Pharmacogenomics in Clinical Therapeutics

Edited by Amitava Dasgupta
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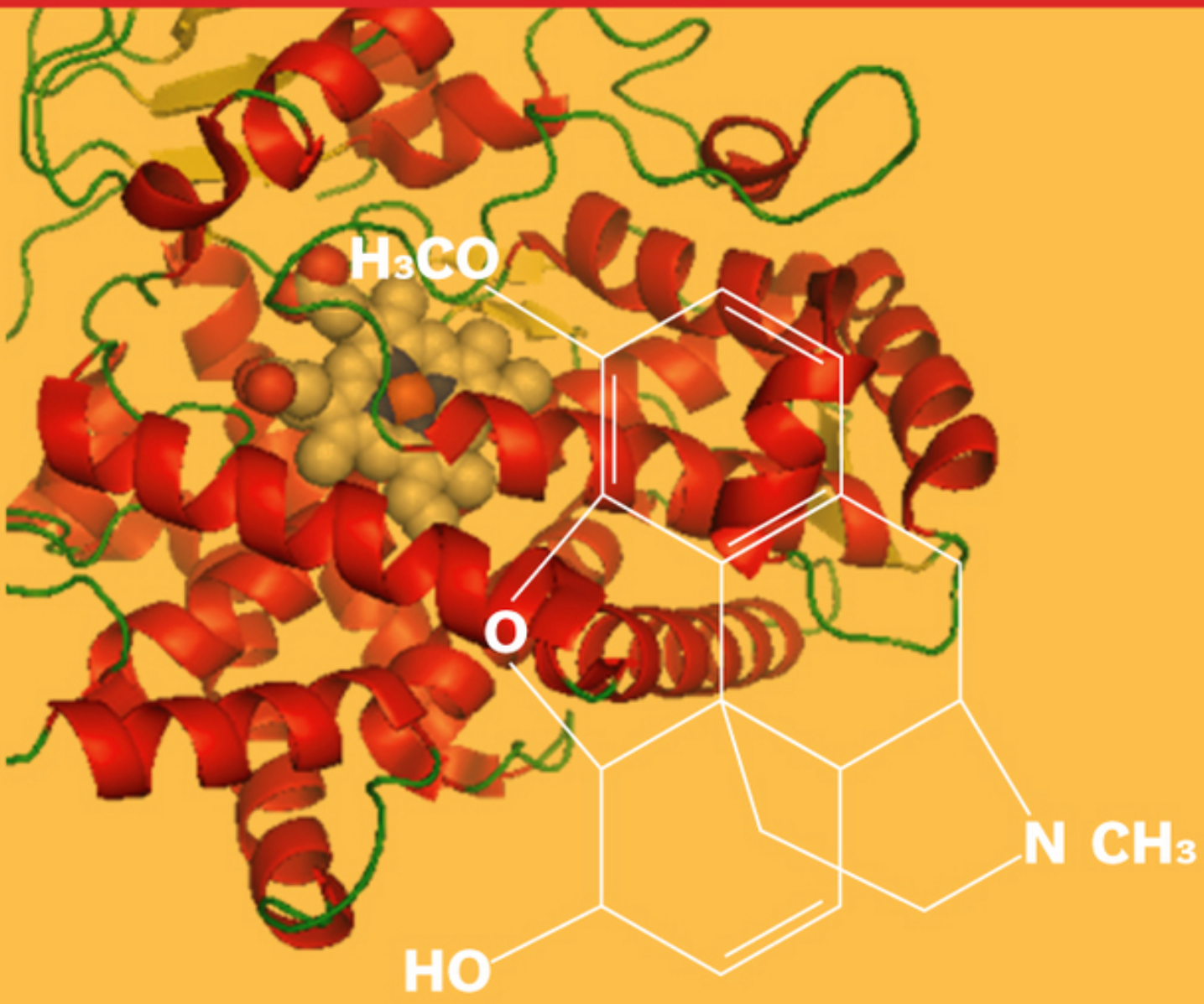

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Preface

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.

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

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 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

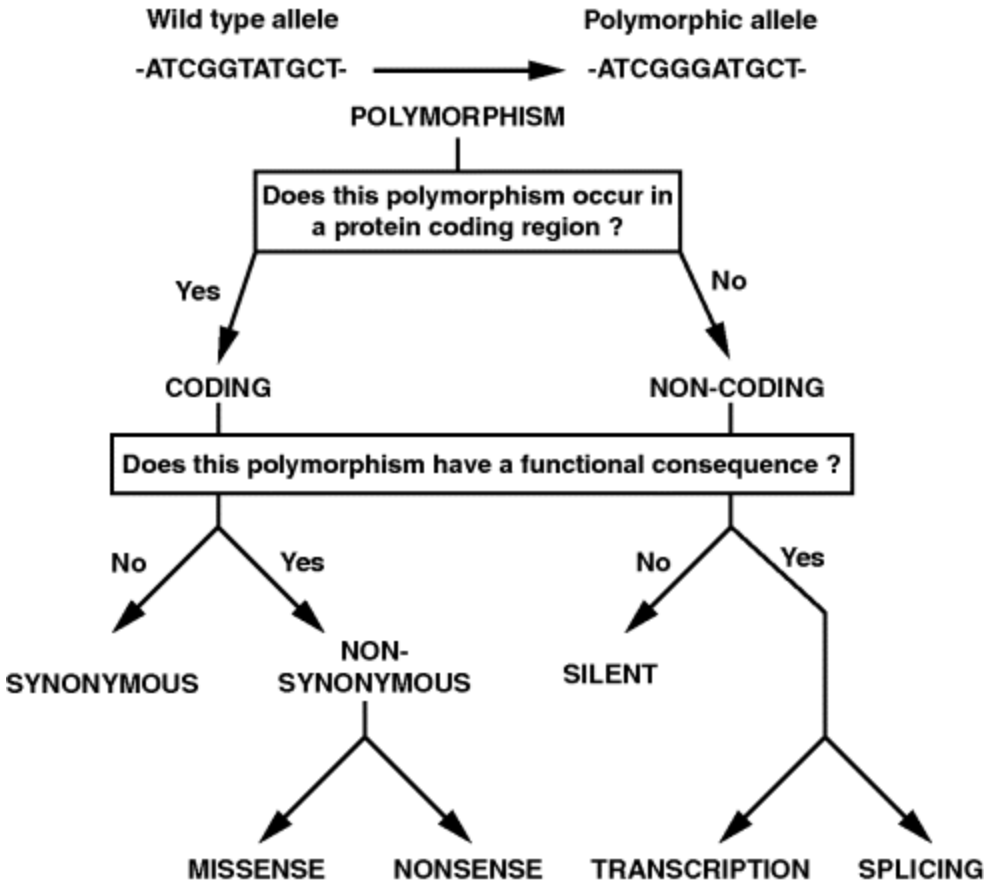
Patient genotype can account for a large proportion of drug response variability. Unfortunately, most physicians do not have knowledge of their patients' genotypes before prescribing medications. However, it is important to remember that genetic variation interacts with all the other sources of variability 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 interindividual 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