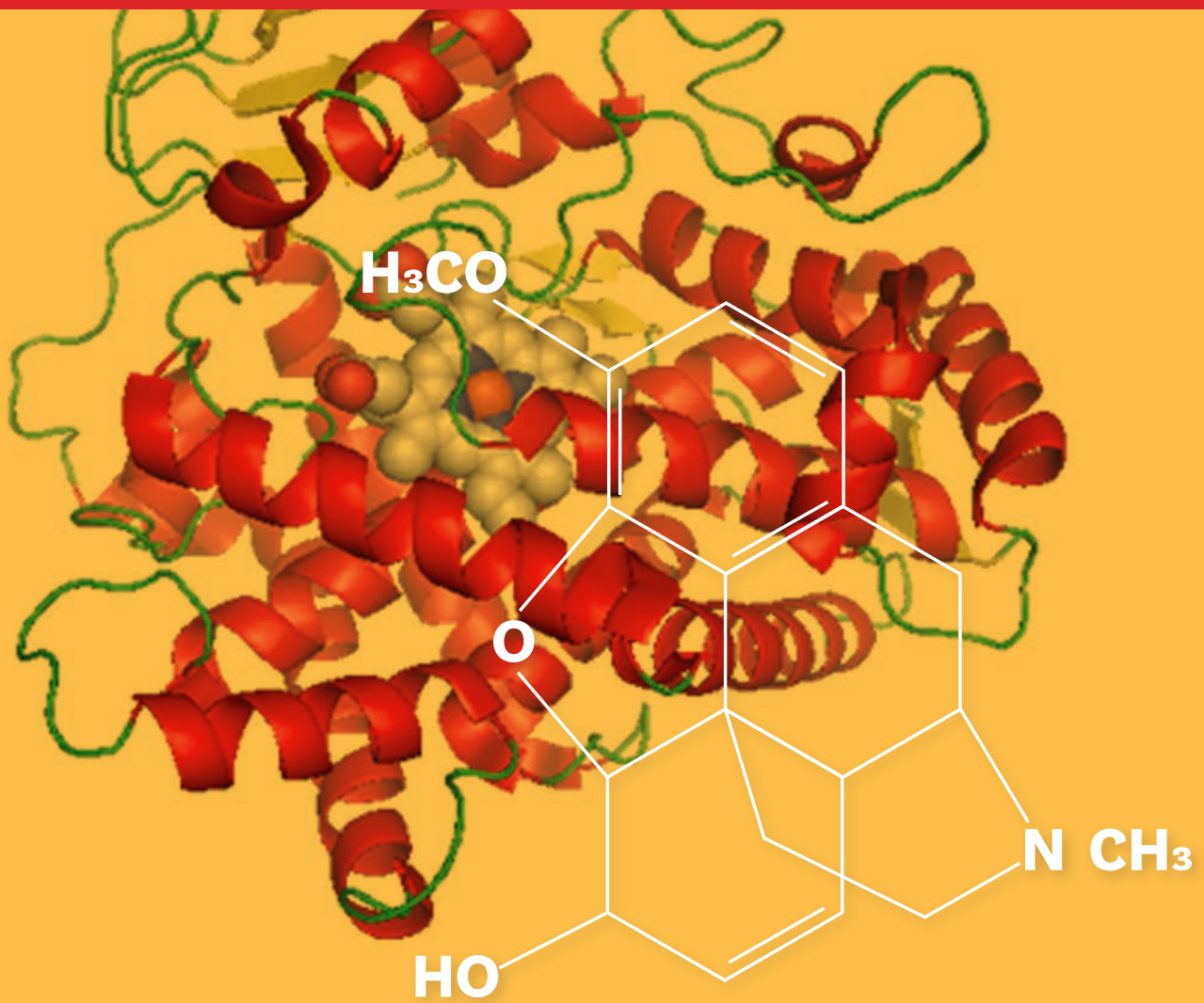
Pharmacogenomics in Clinical Therapeutics

Edited by
Loralie J. Langman and Amitava Dasgupta



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Preface

In recent years the media have focused on personalized medicine, thus increasing the general public's awareness regarding better therapy for various illnesses. After complete characterization of the human genome, there were expectations of translating these findings into better treatment of diseases as well as magic cures for genetically inherited diseases. In reality, there is always a significant time gap between discovery in basic science and its translation into application. The field of pharmacogenomics is no exception. In the 1970s, expansion of traditional therapeutic drug monitoring (TDM) services for drugs with narrow therapeutic indices certainly improved patient management by reducing incidences of drug toxicity by achieving personalized dosage of a particular drug for an individual. Pharmacogenomics is conceptually a step forward toward personalized medicine over TDM because it might be possible to predict the correct dosage. A good example is warfarin, where dosage based on a polymorphism of CYP2C9 and VCKOR1 has been stated in the package insert. Currently, in addition to warfarin, there is evidence that pharmacogenomics may be helpful in therapy with various antidepressants, immunosuppressants, cardioactive drugs, anesthetics, and analgesics. In addition, pharmacogenomics testing as well as TDM are both useful in managing patients infected with human immunodeficiency virus (HIV).

There are conflicting reports regarding the roles of pharmacogenomics in affecting therapy. This is particularly a problem with various psychoactive drugs because both genetic and environmental factors are known to affect the outcome of therapy. Other problems of pharmacogenomics testing are the costs of tests and reimbursement issues, especially from the federal government. Finding qualified technologists to perform these specialized tests is also challenging.

The goal of this book is to provide a comprehensive platform for readers to become familiar with the current state of pharmacogenomics in pharmacotherapy. Each chapter is written by experts in their field, covering all aspects of pharmacogenomics in clinical therapeutics which will be helpful for pharmacologists, toxicologists, clinical laboratory scientists, pathologists, and clinicians. Basic aspects of pharmacogenomics are discussed in Chapter 1 and also reviewed in each chapter as appropriate for the drugs discussed in these chapters for treating certain conditions. Therefore, readers do not need a background in pharmacogenomics to follow this book. However, readers with a background in pharmacogenomics will also be able to utilize this book as a quick handbook or reference, since at the end of each chapter, there is an extensive list of references for further advanced studies in this field.

We hope you enjoy reading this.

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CHAPTER 1
Pharmacogenomics Principles: Introduction to Personalized Medicine

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Introduction

Interindividual variability in drug response is a clinical reality, and one that has been long recognized by physicians and healthcare professionals. The essence of personalized medicine is the act of tailoring a treatment regimen to an individual based on their unique characteristics. However, our increasing understanding and sophistication in elucidating the causes of variability provide a new opportunity for an integrative and holistic personalized medicine – one that can synchronize all these factors together to deliver the right treatment, at the right dose, for every patient.

Although medications are typically marketed based on standard doses that are associated with safe and efficacious profiles in controlled clinical trials, these trials are not always representative of the clinical setting. In reality, patients differ widely in their response to treatment; while many may benefit from drug therapy, a proportion of individuals may be nonresponders, while others may develop adverse drug reactions. To truly deliver personalized medicine, one must have a grasp on the factors that contribute to variable outcomes in patients (Table 1.1) and how these factors may interact together in an individual. In the following sections, we will consider these sources of variation in more detail.

Factors that contribute to variability in drug response

Adherence, the extent to which a person's behavior – taking medication, following a diet, and/or executing lifestyle changes – corresponds with agreed recommendations from a health care provider (World Health Organization, 2003) (1), is a major, sometimes unrecognized, source of variability in the clinical setting. The term *adherence* is preferred over *compliance*, which denotes a passiveness on the part of the patient to follow the doctor's orders rather than establish a therapeutic alliance with their physician (1, 2). However, in many circumstances, the two words may be used interchangeably. Most physicians are unable to recognize nonadherence in their patients (2). Poor medication adherence accounts for 33–69% of all medication-related hospital admissions, and costs approximately $100 billion a year in the United States alone (2).

Of the different disease modalities, adherence to medications in chronic conditions is particularly low. For example, survey results in North America, the United Kingdom, and Western European countries indicate that no more than 30% of patients maintain target blood pressure levels despite receiving pharmacotherapy. Using a pill container with a computerized microchip to record the date and time the container was accessed, researchers were able to

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Table 1.1 Factors Contributing to Variability in Drug Response

Adherence
Age of the patient
Disease state
Drug–drug interactions
Food–drug interactions
Formulation
Gender
Genetics
Pollutants (smoking, etc.)
Pregnancy
Route of administration

demonstrate that up to half of the "failures" in reaching these target blood pressure levels could be associated with inconsistent patterns of medication use, which was different from what was prescribed (3). Interestingly, these lapses were often unrecognized by patients. Similarly in India, more than half of type 2 diabetic patients in one study were nonadherent with their oral hypoglycemic treatment regiments. Considering that India has the highest number of people affected by diabetes in the world (expected to reach 79 million individuals by the year 2030), this is a substantial problem (4).

Clearly, adherence to pharmacotherapy is an international issue (4, 5). An essential step in this direction is to understand the factors that influence adherence in the first place. Some of these predictors are summarized in Table 1.2. These predictors could be social and economic factors, the health care team or system, characteristics of the disease and disease-related therapies, and patient-related factors (1). Going back to our example of antihypertensive medications, one study in a cohort of over 80,000 Chinese patients prescribed antihypertensive identified the following factors that were associated with better adherence amongst patients: advanced age, female gender, payment of fees, adherence for attending appointments (i.e., attendance to specialist clinics and follow-up visits), and certain concomitant medications but not others (5). Overall, Chinese patients were more adherent to their antihypertensive medications (85% good compliance) than previously reported in studies of patients of Caucasian descent.

Table 1.2 Predictors of Poor Adherence

Asymptomatic disease
Cognitive impairment
Complexity of treatment
Cost of medications
Inadequate follow-up or discharge
Patient lack of belief in the treatment
Psychiatric illness
Poor provider–patient relationship
Side effect of medication

It has been postulated that increasing the effectiveness of adherence may have a far greater impact on population health than an improvement in a single area or specific treatment (6). Osterberg and Blaschke outlined four broad types of interventional methods to improve adherence: patient education (clear instructions that simplify the regimen, and information on the value of the treatment, side effects to be expected, and the effects of adherence toward achieving the health outcome), improved dosing schedules (minimizing total number of daily doses, and using medications with long half-lives or extended release formulations), increased accessibility to health care providers (longer clinic hours, shorter wait times, and removal of cost barriers), and improved communication between physicians and patients (2). Patient-tailored interventions that target adherence must be developed as part of the "personalized medicine" regimen.

Age is another important factor to consider in regard to variability in drug response. Throughout our life span, age-related physiological changes may affect the pharmacokinetics (absorption, distribution, metabolism, and elimination) of medications. Similarly, patients' response to medications (pharmacodynamics) may differ depending on age. The field of pediatric clinical pharmacology focuses on the developmental changes which influence pharmacokinetic profiles and drug response in infants and children. There are now many examples supporting the notion that children are not simply "small adults" when it comes to medication dosing requirements and response. For example,

developmental changes in the gastrointestinal tract can influence the rate and extent of bioavailability (7). Gastric acidity does not reach that of adult capacity until around 3 years of age, resulting in relatively increased absorption of acid-labile drugs such as penicillin and ampicillin in neonates (8). On the other hand, neonates may require larger oral doses of drugs that are weak acids, such as phenobarbital, in order to achieve therapeutic plasma levels (7).

The ontogeny and expression profiles of transporters and drug-metabolizing enzymes, key determinants of drug distribution and metabolism respectively, are also important factors to consider in children (7). One well-studied example is the commonly prescribed opioid morphine. Age-related development in morphine glucuronidation and clearance has been shown to correspond to progressive functional maturation of the liver and kidney (9). The mean plasma morphine clearance rate is about 4–5 times higher in children as compared to neonates (10, 11), while the average rate of glucuronidation is about 6–10 times higher in the adult livers as compared to liver from second trimester fetuses (12). The expression of the primary enzyme involved in morphine glucuronidation, uridyl glucuronyl transferase 2B7 (UGT2B7) (13, 14), is expected to reach adult levels at 2 to 6 months of age (15–17). Similar developmentally regulated ontogenically profiles have been reported for transporters (such as p-glycoprotein), and other drug-metabolizing enzymes such as cytochrome P450 2D6 and 3A4.

Clearly, extrapolation from adult dose regimens to children (on mg/kg bases) is often not appropriate. Given the widening gap between the number of adult clinical trials and pediatric clinical trials (18), there are a number of new incentives and international advocacy groups that are devoting their attention to increasing the number of high-quality pediatric drug trials in children. The ultimate goal is to develop pediatric-specific data that will result in age-appropriate diagnostics and guidelines for children, while decreasing the current practice of off-label and/or unlicensed use of medications in the pediatric setting.

Underrepresentation in clinical trials also poses similar problems in the elderly population, who will account for over 20% of the U.S. population by the year 2050. Problems related to polypharmacy, affecting more than 40% of the geriatric population (19), contributes to a disproportionately high incidence of adverse drug reactions in this age group. The relative contribution of physiological changes associated with the normal aging process in these adverse outcomes is not clearly defined. Factors such as declining hepatic drug-metabolizing enzyme functionality and neuronal changes with aging (20) may account for some of the differences in medication response as compared to younger adults. The sensitivity to drug-related side effects also increases with older age, with poor tolerability and adherence issues interfering with the benefits of treatment (21). Geriatrics-oriented clinical pharmacology will be a pivotal component of the personalized medicine toolbox for future health care professionals.

A third important variable to consider is drug–drug interactions. These interactions can affect the absorption, distribution, biotransformation, or excretion of one drug by another, and/or have consequences on drug action and effectiveness depending on the therapeutic window of the substrate. Sometimes drug interactions are intentional and beneficial, such as inhibiting an efflux transporter at the blood–brain barrier by one drug to allow the therapeutic drug to reach its target. Most often however, the consequences of drug interaction are unintentional and unfavorable, and can be associated with serious clinical consequences, such as transplant rejection (22). About 50–75% of medications are substrates of the cytochrome P450 (CYP) 3A4 enzyme, 2C9, and/or 2D6 metabolizing enzymes. Therefore, knowledge of how and which drugs are subject to metabolism by the cytochrome P450 pathway is an important way to predict potential problems if certain drugs are coadministered (23, 24). Drug interactions which affect the activity of transporters, whose role is to modulate the uptake and efflux of medications into and out of cells, may also have important clinical consequences. Interestingly, there is a profound overlap (in terms of substrates and modulators) between CYP3A and the ubiquitously expressed P-efflux transporter (25). It is also important to keep in mind that CYP3A substrates are not limited to medications, but can include

food products. The classic example of a food–drug interaction is that of felodipine with grapefruit juice, resulting in a clinically significant increase in plasma felodipine concentrations due to grapefruit juice's inhibitory effect on CYP3A4 (26). Smoking and exposure to pollutants, such as occupational exposure to pesticides, may also affect drug pharmacology due to the induction of drug-metabolizing enzymes.

In addition to adherence, age, and drug interactions, Table 1.1 lists other important sources of variation. The underlying disease or pathology is an important consideration as it may necessitate dosage adjustments depending on the scenario. Impaired renal function, for example, may result in toxicity with medications that rely primarily on renal clearance, such as digoxin (27). On the other hand, variances in drug formulations and manufacturing processes can affect the rate of drug entry *into* the system. Extended-release medications, for example, may rely on bead-based formulations that allow gastrointestinal fluid to dissolve and diffuse the drug out of the beads at a predetermined rate (28). The pharmacokinetic profile of such a medication will differ from its standard counterpart. The route of drug administration, whether enteral (oral or rectal) or parenteral (intravenous, intramuscular, inhalation, intradermal, subcutaneous, sublingual, or topical), can also affect the systemic concentration of the drug and its metabolites.

Gender differences refer not only to pharmacokinetic considerations such as differences in intramuscular absorption as a result of blood flow, but also to differences in health and lifestyle behaviors. The physiological changes associated with pregnancy, particularly, can substantially affect drug kinetics and response during the gestational and postpartum periods. Cardiovascular changes are particularly profound, including increases in maternal cardiac output by 30–50%, an increase in blood volume by 50%, increases in blood flow to the uterus and kidney, and increases in the resting heart rate (29). Increases in renal filtration and active drug transport affect the pharmacokinetics of renally cleared drugs such as amoxicillin (30) and digoxin. There are also alterations in the activity of maternal drug-metabolizing enzymes in the perinatal period.

Interindividual genetic variation

Patient genotype can account for a large proportion of drug response variability. Unfortunately, most physicians do not have knowledge of their patients' genotypes before prescribing medications. However, it is important to remember that genetic variation interacts with all the other sources of variability that influence drug response. Therefore, it best viewed as an integrative component of clinical pharmacology and therapeutics.

Genotype refers to an individual's full hereditary information. Genes can be viewed as the molecular blueprints that an individual is born with. *Phenotype* refers to the individual's actual, expressed properties. This could be molecules such as proteins, which are the products derived from the genetic blueprints. Phenotype can also refer to behaviors, actions, and diseases. Genetic studies try to deduce the associations between genotype and phenotype. While the majority of human genetic sequence is conserved between individuals, genetic variability does exist due to single-nucleotide polymorphisms (SNPs) and copy number variation (CNV).

A SNP is a base change in the genetic code that occurs at a population frequency above 1%. At the single nucleotide level, every two humans differ at 0.5–10 in 1000 bp (about 1 million SNP differences between individuals). Nucleotide base changes that occurs less commonly ($< 1\%$) are referred to as mutations. SNPs occur throughout the genome in both coding and noncoding regions. A SNP that occurs in the coding region may have functional consequences if the polymorphism changes amino acid composition (missense) or induces premature stop codons (nonsense), thereby affecting protein function. In contrast to these types of nonsynonymous polymorphisms, some SNPs in the coding region may not alter amino acid sequence and are thus silent (synonymous). Interestingly, it has been recently shown that even some synonymous SNPs can alter protein function and folding by altering the rate of ribonucleic acid (RNA) translation (31). In general, the majority of SNPs that occur in noncoding regions are silent, although they may affect gene expression (promoter SNPs) or RNA splicing (Figure 1.1).

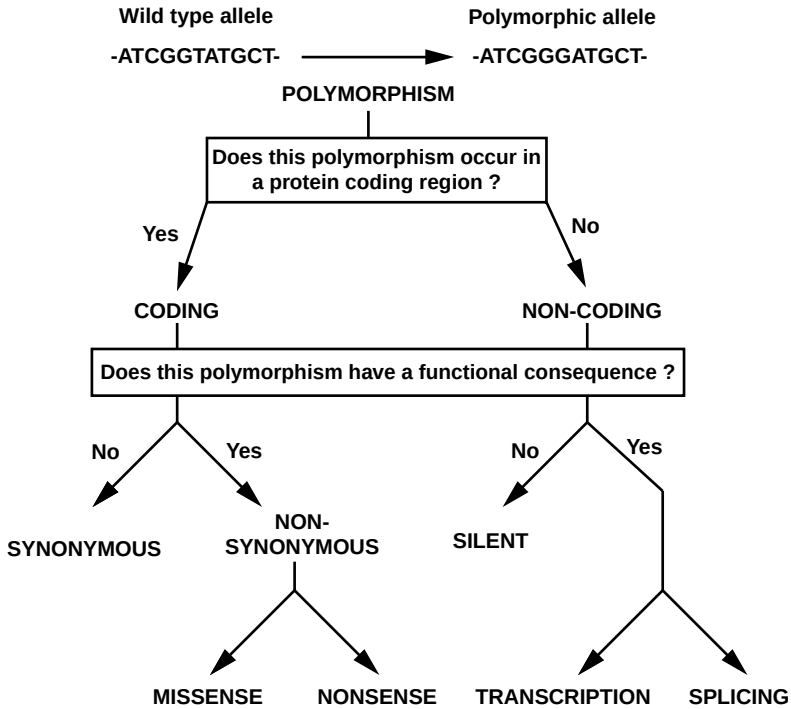

Figure 1.1 Single-nucleotide polymorphisms. A single point change of a nucleotide, here of the wild-type thymine (T) to a guanine (G), can occur throughout the genome in both coding and noncoding regions. More commonly, the polymorphism will not have a functional consequence (synonymous, or silent). However, functional consequences may arise if the polymorphism alters protein structure and function, or in noncoding regions, affects gene expression and splicing.

Copy number variation refers to DNA segments greater than 1,000 bases that are present at variable copy number (in comparison to the reference genome). CNVs are considered a substantial source of human genetic variation. Covering an estimated 12% of the human genome (32), the number of base pairs affected by CNVs is greater than the sum of all the SNPs across the genome combined (33). CNVs can consist of deletions and duplications which may arise from unequal crossover events during homologous recombination. There has been some success in our understanding of the clinical significance of certain CNVs with diseases, such as autism. Certainly in pharmacogenomics, CNVs have been identified in an important drug-metabolizing enzyme affecting the metabolism of many medications, as will be described below. However, complex disease–CNV associations are generally complicated by the fact that novel CNVs are found ubiquitously in each healthy control and patient that is genetically characterized. Therefore, the idea of the "reference genome" is constantly evolving. International repositories for human CNV data (such as the Database for Genomic Variants, hosted online by the Centre for Applied Genomics in Toronto, Canada) have been established to aid in this matter.

The field of pharmacogenetics and pharmacogenomics utilizes genetic information to predict both drug action (pharmacokinetics) and drug response (pharmacodynamics) for an increasing number of xenobiotics. While pharmacogenetic studies have traditionally focused on a single gene or several genes along a drug pathway, pharmacogenomic studies now more broadly utilize the entire or significant proportions of the genome. However in many contexts, the terms are used interchangeably.

Pharmacogenomics: history and current state

Despite the recent advancements in pharmacogenetics and genomics, the main principle guiding this field – that genetic variability can account for inter-individual differences in drug response and toxicity– was established decades before the first human genome was sequenced (34). These early pharmacogenetic studies aimed to elucidate the molecular and functional mechanisms of variability between individuals. These early studied formed the basis and rational behind therapeutic drug monitoring of certain drugs.

One of the first and classical pharmacogenetic examples is the antituberculosis drug isoniazid and *N*-acetyltransferase 2 (NAT2) variability in individuals. In the 1950s, isoniazid was introduced as a breakthrough drug in the treatment of tuberculosis. Shortly after its introduction, a heritable difference in the rate of isoniazid metabolism was observed (35). This variability was due to a liver enzyme that remained in the supernatant after centrifugation – this enzyme was discovered to be an acetyltransferase (36, 37), later identified as NAT2 (N-acetyltransferase 2). Peripheral neuropathy was frequently observed in "slow acetylators" of isoniazid. Mechanistically, it was shown that isoniazid competed for an enzyme involved in the pyridoxine (vitamin B6) pathway, and that administration of pyridoxine could prevent and reverse isoniazid-induced peripheral neuropathy (38).

Currently, the most severe and delimiting adverse drug reaction associated with isoniazid is not peripheral neuropathy but hepatotoxicity. While the exact mechanism of how isoniazid induces hepatotoxicity is not known, there is some evidence that slow acetylation status may play a role in shifting the metabolism of isoniazid into an elimination pathway that favors the production of toxic metabolites. Genetic polymorphisms, as well as nongenetic factors such as age, concomitant medications, and underlying liver disease, have also been shown to potentiate the hepatotoxic effects of isoniazid treatment. Nonetheless, isoniazid remains an important and globally used medicine today. Knowledge of the factors which contribute to variability in isoniazid response, prior to the administration of the medication, will be useful in maximizing the benefits of this therapy while minimizing the risk of liver toxicity.

Another globally important pharmacogenetic discovery that started its roots in the 1950s was the observation that hemolytic anemia developed in a minority of patients that were administered the antimalarial drug primaquine. This hemolysis was subsequently attributed to a deficiency in the glucose 6-phosphate dehydrogenase (G6PD) enzyme (39, 40). Over time, it was shown that not only primaquine but also other medications such as dapsone, methylthioninium chloride (methylene blue), nitrofurantoin, phenazopyridine, rasburicase, and tolonium chloride (toluidine blue) caused red blood cell destruction in G6PD-deficient individuals (41). While the exact mechanism of drug-induced hemolytic anemia is not known, primaquine and the other medications mentioned in the last sentence are chemical oxidants. The erythrocyte is the most susceptible cell type to oxidative stress, because the G6PD–NADPH pathway is the only source of reduced glutathione, an important endogenous antioxidant. In G6PD-deficient individuals, this pathway is blocked and oxidative stress resulting in hemolysis occurs. Sporadic hemolytic crises are also caused by certain infections and the ingestion of the fava bean (favism) in individuals with the "Mediterranean" enzyme variant. Numerous biochemical and genetic studies to date have identified over 300 abnormal G6PD variants resulting from approximately 100 diverse mutations. G6PD deficiency is the most common enzymopathy in the world, affecting approximately 400 million people. Presumably, one of the reasons for its widespread frequency, particularly in endemic parts of the world, is its conferred resistance to malaria.

In the late 1950s, Kalow and colleagues, observing marked variability in drug action among individuals that had received the muscle relaxant succinylcholine, identified the basis for a third pharmacogenetic association. In most individuals, the effect of succinylcholine after injection would last for several minutes before rapid degradation of the drug by plasma cholinesterase. However in a minority of patients, this paralysis effect was observed for hours (referred to as *succinylcholine apnea*). Using the ultraviolet

spectrophotometer, Kalow was able to demonstrate that in those patients who experienced succinylcholine apnea, there was a reduced cholinesterase binding affinity for its substrates, arising from a genetic alteration. Familial studies were also in line with this hypothesis of a genetic defect resulting in poor cholinesterase–succinylcholine binding interactions (42–47). This phenomenon was later linked to several functional variants in the butyrylcholinesterase gene (48, 49). The biochemical test Kalow developed to identify individuals susceptible to succinylcholine apnea is still used today (50).

The next wave of important pharmacogenetic studies came forth in the late 1970s and early 1980s. In Germany, Eichelbaum and colleagues were conducting pharmacokinetic studies on sparteine, an antiarrhythmic and uterine contractile (oxytocic) agent. In their study, two participants developed diplopia, blurred vision, dizziness, and headache following sparteine administration. Coincidentally, the plasma levels of sparteine in these two patients was several-fold higher than all the other subjects who had been administered the same dose of the drug. In addition, drug metabolites were not present in their urine and plasma, indicating minimal metabolism (51). Around the same time in Britain, Smith and colleagues were conducting pharmacokinetic studies on the new antihypertensive drug debrisoquine. In their study, it just so happened that the investigator, Smith, was also a study participant who took a standard oral dose of debrisoquine along with four other volunteers. Within 2 hours of drug administration, only Smith became dizzy, faint, and unable to stand, with blood pressure dropping to as low as 70/50 mmHg. While most symptoms improved, cardiovascular effects remained for several days after these events. Urine analysis revealed that Smith alone eliminated debrisoquine almost entirely as the parent compound with minimal metabolism, while the other subjects excreted the drug mainly in its metabolite form (52). These early studies illustrated the concept that the "dose of a drug was a poor predictor for patient response." Over the next several years, it was confirmed that sparteine and debrisoquine were metabolized by the same enzyme, aptly named sparteine/debrisoquine hydroxylase. Teams of researchers identified that this enzyme was a cytochrome P450, and that enzymatic deficiencies were inherited in an autosomal recessive fashion – the gene traced to chromosome 22 (53, 54). Today, this enzyme is more commonly referred to as cytochrome P450 2D6 (CYP2D6).

CYP2D6 is involved in the metabolism of a wide variety of medications (an estimated 25% of all drugs on the market) and has several recently suggested endogenous (55, 56) substrates (Table 1.3). However, CYP2D6 genetic variation has variable consequences for each of these listed substrates. Factors such as the reliance of the drug on the CYP2D6 pathway (i.e., the absence of other compensatory metabolic pathways), the therapeutic

Table 1.3 Substrates and Inhibitors of CYP2D6

CYP2D6 Substrates
For treatment of psychiatric and neurological disease Amitriptyline, atomoxetine clomipramine, clozapine, desipramine, duloxetine, fluoxetine, fluvoxamine, haloperidol, imipramine, levomepromazine, mirtazapine, nortriptyline, olanzapine, paroxetine, risperidone, sertraline, tetrabenazine, venlafaxine
For treatment of cardiovascular disease and/or eye disease Alprenolol, amiodarone, atenolol, bufuralol, bupranolol, debrisoquine, flecainide, indoramin, metoprolol, nimodipine, oxprenolol, propafenone, propranolol, quinidine, timolol
For treatment of pain Codeine (metabolite is active), hydrocodone (metabolite is active), oxycodone (metabolite is active), tramadol (metabolite is active)
Others Chlorpropamide, dextromethorphan, flunarizine, ondansetron, tamoxifen (metabolite is active), tropisetron, sparteine, 3,4-methylenedioxymethamphetamine ("Ecstasy"), amphetamine, methamphetamine
Endogenous substrates 5-methoxytryptamine (5-MT), 5-methoxy-*N,N*-dimethyltryptamine (5-MDMT), 6-methoxy-1,2,3,4-tetrahydro-β-carboline
CYP2D6 inhibitors Fluoxetine, fluvoxamine, paroxetine, quinidine, sertraline

window of the drug, and the relationship between drug plasma concentration and drug effect are all predictors of the importance of CYP2D6 perturbations for each drug action.

While the discovery of the polymorphic nature of CYP2D6 was partly based on the administration of a probe drug and urine collection to determine drug and metabolite concentrations over time "phenotyping," there has been much effort to infer phenotype directly by genotyping, prior to administering the drug to the individual (Figure 1.2). A direct genotyping approach certainly has its advantages: the administration of the probe drug may be dangerous for some patients that cannot be identified beforehand, genotyping is less labor intensive on the part of the patient, and genotyping only needs to be performed once in a person's lifetime and is not subject to temporary sources of variation such as concomitant drug administration. On the other hand, CYP2D6 genotype to phenotype correlations is complicated by the highly polymorphic nature of CYP2D6, with over 70 alleles identified, and by gene duplication events resulting in as many as 13 CYP2D6 copy number variants. In addition, two individuals with the same genotype may not have the same phenotype due to basal differences in gene expression, dietary and ethnic influences, and the presence of yet undiscovered novel variants. However, clinical tools such as the CYP2D6 activity score have been developed and validated in some populations to optimize such correlations (57).

Although genotyping provides a long-term source of information pertaining to an individuals' metabolic capacity, genotype interpretations should always be made with the consideration of concomitant medications that the patient may be taking at the current time. Particularly, prescribers should be aware that drug interactions may interfere with the genotype to phenotype prediction, in a phenomenon referred to as phenocopying (a drug–drug

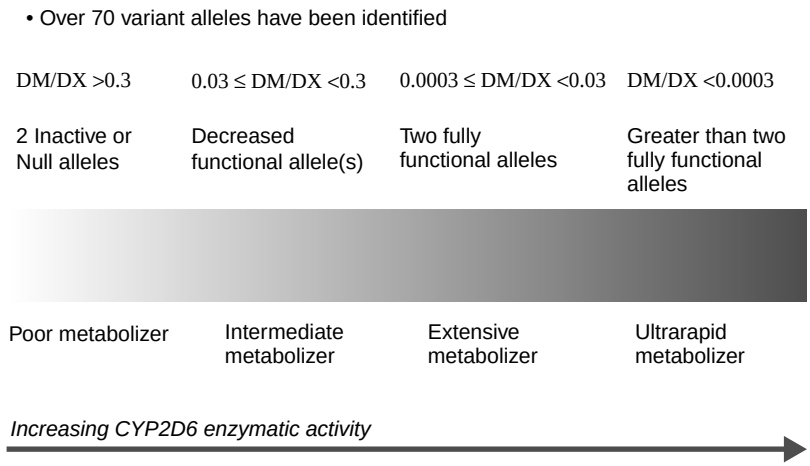

Figure 1.2 A gradient of CYP2D6 enzymatic activity – characterizing activity levels by phenotyping or genotyping. Characterization of CYP2D6 enzymatic activity may be achieved by phenotyping an individual. Here, a CYP2D6 specific probe drug such as dextromethorphan is administered, and the relative ratio between the concentration of probe drug (dextromethorphan: DM) and its CYP2D6-specific metabolite (dextorphan: DX) is obtained. Those with higher DM:DX ratios have a higher proportion of unmetabolized parent drug to metabolite, thus exhibiting limited CYP2D6 metabolic capacity (poor metabolizers). Conversely, very small DM:DX ratios depict extensive CYP2D6 metabolic capacity in the individual (extensive to ultra-rapid metabolizers). Alternatively, one may indirectly infer enzymatic activity by genotyping the individual and assigning the individual to a metabolizer category (poor, intermediate, extensive, or ultra-rapid) based on the inferred functional consequence of their genotype. Although four phenotypic classes are commonly used, the cutoffs between the categories are not distinct, as there is a gradient of activity levels in the population. *Note*: DM: dextromethorphan, a CYP2D6 substrate. DX: dextrorphan, a CYP2D6-specific metabolite of dextromethorphan.

interaction can inhibit the CYP2D6 enzyme such that an individual with a genotype associated with extensive CYP2D6 metabolism will be phenotypically similar to an individual who has a poor metabolizer genotype).

The other important pharmacogenetic discovery that arose in the early 1980s was that of thiopurine *S*-methyltransferase (TPMT) polymorphisms. TPMT is a cytosolic enzyme involved in the metabolism of thiopurine drugs such as 6-mercaptopurine and azathioprine. Analyzing TPMT enzymatic activity from the red blood cells (RBC) of 298 randomly selected and unrelated Caucasian subjects, Weinshilboum and Sladek (58) reported three distinct activity profile cohorts (high-activity individuals [88.6%], intermediate-activity ones [11.1%], and those of undetectable activity [0.3%]). The authors also confirmed, via familial studies, that this variability in TMPT enzymatic activity was an inherited trait. Subsequently, it was shown that undetectable or low TPMT activity, occurring in about 1/300 individuals, was a major risk factor for the development of life-threatening azathioprine-induced myelosuppression in patients receiving "standard" doses of the drug (59). Similar adverse drug reactions were also reported in TMPT deficient patients receiving 6-mercaptopurine (60). Mechanistically, these adverse drug reactions develop as a result of an accumulation of cytotoxic thioguanine nucleotides, which are normally responsible for the therapeutic effect of thiopurines, but at high concentrations may cause severe toxicity. Thus, TMPT-deficient individuals require 10–15-fold lower doses than in those who possess high-functioning TMPT enzyme activity. Since the 1990s, the red blood cell (RBC) TPMT activity assay (61) and/or the RBC 6-thioguanine assay (which is inversely correlated to the RBC TMPT assay) (62) have been used in some institutions as a method to help determine optimal thiopurine doses in patients.

Following the cloning and characterization of the *TMPT* gene in the early 1990s, the molecular basis for the observed phenotypic variations has been better defined. Currently, there have been 30 SNPs identified in *TPMT* (63), most of which have been associated with decreased enzymatic activity. The frequency of these SNPs varies amongst different ethnic groups; TPMT*3A, for example, is the most common low activity variant in Caucasians (5% frequency), while the *3C is the major variant allele in African Americans and East Asian populations (64). In Caucasians, over 95% of cases of inherited TPMT deficiency can be detected by assaying for the *TMPT*3A, *3C*, and *2*. Interestingly, trinucleotide repeat variants in the promoter of *TPMT* have recently been identified in a subset of individuals (1–2%), that exhibit extremely high enzyme activity (65). These individuals may actually require higher than standard thiopurine doses to achieve therapeutic effect.

Despite the well-established clinical significance of *TPMT* polymorphisms, the validated biomarkers for *TPMT* testing, and the safety information included on the FDA drug label, the uptake of *TPMT* genetic testing to determine optimal treatment for acute lymphocytic leukemia has been variable in the United States (66). Specifically in regard to *TMPT*, the rarity of the potentially fatal adverse drug reaction (1 in 300 to 400 patients) may mean that some physicians will never have a patient that experienced such toxicity in the absence of testing (66). Moreover, some oncologists argue that the pharmacodynamic response, measured as a decrease in leukocyte counts, has the same sensitivity and predictive value. Factors such as financial or logistical roadblocks to accommodate pharmacogenetic testing and patient and prescriber education in regard to treatment management options may contribute to this variable uptake and will be discussed in the subsequent section.

Pharmacogenomics and translational approaches

Although the terms *pharmacogenetics* and *pharmacogenomics* are used interchangeably, on a philosophical level, the word pharmacogenomics represents a more comprehensive way of thinking about the influence of genes on drug response. From the clinical pharmacology perspective, since the 1960s, when Dr. Werner Kalow wrote the first textbook in pharmacogenetics entitled *Pharmacogenetics: Heredity and the Response to Drugs*, to five decades later in the new millennium, our understanding of the multifactorial nature of drug response and

variability has become more apparent. In the words of Kalow, "Pharmacogenetics arose with studies of single genes, which had major effects on the action of particular drugs. It turned into pharmacogenomics through realization that the controls of most drug responses are multifactorial" (67). From the genomics perspective, such realization was inextricably tied with the rapid advances in molecular biology based on user-friendly technological platforms that could scan large or complete proportions of the genome in a rapid and increasingly cost-effective manner.

Moving away from single gene studies, current approaches may involve genetic markers along an entire drug metabolism and response pathway. The polymorphisms in the major human drug-metabolizing enzymes and their pharmacokinetic effects have been well studied; the emphasis now is on the characterization of drug transporter and receptors polymorphisms and the synergistic effects of variation amongst the entire drug pathway. Another approach is genome-wide association studies (GWAS), which can assess over a million SNPs depending on the assay platform. This hypothesis-generating technique has contributed to over 800 unique SNP–trait associations for common diseases within the past decade (68). However, GWAS studies have traditionally been performed in those with European descent, and most commercially available SNP microarrays cannot capture variation in non-Caucasian ethnicities. Furthermore, rarer and novel variants are less likely to be identified. Thus, the more attractive, increasingly feasible, and comprehensive option, which is at the cutting edge of genomic science, is whole exome sequencing or whole genome sequencing.

By most predictions, as a consequence of the rapidly decreasing costs, whole genome sequencing will become routinely available within the next 5 to 10 years. Already, it has been demonstrated that clinically meaningful pharmacogenomic and disease risk information can be obtained in a clinic setting using the information derived from the sequencing of one patient's whole genome (69). Such comprehensive genomic techniques are able to identify novel variants and stimulate a revolution in our thinking of disease and drug response (70), though not without its challenges (71) (Table 1.4). In contrast to the mechanistic approaches which assessed the causative effect of genetic polymorphisms in early "phenotype-to-genotype" pharmacogenetic studies, the functional effect of most variants that arise from a whole genome sequence is unknown. Thus, there is a significant diagnostic uncertainty about the meaning of the results. On one hand, the collection of large data sets from carefully phenotyped patients contributes to our knowledge of the clinical significance of these variants, which will ultimately advance our understanding of countless medical conditions in the future. In addition, the patient whose whole genome is sequenced today must be made aware that most sequence information obtained from their genome will be of unknown meaning, and under the pretense of a rapidly changing knowledge base.

This rapidly changing knowledge base will mean that genomic interpretations may change over time.

Table 1.4 Use of Whole Genome Sequence in Clinical Practice

1. The broad scope of the results will require that patients receive complex and detailed information before they decide whether to be tested.
2. Interpretation of genome sequences should take into account the limits of the sequencing method used.
3. Easily accessible and well-curated information about the links between genomic sequences and diseases needs to be created, maintained, and frequently updated.
4. Physicians and patients will have to cope with enormous uncertainty in some results, particularly around variants of unknown importance, which might require analysis of genetic information from family members.
5. Effective ways to convey meaningful information to patients about the many implications of their whole genome sequences need to be developed and training for appropriate specialists to convey this information funded.
6. Whole genome sequences will need to be reviewed regularly to incorporate new information about disease risks, and changes in assessment will have to be conveyed to patients.

Source: From Ormond KE, Wheeler MT, Hudgins L, Klein TE, Butte AJ, Altman RB, *et al*. (2010). Challenges in the clinical application of whole-genome sequencing. *Lancet* 375 (9727):1749–51. Copyright Elsevier, Reprinted with permission.

It also necessitates the need for publically available, user-friendly, and frequently updated databases for prescribers and patients, such as the Pharmacogenomics Knowledge Database (PharmGKB; see www.pharmgkb.org), supported by the National Institute of Health and the National Institute of General Medical Sciences. Such user-friendly translational interfaces are becoming an important component of an overall movement toward wide-scale educational and translational genomic strategies.

Educational and translational genomic strategies are needed in every aspect of science and medicine in this post-human genome sequencing era. Scientists and researchers will need to gain evaluative skills to be able to utilize the large quantities of data that are derived from genome sequencing. The emergent field of translational bioinformatics is uniquely positioned to make major advances in this area. Translational bioinformaticians integrate molecular and clinical data to enable novel translational hypotheses bidirectionally between the domains of biology and medicine (72). According to the American Medical Informatics Association, translational bioinformatics refers to "the development of storage, analytic, and interpretive methods to optimize the transformation of increasingly voluminous biomedical data, and genomic data in particular, into proactive, predictive, preventive, and participatory health." (73)

Major initiatives are currently underway to improve the competency of health care providers in the field of genomics. Traditionally, the field of medical genetics was devoted to the study of relatively rare single-gene or chromosomal disorders in primarily tertiary care settings by specialists. With advances in pharmacogenomics and the elucidation of multiple genomic contributions toward more common and complex conditions, genomic information is moving into the "medical mainstream" (74). However, the interpretation of genomic markers for these more common diseases is not as straightforward as rare, highly penetrant single gene–disease associations, given the interplay between genetic and environmental factors. Recognizing that health care professionals will increasingly use genetic and genomic information to meet the needs of their patients, essential genomic competencies, practice guidelines, and curricular resources in genetics and genomics are being developed across medical disciplines (75).

The literacy of the public in genomics also needs to be improved. The general public has limited knowledge of genetic risk factors as a cause of multifactorial disease and even less knowledge of how and why these factors affect health (76). These initiatives are particularly imperative in the current atmosphere of direct-to-consumer genetic testing companies, which bypass the medical system to deliver and market genetic information directly to the shopper. The public needs to develop an understanding of the limitations of genetic testing in order to critically appraise marketed genetic tests. They also need to be informed on the ethical, social, and legal issues surrounding genetic information and genetic testing.

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