximately 1 in 3500 white persons is homozygous for the variant allele. Patients with the atypical butyrylcholinesterase but receiving a normal dose of the muscle relaxant have higher serum levels of the drug and develop prolonged muscle paralysis and apnea (Kalow and Staron, 1957; Kalow and Gunn, 1959).

N-Acetyltransferases catalyze the acetylation of aromatic amines and hydrazines. Human variability in drug acetylation was discovered more than 50 years ago during the initial clinical trials of isoniazid for the treatment of tuberculosis (Evans et al., 1960). The drug was highly effective in antituberculosis therapy. However, a high percentage of patients receiving isoniazid developed devastating nerve toxicity that correlated with high blood levels of the drug. Two distinct phenotypes of acetylation were identified as "rapid acetylators" and "slow acetylators" (Fig. 2A). The phenotypes were later attributed to differences in the enzymatic activities of NAT1 and NAT2 (Weber, 1987). Among 15 *NAT2* alleles identified in humans, *NAT2*5A*, *NAT2*6A*, and *NAT2*7A* are associated with the slow acetylator phenotype (Zhou et al., 2008). The slow acetylation form is present in up to 90% of some Arab populations, 40 to 60% of whites, and 5 to 25% of East Asians (Green et al., 2000). Because NAT2 metabolizes carcinogenic arylamines, NAT2 polymorphisms are associated with individual susceptibility to certain cancers caused by industrial chemicals, such as α- and β-naphthylamine and benzidine (Stern et al., 1985). Persons with a poor acetylator phenotype have increased risks of lung, bladder, and gastric cancers if exposed to carcinogenic arylamines for a long period of time.

UDP-glucuronosyltransferase (UGT) 1A1 catalyzes the glucuronidation of many commonly used drugs or metabolites, such as the active metabolite [7-ethyl-10-hydroxycamptothecin (SN-38)] of the anticancer drug irinotecan, and endogenous substrates, such as bilirubin (Tukey and Strassburg, 2000). More than 100 *UGT1A1* polymorphisms have been identified. The frequency of *UGT1A1*6* polymorphism is high among Japanese and Chinese (16–23%), whereas it is low (<1%) in whites. The high frequency of the *UGT1A1*6* variant allele may contribute to the high incidence of neonatal hyperbilirubinemia in Asian populations, consistent with a major role of UGT1A1 in the glucuronidation of bilirubin. *UGT1A1* polymorphisms cause three forms of inherited, unconjugated hyperbilirubinemia in humans (Kadakol et al., 2000; Tukey and Strassburg, 2000). The Crigler-Najjar syndrome types I and II are caused by variant alleles in the *UGT1A1* coding region and the Gilbert's syndrome by polymorphisms in the promoter of the *UGT1A1* gene. The severity of the hyperbilirubinemia correlates with the enzymatic activities of the polymorphic UGT1A1. Patients with the Crigler-Najjar type I syndrome completely lack bilirubin glucuronidation, re-

sulting in very high serum levels of unconjugated bilirubin and early childhood death. Patients with the Crigler-Najjar type II syndrome have reduced activity of bilirubin conjugation (~10–30% of the normal level). In patients with the Gibert's syndrome, a genetic polymorphism in the promoter region of the *UGT1A1* gene (*UGT1A1*28*) gives rise to reduced expression of UGT1A1 and, consequently, the syndrome. *UGT1A1*28* is associated with increased toxicity of the anticancer drug irinotecan, because UGT1A1 is responsible for metabolic inactivation of SN-38, the active metabolite of irinotecan (Fig. 5) (see also discussion on irinotecan toxicity in section VII.A).

V. Genetic Polymorphisms of Drug Transporters

Drug transporters modulate the absorption, distribution, and elimination of drugs by controlling the influx and efflux of drugs in cells. Increasing evidence indicates genetic polymorphisms of transporters can have profound impact on drug disposition, drug efficacy, and drug safety.

The *ABCB1* gene encodes the P-glycoprotein (Pgp, ABCB1, multidrug resistance 1) that transports many important drugs out of cells. *ABCB1* is highly polymorphic, and some allelic variants exhibit ethnic-dependent distribution. The SNP C3435T of *ABCB1* occurs with high frequencies in many populations (20–60%) (Zhou et al., 2008) and is thus of particular interest. The functional impact of the C3435T polymorphism on the pharmacokinetic and pharmacodynamic properties of Pgp substrates has not been defined. Conflicting results have been reported on the disposition of digoxin, a substrate of Pgp. In one study, C3435T was shown to be associated with reduced serum digoxin concentrations, whereas in another, it was associated with higher plasma digoxin levels (Sakaeda et al., 2001; Verstuyft et al., 2003). The discrepancy between these two studies may exist because polymorphisms other than C3435T and other genetic traits also influence the expression and function of the Pgp protein.

The breast cancer resistance protein (BCRP) is an ABC transporter (ABCG2) important in the intestinal absorption and biliary excretion of drugs, drug metabolites, and some toxic xenobiotics (Gradhand and Kim, 2008). The C421A variant of *ABCG2*, which causes a mutation in the BCRP protein (Gln141Lys), is widely present in ethnic groups, with frequencies of 30 to 60% in Asians and 5 to 10% in whites and African Americans. Patients with a heterozygous genotype of *ABCG2 C421A* have 300% higher plasma levels of diflomotecan, an anticancer drug, with an intravenous administration of the drug (Sparreboom et al., 2004). ABCG2 plays a significant role in the disposition of rosuvastatin. The *ABCG2 C421A* polymorphism influences the pharmacokinetics and therapeutic effect of rosuvastatin in Chinese and white subjects (Zhang et al., 2006; Keskitalo et al., 2009). In a study of 305 Chinese patients with hypercholesterolemia treated with rosuvastatin at a dose of 10 mg/day, the C421A variant was significantly associated with greater reduction in LDL cholesterol levels in a gene-dose-dependent manner (Tomlinson et al., 2010). In the above two cases, the *ABCG2* variant allele alters drug exposure by reducing the biliary excretion of diflomotecan and rosuvastatin, causing variations in drug effects.

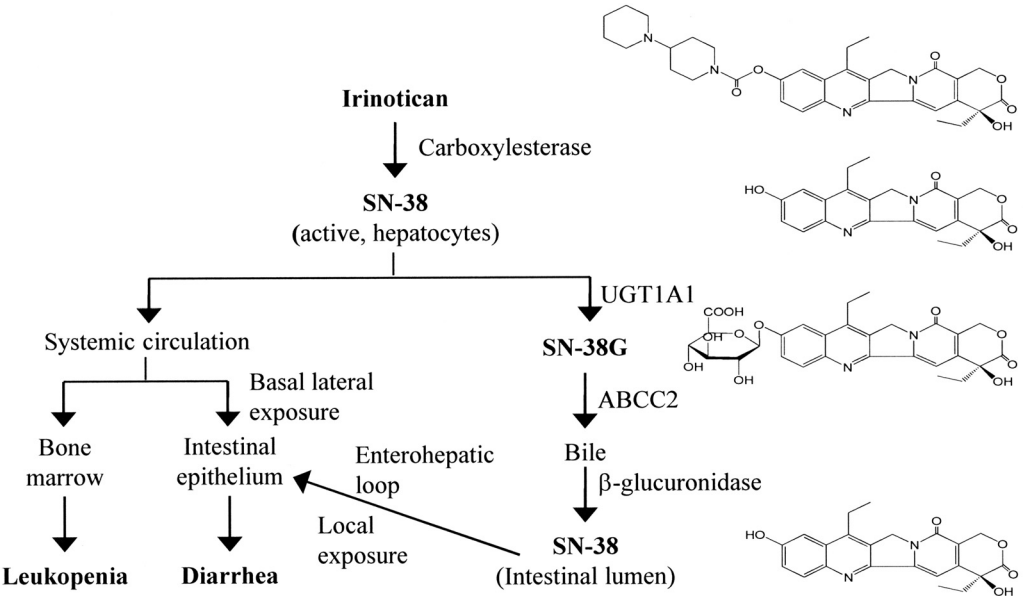

FIG. 5. Irinotecan metabolism and toxicity. Irinotecan is metabolized in the liver to SN-38, which is inactivated via glucuronidation by UGT1A1 (and other UGTs to a lesser extent) and by secretion into the bile through ABCC2 (possibly other transporters as well). The majority of SN-38 glucuronide (SN-38G) in the intestine is reconverted to SN-38 by β-glucuronidase. Intestinal SN-38 is reabsorbed and returns to the liver, forming an enterohepatic circulation loop. In addition to inhibiting topoisomerase I in cancer cells, SN-38 causes severe bone marrow depression and diarrhea, which are affected by polymorphisms of *UGT1A1*28* and *ABCC2*2*. The figure is based on information from Tukey et al. (2002).

OATPs are a large family of membrane transporter proteins for the transport of organic anions, including drugs and metabolites, across the cell membrane. The *SLC21A6* gene encodes OATP-C, a liver-specific transporter important for hepatic uptake of a variety of endogenous and therapeutic compounds. Sixteen *OATP-C* variant alleles were characterized in vitro. *OATP-C*5* and *OATP-C*9* have reduced uptake of OATP-C substrates, such as estrone sulfate and estradiol 17β-D-glucuronide (Tirona et al., 2001). The significance of the alleles on the disposition of OATP-C substrates is highlighted by the high frequency of the variants in human populations: *OATP-C*5* is present in 14% of European Americans and *OATP-C*9* in 9% of African Americans. Persons carrying *OATP-C*5* have high plasma levels of pravastatin, a cholesterol-lowering drug, and repaglinide, an antidiabetic drug, both of which are substrates of OATP-C (Nishizato et al., 2003; Mwinyi et al., 2004; Niemi et al., 2005). OATP1B1 encoded by *SLCO1B1* is critical for hepatic uptake of simvastatin acid, the active metabolite of simvastatin. A polymorphism of *SLCO1B1* (c.521T>C) that is associated with reduced activity of OATP1B1 increases the blood concentration of simvastatin acid and, consequently, increased toxicity and reduced efficacy (see also discussion on statin toxicity in section VII.A) (Pasanen et al., 2006; SEARCH Collaborative Group, 2008).

VI. Genetic Variables Indirectly Affecting Drug Response

Many genetic variables affect drug response by modulating the functions of proteins that are not direct drug targets, drug-metabolizing enzymes, or drug transporters but influence the biological context of a drug response.

In addition to VKORC1 (direct target of warfarin) and CYP2C9 [major P450 metabolizing (*S*)-warfarin], several other enzymes and proteins are important in vitamin K-dependent blood coagulation, including GGCX, which catalyzes the γ-carboxylation of vitamin K-dependent clotting factors; calumenin (Calu), which functions as a chaperone protein to inhibit GGCX in the endoplasmic reticulum membrane; the anticoagulant proteins C, S, and Z; and proteins involved in the transport of warfarin, such as Pgp and apolipoprotein (apo) E (Fig. 3). Polymorphisms of the genes encoding these proteins have been proposed to play a role in the variability of warfarin anticoagulation therapy by affecting the bleeding tendency of patients. However, evidence supporting the notion is limited at the present time. A *GGCX* SNP [reference SNP ID (rs) 12714145 (http://www.ncbi.nlm.nih.gov/snp)] in the intron 2 of *GGCX* was shown to have a small but significant effect on warfarin dose (Wadelius et al., 2005). The *PROC* gene encoding protein C is polymorphic and its genetic variability may contribute to approximately 4% of the variance in warfarin dose (Wadelius et al., 2007). A CALU polymorphism (rs39097 A>G) is associated with higher warfarin dose requirements, independently of other known genetic and nongenetic predictors of warfarin dose in African Americans (Voora et al., 2010).

The *APOE* gene encoding human apo-E is polymorphic with three common alleles, *APOEε2*, *APOEε3*, and *APOEε4*. Apo-E is critical in the modulation of cholesterol and phospholipid transport between cells of different types. Apo-E4 is associated with sporadic and late-onset familial Alzheimer's disease (AD). Genetic polymorphisms of *APOE* may have a predictive value for drug response in the therapy for AD. The apo-E4 allele has a C112R mutation. The effect of the apo-E4 allele on cholinomimetic drug responsiveness was assessed in a group (n = 40) of patients with AD who completed a double-blind, 30-week clinical trial of the cholinesterase inhibitor tacrine (Poirier et al., 1995). More than 80% of apo-E4–negative patients with AD showed marked improvement after 30 weeks, as measured by the AD assessment scale; 60% of apo-E4 carriers had scores that were worse compared with baseline, strongly supporting that apo-E4 plays a crucial role in the cholinergic dysfunction associated with AD and may be a prognostic indicator of poor response to therapy with acetylcholinesterase inhibitors in patients with AD. The interaction between the *APOE* genotype and tacrine treatment of AD seems to be the strongest among women, indicating that gender also contributes to the variability of tacrine efficiency (Farlow et al., 1998).

Idiopathic cerebral vein thrombosis is associated with genetic risk factors, such as the G20210A mutation of the gene encoding prothrombin and the G1691A mutation of the gene encoding factor V, as well as nongenetic risk factors, such as the use of oral contraceptive agents, in women. In one study (Martinelli et al., 1998), the odds ratio of having cerebral-vein thrombosis in patients carrying the prothrombin-gene mutation was found to be approximately 10 compared with controls. Similar results were obtained for the mutation in the factor V gene. The use of oral contraceptives was more frequent among women with cerebral-vein thrombosis than among controls with an odds ratio of 22. The odds ratio for cerebral-vein thrombosis in women who were taking oral contraceptives and who also had the prothrombin-gene mutation rose to 149. Therefore, the use of oral contraceptives is strongly and independently associated with the cerebral-vein thrombosis; the presence of both the prothrombin-gene mutation and oral-contraceptive use further increases the risk of the disease.

VII. Genetic Variables Affecting Adverse Drug Reactions

Adverse drug reactions are highly variable in many cases and thus represent a major limiting factor in drug therapy and drug development. Idiosyncratic adverse

drug reactions characterized by their rare occurrence and requirement of multiple exposures are the most extreme cases of individual variability in drug safety. To date, many drug-related safety issues remain unsolved, largely because of the lack of mechanistic understanding of adverse drug reactions, especially those of an idiosyncratic nature. The key to address drug safety issues is to understand the mechanism of adverse drug response, determine the gene or genes responsible for the adverse events, and develop reliable biomarkers for screening sensitive individuals. A few examples in which genetic variables that influence individual variability of drug toxicity and drug hypersensitivity were identified illustrate the utility of this approach and bring the hope that drug dose or alternative drugs can be chosen according to individual genotypes and phenotypes to minimize adverse drug reactions in patients.

A. Drug Toxicity

Drug toxicity can result from the inhibition or activation of a therapeutic target by a drug or from an interaction between a drug and a target protein different from the therapeutic target of the drug. In the former case, "on-target" toxicity, such as excessive bleeding from high doses of warfarin, is observed; in the latter case, "off-target" toxicity, such as statin-induced myopathy, takes place. All genetic factors that influence drug response—drug targets, drug-metabolizing enzymes, drug transporters, and genes indirectly affecting drug action—can modulate drug toxicity and contribute to its individual variability.

Drug-induced liver injury (DILI) is the most common cause of clinical trial termination of new drugs (~33%) and a main cause of the withdrawal of clinical drugs from the market. The standard incidence rate of symptomatic hepatic adverse drug reaction is 8 in 100,000. Idiosyncratic DILI causes 13% of acute liver failure in the United States, and 75% of the patients either die or require liver transplantation (Ostapowicz et al., 2002). The pathogenesis of most DILI remains unclear. Genetic association of individual susceptibility to DILI is available for a few drugs. Flucloxacillin is widely used for the treatment of staphylococcal infection but is associated with a characteristic cholestatic hepatitis, with an incidence of 8.5 in every 100,000 new users in days 1 to 45 after the start of the drug. In a genome-wide association study, an 80-fold higher risk of flucloxacillin DILI was attributed to a SNP in the major histocompatibility complex (MHC), rs2395029[G] ($p = 8.7 \times 10^{-33}$, odds ratio = 45). rs2395029 is a missense polymorphism in the HCP5 gene and is in complete linkage disequilibrium with a human leukocyte antigen (HLA) gene polymorphism, *HLA-B*5701*, which is also strongly associated with flucloxacillin DILI with an odds ratio of 81 (Daly et al., 2009). *HLA-B*5701* may affect the risk of flucloxacillin DILI by affecting inflammation within the liver; this mechanism differs from that by which it influences abacavir-induced hypersensitivity described in the following section.

Lumiracoxib is a selective cyclooxygenase-2 inhibitor efficacious in the symptomatic treatment of osteoarthritis and acute pain. The risk of developing cardiovascular events by lumiracoxib is similar to nonsteroid anti-inflammatory drugs, but the concern over its hepatotoxicity has led to market withdrawal or nonapproval of the drug worldwide. A recent genomic-wide study identified several SNPs from the MHC class II region that showed strong association with lumiracoxib hepatotoxicity (top SNP rs3129900, $p = 4.4 \times 10^{-12}$) (Singer et al., 2010). Fine mapping identified a strong association to a common HLA haplotype (*HLA-DRB1*1501-HLA-DQB1*0602-HLA-DRB5*0101-HLA-DQA1*0102*, most significant allele odds ratio = 5). It remains possible that *HLA-B*5701* identified for flucloxacillin DILI and the common HLA haplotype for lumiracoxib hepatotoxicity also influence the incidence of DILI by other drugs.

Irinotecan is a potent DNA topoisomerase I inhibitor used for the treatment of colorectal and lung cancers (Tukey et al., 2002). Irinotecan is converted to its active metabolite, SN-38, by carboxylesterase in the liver (Fig. 5). However, high levels of SN-38 lead to severe side effects, including severe myelosuppression in 15 to 20% and severe delayed-type diarrhea in 20 to 25% of patients receiving irinotecan treatment. Both bone marrow depression and diarrhea are likely to result from inhibition of the drug target in fast-dividing bone marrow hematopoietic cells and gastrointestinal epithelial cells by SN-38. SN-38 is metabolized through glucuronidation by UGT1A1 and, to a lesser extent, other UGTs, such as UGT1A6. Conjugated SN-38 is secreted into the bile via ABCC2 (multidrug resistance protein 2) and possibly several other transporters alike. The majority of conjugated SN-38 in the bile is reabsorbed after cleavage by intestinal glucuronidase, forming an enterohepatic circulation loop of SN-38 (Fig. 5).

UGT1A1 is highly polymorphic. Individuals carrying *UGT1A1* variant alleles could face potentially severe bone marrow and gastrointestinal toxicity in irinotecan therapy as a result of reduced glucuronidation of SN-38 and accumulation of the active metabolite to a high level. The *UGT1A1*28* polymorphism carries seven, instead of six, TA repeats in the *UGT1A1* promoter, resulting in a considerable reduction of UGT1A1 expression (~30–80%) and reduced glucuronidation of SN-38 (Kadakol et al., 2000; Zhang et al., 2007). Patients homozygous or heterozygous for the *UGT1A1*28* allele have elevated levels of SN-38 and consequently are susceptible to bone marrow and gastrointestinal side effects of SN-38 if treated with a normal dose of irinotecan for cancer therapy. For this reason, the FDA has recommended that patients be genotyped for the *UGT1A1*28* polymorphism and that the dose adjusted accordingly before irinotecan treatment. In addition to *UGT1A1*,

polymorphisms of *ABCC2* seem to influence the incidence of irinotecan-related diarrhea (de Jong et al., 2007). The haplotype *ABCC2*2* was associated with lower clearance of irinotecan from the blood, possibly as a result of reduced hepatobiliary secretion of SN-38 glucuronide through ABCC2, which reduces the exposure of intestinal epithelial cells to SN-38 in the intestine. A significant reduction of severe diarrhea was noted in patients who carry the *ABCC2*2* allele but no *UGT1A1*28* allele (odds ratio of 0.15). This reduction was not observed in patients who carry at least one allele of *UGT1A1*28* (odds ratio of 1.87). Therefore, the presence of *ABCC2*2* is associated with reduced risk of irinotecan-induced diarrhea.

The statins—simvastatin, pravastatin, and rosuvastatin—inhibit HMG-CoA reductase to reduce LDL cholesterol levels, which reduces the incidence of heart attacks, strokes, and revascularization procedures by approximately one fifth for each reduction of 40 mg/dl in the LDL cholesterol level. On the other hand, statins cause myopathy (muscle pain and weakness associated with elevated creatine kinase levels) in a small number of patients receiving statin therapy. Statin-induced myopathy occasionally develops into rhabdomyolysis (muscle breakdown and myoglobin release) that may cause renal failure and death. The mechanism of statin-induced myopathy is unclear. Statin-induced myopathy is generally rare—typically approximately 1 case per 10,000 patients per year with a standard dose of statins (i.e., 20–40 mg/day), but the incidence increases significantly with a higher dose (80 mg/day) and with concomitant use of other drugs such as cyclosporine or amiodarone (Davidson et al., 1997; SEARCH Collaborative Group, 2008) (Table 2).

Simvastatin is administered as an inactive lactone prodrug that is converted to the active metabolite simvastatin acid in the plasma, liver, and intestinal mucosa via nonenzymatic and carboxylesterase-mediated processes. OATP1B1 encoded by *SLCO1B1* is an influx transporter on the basolateral membrane of hepatocytes. The transporter facilitates the uptake of statins and other drugs, as well as bile acids, from portal blood into hepatocytes. In a study with healthy volunteers, a common polymorphism of *SLCO1B1* (c.521T>C, V174A, or rs4149056) was shown to markedly affect individual variations of statin pharmacokinetics. The plasma $AUC_{0-\infty}$ of simvastatin acid (but not simvastatin) was increased more than 2- or 3-fold in persons with the homozygous c.521CC genotype compared with the TC heterozygous or the TT homozygous genotypes, respectively, as a result of reduced uptake of simvastatin acid into hepatocytes via OATP1B1 in the latter genotypes (Pasanen et al., 2006). Increased plasma concentrations of simvastatin acid in patients carrying the c.521C variant allele may have increased risk of systemic adverse effects and reduced cholesterol-lowering efficiency as a result of reduced intracellular simvastatin acid for inhibition of HMG-CoA reductase in hepatocytes.

In a large randomized SEARCH trial involving 12,064 subjects, 96 participants given 80 mg/day simvastatin and known to have definite or incipient myopathy were selected for a genome-wide association study using 316,184 SNPs in comparison with 96 control subjects that received 80 mg/day simvastatin but had no documented myopathy (SEARCH Collaborative Group, 2008). A single strong association with myopathy was noted with the rs4363657 SNP marker located within the intron 11 of *SLCO1B1* on chromosome 12. The odds ratio for myopathy was 4.3 per copy of the C allele and 17.4 among CC homozygous compared with TT homozygous. Moreover, the rs4363657 was found to be in nearly complete linkage disequilibrium with the rs4149056 polymorphism (c.521T>C, V174A) ($r^2 > 0.95$). The odds ratio of rs4149056 for myopathy was 4.5 per copy of the C allele and 16.9 among CC homozygotes compared with TT homozygotes (Table 5). The study suggested that genotyping of *SLCO1B1* may help screen out those persons with abnormal OATP1B1 activities and thereby, achieve the benefits of statin therapy safely and effectively. As discussed in sections III and V above, genetic variations in *HMGCR* encoding HMG-CoA reductase, *ABCG2* encoding the ABCG2 transporter, and *SLC21A6* encoding the OATP-C transporter also influence the efficacy and/or pharmacokinetics of statins (Chasman et al., 2004; Mwinyi et al., 2004; Tomlinson et al., 2010).

Excessive prolongation of the QT interval, which may develop into life-threatening arrhythmias (*torsades de pointes* or ventricular fibrillation), can occur in response to a variety of cardiac and noncardiac drugs. *KCNE2* encodes MinK-related peptide 1, a subunit of the cardiac

TABLE 5

Cumulative risk of myopathy associated with SLCO1B1 rs4149056 genotypes and simvastatin at 80 mg daily dose

Data from SEARCH Collaborative Group, 2008.

Genotype	Population Frequency	Myopathy Odds Ratio	Year 1		Year 5	
			Total	Proportion Attributable to Genotype	Total	Proportion Attributable to Genotype
				%		%
TT	0.730	1.0	0.34	0	0.63	0
CT	0.249	4.5	1.38	75	2.83	78
CC	0.021	16.9	15.25	98	18.55	97
All	1.000		28.40	63	1.56	60

potassium channel 1 (Kr). KCNE2 polymorphisms are associated with inherited long QT syndromes (LQTS) and some drug-induced LQTS. In a cohort of 98 patients with drug-induced LQTS, a KCNE2 variant (8T>A) was associated with development of a very long QT interval when given trimethoprim-sulfamethoxazole (Sesti et al., 2000). The channels with the SNP were normal at baseline but were inhibited by sulfamethoxazole at therapeutic levels that did not affect wild-type channels. The SNP is found in approximately 1.6% of the general population. Several other mutations were identified and showed diminished potassium flux at baseline. Because of the apparent effect of *KCNE2* variants on drug-induced LQTS and the high risk of cardiac arrest from drug-induced LQTS, screening for KCNE2-blocking activity is routinely carried out for new drugs.

B. Drug Hypersensitivity

Drug hypersensitivity reactions (DHRs) are the adverse effects of drugs that occur at a dose tolerated by typical subjects and clinically resemble allergy. DHR may represent up to one third of adverse drug reactions and concern more than 7% of the general population. DHRs can be life-threatening, require or prolong hospitalization, or entail changes in drug prescription. The pathogenic mechanisms of many DHRs remain unclear. Although DHRs are unpredictable for the most part, genetic polymorphisms of certain genes can predispose patients to drug allergy.

The use of abacavir, a potent HIV-1 nucleoside-analog reverse-transcriptase inhibitor, is complicated by a potentially life-threatening hypersensitivity syndrome. Abacavir hypersensitivity occurs in approximately 5 to 9% of the patients receiving abacavir treatment and is characterized by multisystem involvement. The hypersensitivity was strongly associated with the HLA polymorphism *HLA-B*5701* and its combination with a haplotypic polymorphism of *Hsp70-Hom* (*M493T*) (Mallal et al., 2002; Martin et al., 2004). HLA-B*5701 is an effective antigen-presentation molecule, whereas Hsp70 proteins facilitate antigen presentation, particularly cross-presentation of exogenous antigen to $CD8^+$ T cells. The involvement of HLA-B*5701 in determining a MHC class I-restricted immune response to abacavir is consistent with $CD8^+$ T-cell-dependent production of tumor necrosis factor α (TNFα) and a prominent $CD8^+$ cell infiltrate in patch testing. It is speculated that *HLA-B*5701* and *Hsp70-Hom* cooperate during antigen presentation to confer susceptibility to abacavir hypersensitivity. A double-blind, prospective, randomized study involving 1956 patients from 19 countries revealed that prospective screening for *HLA-B*5701* reduced the risk of hypersensitivity reaction to abacavir (Mallal et al., 2008). A haplotypic polymorphism within the *TNF* promoter region (*TNF-238A*) may also influence the severity of abacavir reactions by affecting TNF production (Mallal et al., 2002; Martin et al., 2004).

Carbamazepine (CBZ), a commonly prescribed first line anticonvulsant for the treatment of seizures, frequently causes cutaneous DHRs including maculopapular eruption, hypersensitivity syndrome, Stevens-Johnson syndrome (SJS), and toxic epidermal necrosis (TEN). CBZ-induced SJS/TEN was strongly associated with a HLA polymorphism *HLA-B*1502* in Han Chinese. In one study, the incidence of this allele was 100% in Han Chinese with CBZ-induced severe bullous skin reactions (Chung et al., 2004). In a separate study with a larger patient population, the *HLA-B*1502* allele was strongly associated with CBZ-SJS/TEN (odds ratio = 1357), but not CBZ-induced maculopapular eruption and hypersensitivity syndrome, in Han Chinese (Hung et al., 2006). The *HLA-B*1502* allele is present in 8% of Han Chinese but only 1 to 2% of white persons; this differential allelic distribution may explain the lower incidence of CBZ-SJS in white persons compared with Han Chinese (Chung et al., 2004). CBZ-induced hypersensitivity reactions were also associated with a *TNFα* promoter polymorphism (*−308TNFα*) and with a number of *HSP70* gene variants for more severe CBZ hypersensitivity reactions (Pirmohamed et al., 2001; Alfirevic et al., 2006).

Asparaginase is an effective antileukemia drug used in the treatment of childhood acute lymphoblastic leukemia. Hypersensitivity reactions to therapeutic asparaginase isolated from *Escherichia coli* or *Erwinia chrysanthemi* are common, occurring in up to 45% of patients, much higher in incidence than hypersensitivity reactions to other drugs, including carbamazepine and abacavir. Many of the asparaginase allergic reactions are typical of type I hypersensitivity, which is also a hallmark of asthma. Allergy to asparaginase may also attenuate the pharmacological effect of the drug. In a genome-wide screening of 485 children with acute lymphoblastic leukemia, more than 500,000 SNPs were analyzed (Chen et al., 2010). One SNP (rs4958381) in *GRIA1* on chromosome 5q33 and four additional SNPs annotated to *GRIA1* were significantly associated with allergy to asparaginase. Chromosome 5q31-33 contains a cluster of cytokine and other immune-related genes and has been mapped as a susceptibility locus for several inflammatory and autoimmune diseases such as asthma. *GRIA1* encodes a subunit of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, a tetrameric ligand-gated ion channel transmitting glutamatergic signals in the brain. Glutamate may have a role as an immunomodulator in addition to being a neurotransmitter. The findings support the notion that polymorphisms in 5q33 represent inherited variation in the risk of asparaginase allergy, and drug allergy and asthma may share a range of genes as the cause of the reactions.

VIII. Translating Pharmacogenomics into Clinics: Individualized Medicine

A. Warfarin Anticoagulation As a Model of Individualized Medicine

Although widely used for the treatment and prevention of thromboembolic diseases, warfarin is well noted for its narrow therapeutic index, large individual variability in clinical response, high rates of adverse—sometimes lethal—events, and the requirement for careful selection and frequent adjustment of therapeutic dose for individual patients. Furthermore, genetic polymorphisms of a number of enzymes involved in warfarin drug action and pharmacokinetics have been shown to be important in determining individual variability in warfarin therapy. From these perspectives, warfarin anticoagulation therapy is considered well suited as a model for studying individualized medicine (Rettie and Tai, 2006).The standard dose of warfarin is between 4 and 6 mg/day. However, the effective daily dose varies from 0.5 to more than 30 mg from patient to patient in a large population. Patients receiving an insufficient warfarin dose are at risk of failing to control blood clotting, whereas patients given too high a dose can experience excessive and uncontrolled bleeding. Frequent monitoring of the patients during warfarin therapy is commonly practiced, in which the "prothrombin time," a blood test that measures the time required for blood to clot after medication, is used by physicians to adjust the warfarin dose so that a desired balance between the therapeutic effect and bleeding risk can be achieved. The goal of warfarin dose adjustment is to maintain the standardized prothrombin time known as INR in a range between 2 and 3. The time required to reach this INR range after warfarin treatment may differ significantly among patients, ranging from several days to several months. Nevertheless, the prothrombin time has served as a simple and relatively economical way for clinicians to treat patients with individualized warfarin dose in the past.

The target protein of warfarin anticoagulation and factors that determine warfarin pharmacokinetic properties have been identified (Fig. 3). Genetic polymorphisms of these genes have been shown to affect individual variability of warfarin therapy to various extents. These include polymorphisms of VKORC1, CYP2C9, and several genes that indirectly influence warfarin anticoagulation, such as GGCX, CALU, and PROC (see also discussions in sections III, IV, and VI). For example, persons carrying the 1173T/T allele of *VKORC1* require approximately half of the warfarin daily dose compared with patients with the wild-type 1173C/C allele, whereas polymorphisms in the coding regions of *VKORC1* often lead to varying degrees of warfarin resistance, necessitating high doses of warfarin to reach a desired INR value in these patients (Table 4). On the other hand, patients carrying the *CYP2C9*2* and *-*3* alleles have reduced capacity for metabolizing (*S*)-warfarin, resulting in increased plasma area-under-the-curve for (*S*)-warfarin. These patients have a higher risk of developing side effects than those carrying the wild-type *CYP2C9*1* allele and should receive a reduced daily dose of warfarin. Therefore, genotyping of patients before warfarin therapy is believed to reduce warfarin adverse events and facilitate achieving stable INR efficiently in patients with genetic variations of *CYP2C9* and *VKORC1* (Gage and Lesko, 2008; Kim et al., 2009).

Given the large variation and difficulty in achieving effective, safe anticoagulation and the consistent predictive values of *VKORC1* and *CYP2C9* genotyping in warfarin anticoagulation therapy, it is hoped that more accurate prediction of the initial dose of warfarin for better control of anticoagulation can be achieved based on the pharmacogenetics of individual patients. In promoting the use of genetic information in warfarin therapy, the FDA revised the warfarin labeling to include pharmacogenetic information in August 2007. However, shortly after, the FDA issued a brief press release, as a response to a negative feedback from the medical community, indicating that the new labeling was not a mandatory requirement for clinicians to genotype patients before initiating a warfarin therapy. It was argued that, although the influence of *CYP2C9* and *VKORC1* genotypes on warfarin dose requirements has been consistently demonstrated in observational studies and randomized clinical trials, evidence from prospective, controlled studies has not yet demonstrated an added benefit of incorporating genotype-guided therapy in improving anticoagulation control or in preventing or reducing adverse effects. Therefore, the evidence currently available does not support the routine use of *CYP2C9* and *VKORC1* genotyping in the general patient population of those beginning warfarin therapy (Limdi and Veenstra, 2008).

Variability in warfarin therapy is a complex issue that involves more than just the genotypes of *CYP2C9* and *VKORC1*. The contribution of variable warfarin metabolism by CYP2C9 is rather small, estimated to be approximately 10% of warfarin dose variations. The contribution of *VKORC1* genotypes to dose variations is approximately 25%, whereas clinical factors, such as age, sex, diet, drugs, and body mass index, contribute another 20%. Thus, the identified factors that contribute to variable warfarin responses account for approximately 50%. The other, as-yet-unidentified factors reach almost 50%. Perhaps this is the major reason that many clinicians do not believe that the time is ripe for incorporating genotyping testing in warfarin therapy, particularly when the "prothrombin time" test, although not perfect, is available clinically to provide reasonable information for dose adjustment (Bussey et al., 2008). The International Warfarin Pharmacogenetics Consortium (2009) has used clinical data (age, height, weight, and race) and genetic data (*CYP2C9*1*, *-*2*, and *-*3*, and

VKORC1 variants) of 4043 patients from 9 countries to estimate the warfarin doses to achieve targeted INR. The pharmacogenetic algorithm accurately identified that 50% of the patients require ≤3 mg/day warfarin, and 25% require ≥7 mg/day to achieve the target dose. Thus, despite the impressive progress made in identifying *CYP2C9* and *VKORC1* variants, the successful prediction of a target clinical warfarin daily dose based on clinical and genetic data are 25 to 50%, which is consistent with the fact that almost 50% of the factors responsible for variable warfarin responses remain unknown.

The warfarin story reveals that prospective clinical trials demonstrating that incorporation of genetic testing can indeed benefit the selection of appropriate therapeutic agent and drug dose for individual patients to improve therapeutic response, reduce adverse drug effects, and reduce overall healthcare cost are critical for wide clinical acceptance of pharmacogenetic testing. Research efforts designed to evaluate the effectiveness of genotype-guided warfarin therapy in improving therapeutic outcomes are under way. Definitive clinical outcomes in such studies would certainly be welcome news for the new era of individualized medicine.

B. The Reality of Achieving Individualized Drug Therapy

The seemingly wide chasm between the promise of pharmacogenomics and the clinical practice of personalized medicine at present raises the question of whether individualized medicine can ever be achieved. The answer to this question varies from optimistic to pessimistic depending on the disease and drug involved and upon what one considers a success in achieving the goal of individualized drug therapy. Individualized medicine could be achieved when the variable factor affecting the efficacy and side effect of a drug is simple and well defined. The clinical use of psychiatric drugs, many of which are metabolized by CYP2D6, illustrates the practicality of using CYP2D6 genotyping for dose selection in individual patients. Individual patients carrying *CYP2D6*3*, *-*4*, *-*5*, or *-*6* variants should be given reduced doses of antidepressants to avoid or reduce drug side effects. In this scenario, metabolism of the drugs is the major factor affecting drug response and safety, and genetic variations are well established. For this reason, psychiatrists at the Mayo Clinic have begun to request that the CYP2D6 genotype information be made available before psychiatric drug therapy is begun (Weinshilboum, 2003b).

In many cases, variability of drug response involves multiple factors, such as in the case of warfarin anticoagulation discussed above. Achieving individualized medicine for many diseases requires one to understand the pathogenesis of the disease, to identify the gene(s) responsible for the disease, and to establish the role of genetic polymorphisms of drug targets, transporters, and drug-metabolizing enzymes in drug response variations. It is particularly challenging to achieve these goals if disease genes are unidentified. A major goal of clinical pharmacology and pharmacogenomics now is to establish phenotype-genotype associations through genetic tests that reveal genetic predispositions to a disease and drug toxicity. The practical purpose is to identify patients who are drug responders and patients who are prone to drug toxicity. Although some success has been achieved in recent years in establishing phenotype-genotype associations for monogenic disorders, this task seems to be far more challenging than originally anticipated because of the complexity of the human genome and diseases. Based on literature data, it was argued that, for complex diseases involving multiple genes, it would be very difficult to determine unequivocally an exact phenotype or genotype (Nebert and Vesell, 2006; Nebert et al., 2008). In addition, many nongenetic factors that influence drug efficacy and drug toxicity but are not reflected in genomic information contribute to variability of drug response. Therefore, it remains questionable whether individualized drug therapy will ever be achievable by means of DNA testing alone.

Metabolomics provides a quantitative measurement of time-related, multiparametric metabolic responses of individuals to genetic modifications and to pathophysiological and environmental stimuli. Available evidence supports the notion that metabolomics, together with other "-omics" approaches, such as proteomics and pharmacoepigenetics, may be used to complement pharmacogenomics in achieving individualized drug therapy. This approach is particularly attractive in cases in which genetic association with a disease is weak or has not yet been established. DILI occurs in only a small fraction of patients but is the major adverse event leading to regulatory actions on drugs. Genetic association to individual susceptibility for most DILI cases is currently not available. In a clinical study of healthy adults receiving 4 g/day acetaminophen for 7 days, it was found that urine metabolite profiles obtained shortly after the start of treatment, but before serum alanine aminotransferase elevation, could distinguish responders from nonresponders for liver injury. The predictive metabolites include those derived from the toxic metabolite, *N*-acetyl-*p*-benzoquinone imine. Inclusion of endogenous metabolites is required for significant prediction (Winnike et al., 2010). Some polymorphisms of the *HLA* genes are known to influence DILI. Thus, this example provides proof-of-concept evidence demonstrating the value of an early intervention pharmacometabolomics approach in complimenting pharmacogenomics to achieve individualized therapy for potentially hepatotoxic drugs.

There are several reasons that genomic medicine has not moved as fast as hoped. In addition to scientific difficulties discussed above, economic, ethical, social, and regulatory issues are also very challenging. Although the cost of DNA sequencing is dropping dramat-

ically, data on cost-effectiveness of genomic medicine is currently limited. At the present, racial or ethnic minorities are under-represented in most biobanks in the United States. Persons or groups that carry genetic variations associated with altered pathophysiology can be at a disadvantage for equal access to health care and insurance. Considerable education to the medical and lay communities is much needed to advance genomic medicine in clinical practice. Even for well defined genetic variants that have reproducible and significant consequences for drug therapy, wide acceptance by the medical community can be slow. Regulation of genotyping test and how pharmacogenomics can be incorporated into new drug development, drug approval, and drug labeling are examples of regulatory issues.

IX. Pharmacogenomics in Drug Development

Pharmacogenomics can be used to improve drug discovery and drug development in at least two ways: development of new drugs to overcome drug resistance or target new drug targets, and optimization of drug metabolism and pharmacokinetics (DMPK) to minimize variations in drug levels.

A major challenge in targeted cancer therapy is the rapid development of resistance to targeted anticancer agents as a result of frequent mutations of drug targets in cancer cells. The anticancer drug imatinib inhibits BCR-ABL tyrosine kinase and receptor kinases mast/stem cell growth factor receptor (SCFR, CD117) and platelet-derived growth factor receptor (PDGFR). BCR-ABL is required for CML cancer cell growth, whereas, oncogenic mutations of SCFR and PDGFR are over-represented in gastrointestinal stromal tumors (GISTs) (80% for SCFR and 35% for PDGFR). Clinical studies showed that more than 90% of patients with CML and 75 to 90% of patients with GIST responded to imatinib anticancer therapy. However, a small number of the patients suffered relapse because of the development of resistance to imatinib through mutations of BCR-ABL kinase in patients with CML or SCFR kinase in patients with GIST (Druker, 2008; Stegmeier et al., 2010). In the case of BCR-ABL, at least 40 mutations were found in the ABL kinase domain. Mutations such as T315I and F359V directly affect the contact between imatinib and the ABL kinase domain, whereas others, such as those in the P-loop that bridges the ATP-binding pocket of the kinase domain, affect the conformation required for imatinib binding (Druker, 2008). Genetic and crystal structural information on mutated BCR-ABL facilitated modification of imatinib to improve drug binding to ABL mutants. As a result, nilotinib showed 10- to 30-fold higher potency over imatinib against the major resistant mutants but retained the kinase specificity profile with inhibition confined to ABL, SCFR, and PDGFR. Because nilotinib and several other second-generation-kinase-inhibitor-anticancer drugs, such as nasatinib, bind to the kinase domain in a fashion similar to that of imatinib, resistance due to the emergence of the T315I mutation that directly affects drug binding remains an issue for these drugs. New drugs that inhibit the T315I mutant are now being developed.

Antithrombotic medications are frequently used for the treatment and prevention of heart attack, stroke, and peripheral vascular diseases. Warfarin has been the first-line anticoagulant drug for many years because of the lack of alternatives. Inhibition of VKORC1 by warfarin is limited by a narrow therapeutic index, potentially lethal side effects, and many genetic variations of VKORC1 that confer either hypersensitization or true resistance. Moreover, warfarin has complex pharmacokinetics that is influenced by polymorphisms of CYP2C9. The emergence of new oral anticoagulants that inhibit blood coagulation by blocking ADP-dependent platelet aggregation [i.e., clopidogrel (Plavix) and prasugrel] or the function of clotting factor Xa (i.e., apixaban) provides alternatives to warfarin. New anticoagulants are characterized by simpler, more predictable pharmacodynamics and pharmacokinetic and by reduced need for monitoring. It is noteworthy that clopidogrel is now the second-highest selling drug in revenue, surpassed only by atorvastatin. Moreover, the use of combination of drugs that inhibit different targets of blood coagulation allows reduced doses and, consequently, reduced on-target side effects of individual anticoagulants.

The development of antiplatelet and factor Xa inhibitor drugs illustrates the feasibility of DMPK optimization to reduce variability in drug levels. Clopidogrel exhibits perhaps the most complex pharmacokinetics and most variable drug response among marketed antiplatelet drugs. As a prodrug, clopidogrel requires two sequential P450-mediated oxidations to generate its active metabolite: formation of 2-oxo by CYP2C19, -1A2, and -2B6, followed by formation of active thiol metabolite by CYP3A4, -2B6, -2C19, and -2C9 (Fig. 6A) (Rocca and Petrucci, 2010). Contributions by CYP2C19 and -3A4 are more important than those by other P450s for bioactivation of clopidogrel. The active metabolite is a reactive and short-lived thiol derivative that irreversibly inactivates the platelet P2Y12 receptor to block ADP-dependent platelet aggregation. The hepatic carboxyl esterase 1 inactivates 90% of the administered drug and esterase inactivates 40% of the intermediate thiolactone metabolite. Therefore, generation of active metabolite from clopidogrel depends on the enzymatic balance between bioactivation by P450s and bioinactivation by hepatic carboxyl esterase 1 and esterase, which is influenced by genetic polymorphisms of the P450s and drug-drug interactions. Individual variability in the response to clopidogrel is well recognized. Patients having coronary artery disease but with lesser degrees of platelet inhibition by clopidogrel are at increased risk of cardiovascular events (Matetzky et al., 2004; Angiolillo et al., 2007). The *CYP2C19*2* polymorphism has a frequency of

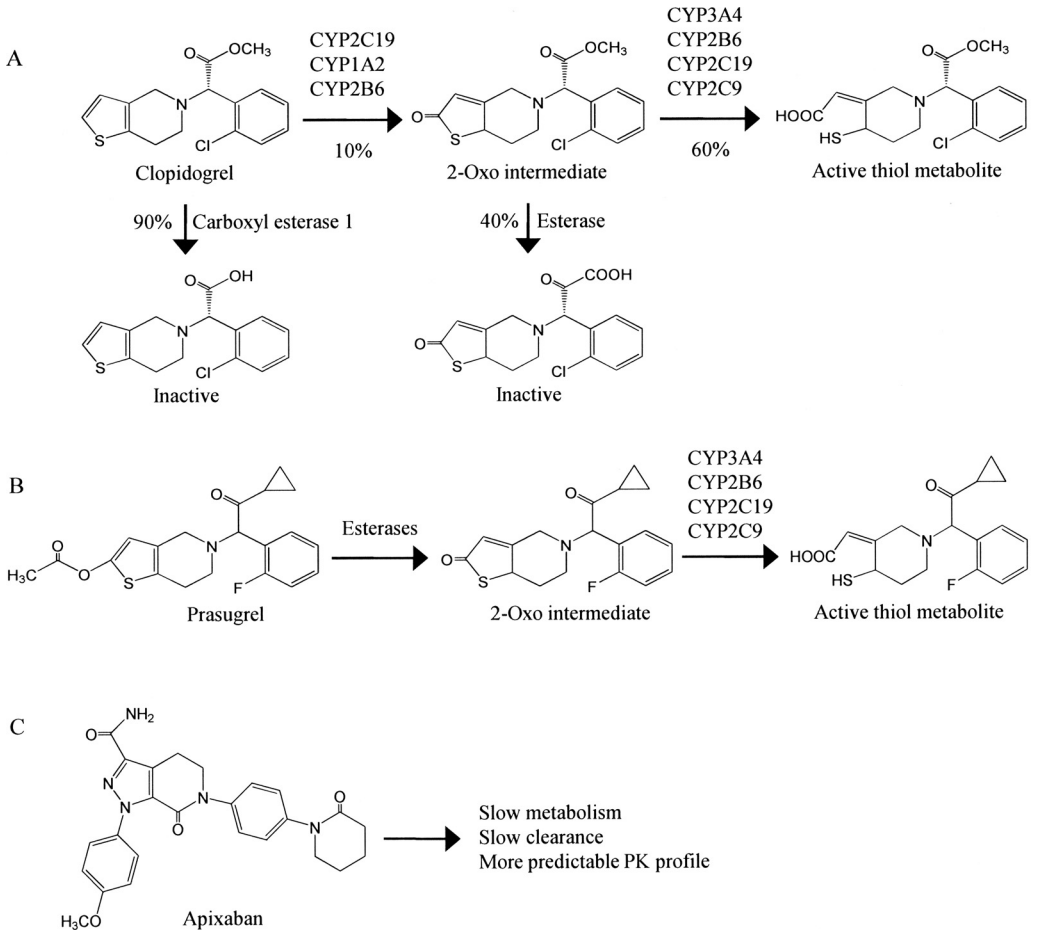

FIG. 6. Metabolism and DMPK optimization of thionopyridines and apixaban. Shown are the structures and metabolism of clopidogrel (A), prasugrel (B), and apixaban (C), based on information from Rocca and Petrucci (2010) and Mega et al. (2009). Clopidogrel undergoes two-step metabolic activation by multiple P450s and inactivation by esterases. The *CYP2C19*2* polymorphism accounts for approximately 12% of variation in clopidogrel therapy. Removal of P450-mediated activation at the first metabolic step of prasugrel results in much less variability in the formation of the 2-oxo and the active thiol metabolites. Apixaban exhibits almost perfect DMPK properties, with slow metabolism, slow clearance, and more predictable pharmacokinetic profile.

2% in humans for homozygotes and accounts for approximately 12% of the variable responses to clopidogrel. The FDA-issued black box warning of clopidogrel states that clopidogrel "at recommended doses forms less of that metabolite and has a smaller effect on platelet function in patients who are CYP2C19 poor metabolizers. Poor metabolizers with acute coronary syndrome or undergoing percutaneous coronary intervention treated with Plavix at recommended doses exhibit higher cardiovascular event rates than do patients with normal CYP2C19 function" (http://products.sanofi-aventis.us/plavix.html).

Prasugrel is a third generation thienopyridine antiplatelet drug that undergoes a much simpler metabolic activation than clopidogrel. In this case, P450-catalyzed oxidation in the first step is replaced by deacetylation of prasugrel by esterase to generate a 2-exo intermediate (Fig. 6B). Elimination of the activation step by P450 results in much less variability in 2-oxo and thiol formation with individual human liver microsomes: <3- versus 50-fold variations in the formation of thiolactone and 150- versus 10,000-fold variations in the formation of active thiol metabolite between prasugrel and clopidogrel (Hagihara et al., 2009). In patients treated with prasugrel, common functionalP450 genetic variants did not affect the active metabolite levels, the inhibition of platelet aggregation, or the clinical cardiovascular event rates (Mega et al., 2009). Prasugrel therapy resulted in greater platelet inhibition and less variability than did clopidogrel (Wiviott et al., 2007). The lack of effect of P450 genetic polymorphisms on prasugrel's active metabolite formation is responsible, in part, for the differential pharmacological and clinical responses to prasugrel and clopidogrel.

Apixaban, a novel and highly selective inhibitor of factor Xa, is a drug with almost perfect DMPK properties. Apixaban exhibits favorable in vitro characteristics: slow turnover by human liver microsomes with <5% metabolism by CYP3A4, CYP1A2, and, to a lesser extent, several other P450s; little or no induction of P450s with up to 20 μM concentrations of the drug; weak inhibition of major P450s with IC_{50} >20 μM and no time-dependent inhibition; a substrate of both Pgp and BCRP for excretion; and finally, very low potential for

drug-drug interaction because of multiple clearance pathways and fractional metabolism by a single P450 that is far less than 0.5 (Wang et al., 2010). In humans, apixaban is well tolerated and efficacious, requiring ≤5 mg for therapy; metabolism is slow, most unchanged parent drug appearing in the plasma, urine, and feces in all species; metabolic profiles are similar in humans and safety animals at high concentrations; clearance is slow with less than 5% blood flow rate, 50% bioavailability, linear pharmacokinetics from 2.5 to 25 mg oral dose, and a half-life of 13 h; elimination is mediated through multiple routes and urinary, biliary, and intestinal clearance is more important than metabolic clearance (Raghavan et al., 2009; Zhang et al., 2009). Apixaban is currently under development for the treatment and prevention of thromboembolic disorders, prevention of stroke in patients with atrial fibrillation, and secondary prevention in patients with acute coronary syndromes (APPRAISE Steering Committee and Investigators, 2009). The example of clopidogrel, prasugrel, and apixaban demonstrates that DMPK optimization is a practical and effective approach in developing orally active anticoagulants that have predictable pharmacokinetic profiles and can be administered with reduced need for monitoring and dose adjustment in anticoagulation therapy.

X. Perspectives and Conclusions

The promise of pharmacogenomics to unravel the genetic basis of individual variability in drug response is built upon the success of pharmacogenetics in establishing causal relations between single-gene polymorphisms and some individual drug responses, the rapid accumulation of knowledge on human genomic variations from genomic sequencing, and the power of large-scale, population-based, and multiparameter genetic analyses of the post-genomic era. Nevertheless, with a few exceptions, the impact of the vast number of genomic variations on pharmacokinetics and clinical outcomes of drug therapy remains to be elucidated. The utility of pharmacogenomics in drug therapy also manifests in its potential to translate into individualized medicine, drug development, and drug regulation, which, like pharmacogenomics itself, need to cope with individual variability in drug therapy and are only at the beginning of meeting this difficult and complex challenge.

The goal of individualized medicine is for physicians to prescribe an appropriate medication to the right target of the disease at the right dose for individual patients to achieve maximal therapeutic benefit with minimal, tolerable adverse effects. The achievements so far have been limited. Good clinical data to support the use of genetic testing for drug treatment for most diseases are still not yet available. In the case of warfarin dosing, despite excellent research and clinical importance, therapeutic dose can only be predicted for 25 to 50% of the patients based on genetic and clinical information, whereas up to 50% of the variation in warfarin dosing remains random.

The challenge for achieving individualized drug therapy is manyfold. Knowledge on genetic determinants of important disease pathogenesis, drug action, and drug pharmacokinetics, especially those of complex disease and drug response, is not always available. Establishing the relation of the many SNPs and other gene variations derived from genomic sequencing to clinical phenotypes of drug therapy may not be straightforward. Large scale, prospective clinical studies are often very challenging but much needed for establishing causal associations between genetic variations and drug response phenotypes and for evaluating the utility and cost-effectiveness of genotyping and individualized medicine. Economic, ethical, social, and regulatory issues associated with genomic medicine are also complex and challenging.

Based on limited success, it is recognized that, perhaps, instead of asking for a full package to address all questions, the challenges and obstacles facing individualized drug therapy can be addressed individually, one at a time, at different stages in the quest for answers. From this prospect, several important achievements can be highlighted at what is the starting point in this long journey. Genotyping of *SLCO1B1* in patients undergoing statin therapy potentially helps achieve the benefit of cholesterol-lowering drugs for safer and more effective therapy. The doses for antidepressant drugs can be adjusted in patients based on their CYP2D6 phenotypes to minimize drug toxicity. Genotyping of *UGT1A1*28* in patients with colon cancer before irinotecan treatment is an important measure to reduce the gastrointestinal and bone marrow toxicities of irinotecan. Target therapies with imatinib and gefitinib for cancer patients of certain genotypes and development of new inhibitors that overcome drug resistance to imatinib and gefitinib have pointed out the direction for future cancer therapy. DMPK optimization has practically assisted the development of orally active anticoagulants that have more predictable pharmacokinetic profiles and can be administered with reduced need for monitoring and dose adjustment.

A few lessons can be learned from these successful examples to guide future research in pharmacogenomics and its application in drug therapy. Basic research shall continue to be critical in understanding mechanism of disease pathogenesis and role of genetic variations of disease genes, drug targets, and proteins important for drug disposition in drug response variations. Integration of genomics, proteomics, metabolomics, and epigenetics in genome-wide association studies is likely to facilitate identification of predisposing genetic factors associated with multifactorial diseases and drug response. Prospective clinical trials that evaluate the utility and cost-effectiveness of genotyping and individualized medicine are critical in guiding clinical practice of

genetic testing and individualized drug therapy. Finally, pharmacogenomics-guided drug development and drug regulation open doors for new and targeted drug development and drug regulation to promote effective, safe, and cost-effective individualized drug therapy.

Acknowledgment

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

Authorship Contributions

Performed data analysis: Ma and Lu.

Wrote or contributed to the writing of the manuscript: Ma and Lu.

References

Alfirevic A, Mills T, Harrington P, Pinel T, Sherwood J, Jawaid A, Smith JC, March RE, Barratt BJ, Chadwick DW, et al. (2006) Serious carbamazepine-induced hypersensitivity reactions associated with the HSP70 gene cluster. *Pharmacogenet Genomics* **16:**287–296.

Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Alfonso F, Macaya C, Bass TA, and Costa MA (2007) Variability in individual responsiveness to clopidogrel: clinical implications, management, and future perspectives. *J Am Coll Cardiol* **49:**1505–1516.

APPRAISE Steering Committee and Investigators, Alexander JH, Becker RC, Bhatt DL, Cools F, Crea F, Dellborg M, Fox KA, Goodman SG, Harrington RA, et al. (2009) Apixaban, an oral, direct, selective factor Xa inhibitor, in combination with antiplatelet therapy after acute coronary syndrome: results of the Apixaban for Prevention of Acute Ischemic and Safety Events (APPRAISE) trial. *Circulation* **119:**2877–2885.

Au N and Rettie AE (2008) Pharmacogenomics of 4-hydroxycoumarin anticoagulants. *Drug Metab Rev* **40:**355–375.

Bell RG (1978) Metabolism of vitamin K and prothrombin synthesis: anticoagulants and the vitamin K–epoxide cycle. *Fed Proc* **37:**2599–2604.

Bodin L, Horellou MH, Flaujac C, Loriot MA, and Samama MM (2005) A vitamin K epoxide reductase complex subunit-1 (VKORC1) mutation in a patient with vitamin K antagonist resistance. *J Thromb Haemost* **3:**1533–1535.

Borges S, Desta Z, Li L, Skaar TC, Ward BA, Nguyen A, Jin Y, Storniolo AM, Nikoloff DM, Wu L, et al. (2006) Quantitative effect of CYP2D6 genotype and inhibitors on tamoxifen metabolism: implication for optimization of breast cancer treatment. *Clin Pharmacol Ther* **80:**61–74.

Bussey HI, Wittkowsky AK, Hylek EM, and Walker MB (2008) Genetic testing for warfarin dosing? Not yet ready for prime time. *Pharmacotherapy* **28:**141–143.

Chasman DI, Posada D, Subrahmanyan L, Cook NR, Stanton VP Jr, and Ridker PM (2004) Pharmacogenetic study of statin therapy and cholesterol reduction. *JAMA* **291:**2821–2827.

Chen SH, Pei D, Yang W, Cheng C, Jeha S, Cox NJ, Evans WE, Pui CH, and Relling MV (2010) Genetic variations in GRIA1 on chromosome 5q33 related to asparaginase hypersensitivity. *Clin Pharmacol Ther* **88:**191–196.

Chung WH, Hung SI, Hong HS, Hsih MS, Yang LC, Ho HC, Wu JY, and Chen YT (2004) Medical genetics: a marker for Stevens-Johnson syndrome. *Nature* **428:**486.

Dahl ML, Johansson I, Bertilsson L, Ingelman-Sundberg M, and Sjöqvist F (1995) Ultrarapid hydroxylation of debrisoquine in a Swedish population. Analysis of the molecular genetic basis. *J Pharmacol Exp Ther* **274:**516–520.

Daly AK, Donaldson PT, Bhatnagar P, Shen Y, Pe'er I, Floratos A, Daly MJ, Goldstein DB, John S, Nelson MR, et al. (2009) HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. *Nat Genet* **41:**816–819.

Davidson MH, Stein EA, Dujovne CA, Hunninghake DB, Weiss SR, Knopp RH, Illingworth DR, Mitchel YB, Melino MR, Zupkis RV, et al. (1997) The efficacy and six-week tolerability of simvastatin 80 and 160 mg/day. *Am J Cardiol* **79:**38–42.

de Jong FA, Scott-Horton TJ, Kroetz DL, McLeod HL, Friberg LE, Mathijssen RH, Verweij J, Marsh S, and Sparreboom A (2007) Irinotecan-induced diarrhea: functional significance of the polymorphic ABCC2 transporter protein. *Clin Pharmacol Ther* **81:**42–49.

Dishy V, Sofowora GG, Xie HG, Kim RB, Byrne DW, Stein CM, and Wood AJ (2001) The effect of common polymorphisms of the beta2-adrenergic receptor on agonist-mediated vascular desensitization. *N Engl J Med* **345:**1030–1035.

Druker BJ (2008) Translation of the Philadelphia chromosome into therapy for CML. *Blood* **112:**4808–4817.

Eichelbaum M, Ingelman-Sundberg M, and Evans WE (2006) Pharmacogenomics and individualized drug therapy. *Annu Rev Med* **57:**119–137.

Evans DA, Manley KA, and McKusick VA (1960) Genetic control of isoniazid metabolism in man. *Br Med J* **2:**485–491.

Evans WE and McLeod HL (2003) Pharmacogenomics–drug disposition, drug targets, and side effects. *N Engl J Med* **348:**538–549.

Evans WE and Relling MV (1999) Pharmacogenomics: translating functional genomics into rational therapeutics. *Science* **286:**487–491.

Evans WE and Relling MV (2004) Moving towards individualized medicine with pharmacogenomics. *Nature* **429:**464–468.

Farlow MR, Lahiri DK, Poirier J, Davignon J, Schneider L, and Hui SL (1998) Treatment outcome of tacrine therapy depends on apolipoprotein genotype and gender of the subjects with Alzheimer's disease. *Neurology* **50:**669–677.

Furuta T, Ohashi K, Kosuge K, Zhao XJ, Takashima M, Kimura M, Nishimoto M, Hanai H, Kaneko E, and Ishizaki T (1999) CYP2C19 genotype status and effect of omeprazole on intragastric pH in humans. *Clin Pharmacol Ther* **65:**552–561.

Gage BF and Lesko LJ (2008) Pharmacogenetics of warfarin: regulatory, scientific, and clinical issues. *J Thromb Thrombolysis* **25:**45–51.

Goetz MP, Rae JM, Suman VJ, Safgren SL, Ames MM, Visscher DW, Reynolds C, Couch FJ, Lingle WL, Flockhart DA, et al. (2005) Pharmacogenetics of tamoxifen biotransformation is associated with clinical outcomes of efficacy and hot flashes. *J Clin Oncol* **23:**9312–9318.

Gradhand U and Kim RB (2008) Pharmacogenomics of MRP transporters (ABCC1–5) and BCRP (ABCG2). *Drug Metab Rev* **40:**317–354.

Green J, Banks E, Berrington A, Darby S, Deo H, and Newton R (2000) N-acetyltransferase 2 and bladder cancer: an overview and consideration of the evidence for gene-environment interaction. *Br J Cancer* **83:**412–417.

Hagihara K, Kazui M, Kurihara A, Yoshiike M, Honda K, Okazaki O, Farid NA, and Ikeda T (2009) A possible mechanism for the differences in efficiency and variability of active metabolite formation from thienopyridine antiplatelet agents, prasugrel and clopidogrel. *Drug Metab Dispos* **37:**2145–2152.

Hesselink DA, van Schaik RH, van der Heiden IP, van der Werf M, Gregoor PJ, Lindemans J, Weimar W, and van Gelder T (2003) Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. *Clin Pharmacol Ther* **74:**245–254.

Hung SI, Chung WH, Jee SH, Chen WC, Chang YT, Lee WR, Hu SL, Wu MT, Chen GS, Wong TW, et al. (2006) Genetic susceptibility to carbamazepine-induced cutaneous adverse drug reactions. *Pharmacogenet Genomics* **16:**297–306.

International Warfarin Pharmacogenetics Consortium, Klein TE, Altman RB, Eriksson N, Gage BF, Kimmel SE, Lee MT, Limdi NA, Page D, Roden DM, et al. (2009) Estimation of the warfarin dose with clinical and pharmacogenetic data. *N Engl J Med* **360:**753–764.

Jacobsen P, Rossing K, Rossing P, Tarnow L, Mallet C, Poirier O, Cambien F, and Parving HH (1998) Angiotensin converting enzyme gene polymorphism and ACE inhibition in diabetic nephropathy. *Kidney Int* **53:**1002–1006.

Jin Y, Desta Z, Stearns V, Ward B, Ho H, Lee KH, Skaar T, Storniolo AM, Li L, Araba A, et al. (2005) CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. *J Natl Cancer Inst* **97:**30–39.

Kadakol A, Ghosh SS, Sappal BS, Sharma G, Chowdhury JR, and Chowdhury NR (2000) Genetic lesions of bilirubin uridine-diphosphoglucuronate glucuronosyltransferase (UGT1A1) causing Crigler-Najjar and Gilbert syndromes: correlation of genotype to phenotype. *Hum Mutat* **16:**297–306.

Kalow W and Gunn DR (1959) Some statistical data on atypical cholinesterase of human serum. *Ann Hum Genet* **23:**239–250.

Kalow W and Staron N (1957) On distribution and inheritance of atypical forms of human serum cholinesterase, as indicated by dibucaine numbers. *Can J Biochem Physiol* **35:**1305–1320.

Kalow W, Tang BK, and Endrenyi L (1998) Hypothesis: comparisons of inter- and intra-individual variations can substitute for twin studies in drug research. *Pharmacogenetics* **8:**283–289.

Keskitalo JE, Zolk O, Fromm MF, Kurkinen KJ, Neuvonen PJ, and Niemi M (2009) ABCG2 polymorphism markedly affects the pharmacokinetics of atorvastatin and rosuvastatin. *Clin Pharmacol Ther* **86:**197–203.

Kim MJ, Huang SM, Meyer UA, Rahman A, and Lesko LJ (2009) A regulatory science perspective on warfarin therapy: a pharmacogenetic opportunity. *J Clin Pharmacol* **49:**138–146.

Kohno M, Yokokawa K, Minami M, Kano H, Yasunari K, Hanehira T, and Yoshikawa J (1999) Association between angiotensin-converting enzyme gene polymorphisms and regression of left ventricular hypertrophy in patients treated with angiotensin-converting enzyme inhibitors. *Am J Med* **106:**544–549.

Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, Watkins PB, Daly A, Wrighton SA, Hall SD, et al. (2001) Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet* **27:**383–391.

Lamba JK, Lin YS, Thummel K, Daly A, Watkins PB, Strom S, Zhang J, and Schuetz EG (2002) Common allelic variants of cytochrome P4503A4 and their prevalence in different populations. *Pharmacogenetics* **12:**121–132.

Lee CR, Goldstein JA, and Pieper JA (2002) Cytochrome P450 2C9 polymorphisms: a comprehensive review of the in-vitro and human data. *Pharmacogenetics* **12:**251–263.

Li T, Chang CY, Jin DY, Lin PJ, Khvorova A, and Stafford DW (2004) Identification of the gene for vitamin K epoxide reductase. *Nature* **427:**541–544.

Lima JJ, Thomason DB, Mohamed MH, Eberle LV, Self TH, and Johnson JA (1999) Impact of genetic polymorphisms of the beta2-adrenergic receptor on albuterol bronchodilator pharmacodynamics. *Clin Pharmacol Ther* **65:**519–525.

Limdi NA and Veenstra DL (2008) Warfarin pharmacogenetics. *Pharmacotherapy* **28:**1084–1097.

Lin JH (2007) Pharmacokinetic and pharmacodynamic variability: a daunting challenge in drug therapy. *Curr Drug Metab* **8:**109–136.

Lin JH and Lu AY (2001) Interindividual variability in inhibition and induction of cytochrome P450 enzymes. *Annu Rev Pharmacol Toxicol* **41:**535–567.

Liu LW, Liu H, Chen GL, Huang YL, Han LL, Xu ZM, Jiang XJ, and Li YS (2010) Angiotensin-converting enzyme gene I/D genotype affected metoprolol-induced reduction in 24-hour average heart rate. *Chin Med J (Engl)* **123:**1382–1386.

Lu AY (1998) Drug-metabolism research challenges in the new millennium: individual variability in drug therapy and drug safety. *Drug Metab Dispos* **26:**1217–1222.

Lu AYH and Ma Q (2010) Pharmacogenetics and individualized medicine, in *ADME-Enabling Technologies in Drug Design and Development* (Zhang D and Surapaneni S eds), in press. Wiley & Sons, New York.

Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, et al. (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* **350:**2129–2139.

Ma Q and Lu AY (2003) Origins of individual variability in P4501A induction. *Chem Res Toxicol* **16:**249–260.

Ma Q and Lu AY (2008) The challenges of dealing with promiscuous drug-metabolizing enzymes, receptors and transporters. *Curr Drug Metab* **9:**374–383.
MacPhee IA, Fredericks S, Tai T, Syrris P, Carter ND, Johnston A, Goldberg L, and Holt DW (2004) The influence of pharmacogenetics on the time to achieve target tacrolimus concentrations after kidney transplantation. *Am J Transplant* **4:**914–919.
Mallal S, Nolan D, Witt C, Masel G, Martin AM, Moore C, Sayer D, Castley A, Mamotte C, Maxwell D, et al. (2002) Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. *Lancet* **359:**727–732.
Mallal S, Phillips E, Carosi G, Molina JM, Workman C, Tomazic J, Jägel-Guedes E, Rugina S, Kozyrev O, Cid JF, et al. (2008) HLA-B*5701 screening for hypersensitivity to abacavir. *N Engl J Med* **358:**568–579.
Martin AM, Nolan D, Gaudieri S, Almeida CA, Nolan R, James I, Carvalho F, Phillips E, Christiansen FT, Purcell AW, et al. (2004) Predisposition to abacavir hypersensitivity conferred by HLA-B*5701 and a haplotypic Hsp70-Hom variant. *Proc Natl Acad Sci USA* **101:**4180–4185.
Martinelli I, Sacchi E, Landi G, Taioli E, Duca F, and Mannucci PM (1998) High risk of cerebral-vein thrombosis in carriers of a prothrombin-gene mutation and in users of oral contraceptives. *N Engl J Med* **338:**1793–1797.
Matetzky S, Shenkman B, Guetta V, Shechter M, Bienart R, Goldenberg I, Novikov I, Pres H, Savion N, Varon D, et al. (2004) Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. *Circulation* **109:**3171–3175.
McLeod HL and Evans WE (2001) Pharmacogenomics: unlocking the human genome for better drug therapy. *Annu Rev Pharmacol Toxicol* **41:**101–121.
Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, Walker JR, Antman EM, Macias WL, Braunwald E, et al. (2009) Cytochrome P450 genetic polymorphisms and the response to prasugrel: relationship to pharmacokinetic, pharmacodynamic, and clinical outcomes. *Circulation* **119:**2553–2560.
Meyer UA (2000) Pharmacogenetics and adverse drug reactions. *Lancet* **356:**1667–1671.
Miyazaki M, Nakamura K, Fujita Y, Guengerich FP, Horiuchi R, and Yamamoto K (2008) Defective activity of recombinant cytochromes P450 3A4.2 and 3A4.16 in oxidation of midazolam, nifedipine, and testosterone. *Drug Metab Dispos* **36:**2287–2291.
Mwinyi J, Johne A, Bauer S, Roots I, and Gerloff T (2004) Evidence for inverse effects of OATP-C (SLC21A6) 5 and 1b haplotypes on pravastatin kinetics. *Clin Pharmacol Ther* **75:**415–421.
Nebert DW and Vesell ES (2006) Can personalized drug therapy be achieved? A closer look at pharmaco-metabonomics. *Trends Pharmacol Sci* **27:**580–586.
Nebert DW, Zhang G, and Vesell ES (2008) From human genetics and genomics to pharmacogenetics and pharmacogenomics: past lessons, future directions. *Drug Metab Rev* **40:**187–224.
Niemi M, Backman JT, Kajosaari LI, Leathart JB, Neuvonen M, Daly AK, Eichelbaum M, Kivistö KT, and Neuvonen PJ (2005) Polymorphic organic anion transporting polypeptide 1B1 is a major determinant of repaglinide pharmacokinetics. *Clin Pharmacol Ther* **77:**468–478.
Nishizato Y, Ieiri I, Suzuki H, Kimura M, Kawabata K, Hirota T, Takane H, Irie S, Kusuhara H, Urasaki Y, et al. (2003) Polymorphisms of OATP-C (SLC21A6) and OAT3 (SLC22A8) genes: consequences for pravastatin pharmacokinetics. *Clin Pharmacol Ther* **73:**554–565.
Ostapowicz G, Fontana RJ, Schiødt FV, Larson A, Davern TJ, Han SH, McCashland TM, Shakil AO, Hay JE, Hynan L, Crippin JS, Blei AT, Samuel G, Reisch J, Lee WM, and U.S. Acute Liver Failure Study Group (2002) Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. *Ann Intern Med* **137:**947–954.
Pasanen MK, Neuvonen M, Neuvonen PJ, and Niemi M (2006) SLCO1B1 polymorphism markedly affects the pharmacokinetics of simvastatin acid. *Pharmacogenet Genomics* **16:**873–879.
Pirmohamed M, Lin K, Chadwick D, and Park BK (2001) TNFalpha promoter region gene polymorphisms in carbamazepine-hypersensitive patients. *Neurology* **56:**890–896.
Poirier J, Delisle MC, Quirion R, Aubert I, Farlow M, Lahiri D, Hui S, Bertrand P, Nalbantoglu J, Gilfix BM, et al. (1995) Apolipoprotein E4 allele as a predictor of cholinergic deficits and treatment outcome in Alzheimer disease. *Proc Natl Acad Sci USA* **92:**12260–12264.
Raghavan N, Frost CE, Yu Z, He K, Zhang H, Humphreys WG, Pinto D, Chen S, Bonacorsi S, Wong PC, et al. (2009) Apixaban metabolism and pharmacokinetics after oral administration to humans. *Drug Metab Dispos* **37:**74–81.
Rettie AE and Tai G (2006) The pharmocogenomics of warfarin: closing in on personalized medicine. *Mol Interv* **6:**223–227.
Rieder MJ, Reiner AP, Gage BF, Nickerson DA, Eby CS, McLeod HL, Blough DK, Thummel KE, Veenstra DL, and Rettie AE (2005) Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose. *N Engl J Med* **352:**2285–2293.
Rocca B and Petrucci G (2010) Personalized medicine, pharmacogenetics, and clopidogrel: unraveling variability of response. *Mol Interv* **10:**12–19.
Roden DM, Altman RB, Benowitz NL, Flockhart DA, Giacomini KM, Johnson JA, Krauss RM, McLeod HL, Ratain MJ, Relling MV, et al. (2006) Pharmacogenomics: challenges and opportunities. *Ann Intern Med* **145:**749–757.
Rost S, Fregin A, Ivaskevicius V, Conzelmann E, Hörtnagel K, Pelz HJ, Lappegard K, Seifried E, Scharrer I, Tuddenham EG, et al. (2004) Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type 2. *Nature* **427:**537–541.
Sachidanandam R, Weissman D, Schmidt SC, Kakol JM, Stein LD, Marth G, Sherry S, Mullikin JC, Mortimore BJ, Willey DL, et al. (2001) A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature* **409:**928–933.
Sakaeda T, Nakamura T, Horinouchi M, Kakumoto M, Ohmoto N, Sakai T, Morita Y, Tamura T, Aoyama N, Hirai M, et al. (2001) MDR1 genotype-related pharmacokinetics of digoxin after single oral administration in healthy Japanese subjects. *Pharm Res* **18:**1400–1404.
Samson M, Libert F, Doranz BJ, Rucker J, Liesnard C, Farber CM, Saragosti S, Lapoumeroulie C, Cognaux J, Forceille C, et al. (1996) Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature* **382:**722–725.
Sata F, Sapone A, Elizondo G, Stocker P, Miller VP, Zheng W, Raunio H, Crespi CL, and Gonzalez FJ (2000) CYP3A4 allelic variants with amino acid substitutions in exons 7 and 12: evidence for an allelic variant with altered catalytic activity. *Clin Pharmacol Ther* **67:**48–56.
Schwab M, Schaeffeler E, Klotz U, and Treiber G (2004) CYP2C19 polymorphism is a major predictor of treatment failure in white patients by use of lansoprazole-based quadruple therapy for eradication of Helicobacter pylori. *Clin Pharmacol Ther* **76:**201–209.
Schwarz UI and Stein CM (2006) Genetic determinants of dose and clinical outcomes in patients receiving oral anticoagulants. *Clin Pharmacol Ther* **80:**7–12.
SEARCH Collaborative Group, Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, Gut I, Lathrop M, and Collins R (2008) SLCO1B1 variants and statin-induced myopathy—a genomewide study. *N Engl J Med* **359:**789–799.
Sesti F, Abbott GW, Wei J, Murray KT, Saksena S, Schwartz PJ, Priori SG, Roden DM, George AL Jr, and Goldstein SA (2000) A common polymorphism associated with antibiotic-induced cardiac arrhythmia. *Proc Natl Acad Sci USA* **97:**10613–10618.
Singer JB, Lewitzky S, Leroy E, Yang F, Zhao X, Klickstein L, Wright TM, Meyer J, and Paulding CA (2010) A genome-wide study identifies HLA alleles associated with lumiracoxib-related liver injury. *Nat Genet* **42:**711–714.
Smith MW, Dean M, Carrington M, Winkler C, Huttley GA, Lomb DA, Goedert JJ, O'Brien TR, Jacobson LP, Kaslow R, et al. (1997) Contrasting genetic influence of CCR2 and CCR5 variants on HIV-1 infection and disease progression. Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC), ALIVE Study. *Science* **277:**959–965.
Sparreboom A, Gelderblom H, Marsh S, Ahluwalia R, Obach R, Principe P, Twelves C, Verweij J, and McLeod HL (2004) Diflomotecan pharmacokinetics in relation to ABCG2 421C>A genotype. *Clin Pharmacol Ther* **76:**38–44.
Stegmeier F, Warmuth M, Sellers WR, and Dorsch M (2010) Targeted cancer therapies in the twenty-first century: lessons from imatinib. *Clin Pharmacol Ther* **87:**543–552.
Stern FB, Murthy LI, Beaumont JJ, Schulte PA, and Halperin WE (1985) Notification and risk assessment for bladder cancer of a cohort exposed to aromatic amines. III. Mortality among workers exposed to aromatic amines in the last beta-naphthylamine manufacturing facility in the United States. *J Occup Med* **27:**495–500.
Tantisira KG, Lake S, Silverman ES, Palmer LJ, Lazarus R, Silverman EK, Liggett SB, Gelfand EW, Rosenwasser LJ, Richter B, et al. (2004) Corticosteroid pharmacogenetics: association of sequence variants in CRHR1 with improved lung function in asthmatics treated with inhaled corticosteroids. *Hum Mol Genet* **13:**1353–1359.
Thervet E, Loriot MA, Barbier S, Buchler M, Ficheux M, Choukroun G, Toupance O, Touchard G, Alberti C, Le Pogamp P, et al. (2010) Optimization of initial tacrolimus dose using pharmacogenetic testing. *Clin Pharmacol Ther* **87:**721–726.
Tirona RG, Leake BF, Merino G, and Kim RB (2001) Polymorphisms in OATP-C: identification of multiple allelic variants associated with altered transport activity among European- and African-Americans. *J Biol Chem* **276:**35669–35675.
Tomlinson B, Hu M, Lee VW, Lui SS, Chu TT, Poon EW, Ko GT, Baum L, Tam LS, and Li EK (2010) ABCG2 polymorphism is associated with the low-density lipoprotein cholesterol response to rosuvastatin. *Clin Pharmacol Ther* **87:**558–562.
Tukey RH and Strassburg CP (2000) Human UDP-glucuronosyltransferases: metabolism, expression, and disease. *Annu Rev Pharmacol Toxicol* **40:**581–616.
Tukey RH, Strassburg CP, and Mackenzie PI (2002) Pharmacogenomics of human UDP-glucuronosyltransferases and irinotecan toxicity. *Mol Pharmacol* **62:**446–450.
Verstuyft C, Schwab M, Schaeffeler E, Kerb R, Brinkmann U, Jaillon P, Funck-Brentano C, and Becquemont L (2003) Digoxin pharmacokinetics and MDR1 genetic polymorphisms. *Eur J Clin Pharmacol* **58:**809–812.
Vesell ES (1989) Pharmacogenetic perspectives gained from twin and family studies. *Pharmacol Ther* **41:**535–552.
Voora D, Koboldt DC, King CR, Lenzini PA, Eby CS, Porche-Sorbet R, Deych E, Crankshaw M, Milligan PE, McLeod HL, et al. (2010) A polymorphism in the VKORC1 regulator calumenin predicts higher warfarin dose requirements in African Americans. *Clin Pharmacol Ther* **87:**445–451.
Wadelius M, Chen LY, Downes K, Ghori J, Hunt S, Eriksson N, Wallerman O, Melhus H, Wadelius C, Bentley D, et al. (2005) Common VKORC1 and GGCX polymorphisms associated with warfarin dose. *Pharmacogenomics J* **5:**262–270.
Wadelius M, Chen LY, Eriksson N, Bumpstead S, Ghori J, Wadelius C, Bentley D, McGinnis R, and Deloukas P (2007) Association of warfarin dose with genes involved in its action and metabolism. *Hum Genet* **121:**23–34.
Wang L, Zhang D, Raghavan N, Yao M, Ma L, Frost CE, Frost CA, Maxwell BD, Chen SY, He K, et al. (2010) In vitro assessment of metabolic drug-drug interaction potential of apixaban through cytochrome P450 phenotyping, inhibition, and induction studies. *Drug Metab Dispos* **38:**448–458.
Weber WW (1987) *The Acetylator Genes and Drug Response*, Oxford University Press, New York.
Weinshilboum R (2003a) Inheritance and drug response. *N Engl J Med* **348:**529–537.
Weinshilboum R (2003b) Richard Weinshilboum: Pharmacogenetics: The future is here! *Mol Interv* **3:**118–122.

Williams JA, Hyland R, Jones BC, Smith DA, Hurst S, Goosen TC, Peterkin V, Koup JR, and Ball SE (2004) Drug-drug interactions for UDP-glucuronosyltransferase substrates: a pharmacokinetic explanation for typically observed low exposure (AUCi/AUC) ratios. *Drug Metab Dispos* **32:**1201–1208.

Winnike JH, Li Z, Wright FA, Macdonald JM, O'Connell TM, and Watkins PB (2010) Use of pharmaco-metabonomics for early prediction of acetaminophen-induced hepatotoxicity in humans. *Clin Pharmacol Ther* **88:**45–51.

Wiviott SD, Trenk D, Frelinger AL, O'Donoghue M, Neumann FJ, Michelson AD, Angiolillo DJ, Hod H, Montalescot G, Miller DL, et al. (2007) Prasugrel compared with high loading- and maintenance-dose clopidogrel in patients with planned percutaneous coronary intervention: the Prasugrel in Comparison to Clopidogrel for Inhibition of Platelet Activation and Aggregation-Thrombolysis in Myocardial Infarction 44 trial. *Circulation* **116:**2923–2932.

Zhang D, Zhang D, Cui D, Gambardella J, Ma L, Barros A, Wang L, Fu Y, Rahematpura S, Nielsen J, et al. (2007) Characterization of the UDP glucuronosyltransferase activity of human liver microsomes genotyped for the UGT1A1*28 polymorphism. *Drug Metab Dispos* **35:**2270–2280.

Zhang D, He K, Raghavan N, Wang L, Mitroka J, Maxwell BD, Knabb RM, Frost C, Schuster A, Hao F, et al. (2009) Comparative metabolism of 14C-labeled apixaban in mice, rats, rabbits, dogs, and humans. *Drug Metab Dispos* **37:**1738–1748.

Zhang W, Yu BN, He YJ, Fan L, Li Q, Liu ZQ, Wang A, Liu YL, Tan ZR, Fen-Jiang, et al. (2006) Role of BCRP 421C>A polymorphism on rosuvastatin pharmacokinetics in healthy Chinese males. *Clin Chim Acta* **373:**99–103.

Zhou SF, Di YM, Chan E, Du YM, Chow VD, Xue CC, Lai X, Wang JC, Li CG, Tian M, et al. (2008) Clinical pharmacogenetics and potential application in personalized medicine. *Curr Drug Metab* **9:**738–784.