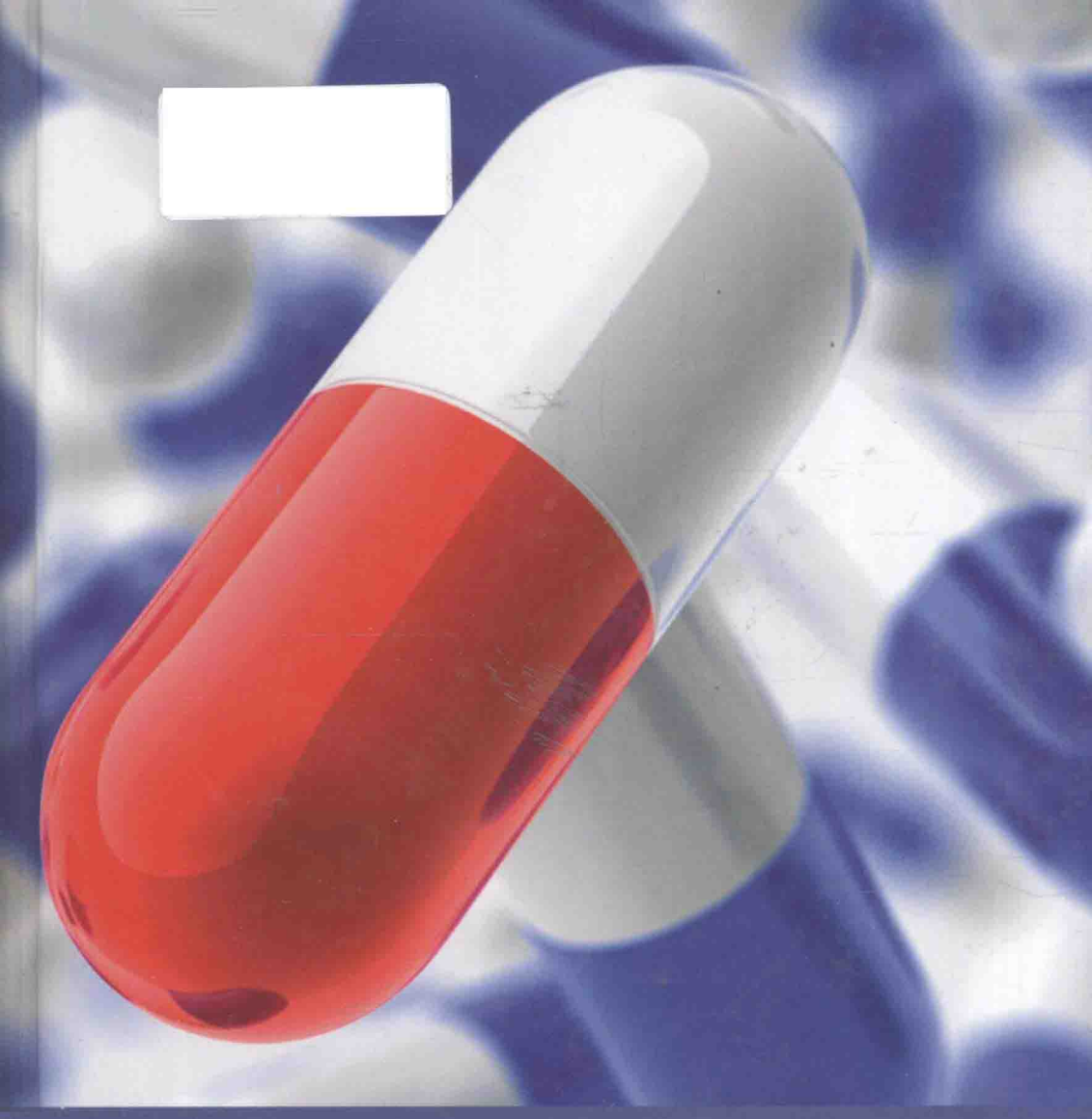

An Integrated Study of Drug Metabolism

Erica Helmer

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Edited by **Erica Helmer**

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Preface

Every book is a source of knowledge and this one is no exception. The idea that led to the conceptualization of this book was the fact that the world is advancing rapidly; which makes it crucial to document the progress in every field. I am aware that a lot of data is already available, yet, there is a lot more to learn. Hence, I accepted the responsibility of editing this book and contributing my knowledge to the community.

Late-stage drug failure can occur due to various factors like unwanted metabolic instability, drug-drug interactions, toxic metabolites, and polymorphic metabolism. To meet the purpose of preventing this failure; sincere effort has been employed by academia as well as the pharmaceutical industry for the development of more effective methods and screening analysis to recognize the metabolic enzymes and profiles involved in drug metabolism. The book elucidates detailed reviews of chosen topics in drug metabolism. The important topics that have been described in this book are: impact of genetic and epigenetic factors on drug metabolism; use of new microdosing methods and new LC/MS and genomic techniques to conclude the metabolic parameters and profiles of potential novel drug candidates; the interaction between metabolism and drug transport in oral biodiversity; and the effect of disease on metabolism and transport.

While editing this book, I had multiple visions for it. Then I finally narrowed down to make every chapter a sole standing text explaining a particular topic, so that they can be used independently. However, the umbrella subject sinews them into a common theme. This makes the book a unique platform of knowledge.

I would like to give the major credit of this book to the experts from every corner of the world, who took the time to share their expertise with us. Also, I owe the completion of this book to the never-ending support of my family, who supported me throughout the project.

Editor

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Pharmacogenetics and Metabolism: Past, Present and Future

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1. Introduction

Throughout history, humanity has referred to toxic reactions in response to food, plants, and more recently, medications or drugs. **Pythagoras** is thought to be one of the first to observe that some individuals, but not all, would get sick after eating fava beans. Introduction of the complex term *pharmakon* (a word used to designate substances that may produce beneficial or harmful effects to human health) by Hippocratic physicians brought with it a paradox: a compound may be served both as a drug and a poison at the same time. As it is currently known, administration of a substance may offer higher risk of toxic effects than when administered at a lower dose. But then, what about a drug dosage that induces toxicity in a patient, treats another well and had no effects on the other? This and other important observations such as the understanding of metabolic variability have started to unravel the mysteries behind these phenomena. For example, the ancient observation why different outcomes were regularly observed after Greek soldiers ate fresh fava beans (Nebert 1999).

The concept of **'variability in metabolism'** claims that biochemical processes within the organism are responsible for transformation of compounds from food or medicines, and that this process could be different among subjects leading to a range of organic responses. Alexander Ure (1841) seems to have been the first to report organism's ability to convert an exogenously administered compound into one or more different metabolites. In the study entitled '*On Gouty Concretions with a New Method of Treatment*', Ure reported that benzoic acid was converted to hippuric acid by humans (Ure 1841). Then, it gradually began to be accepted that living systems have a "physiological chemistry" responsible for the modification of substances, and from the second part of 19th century, a significant number of metabolic pathways have been discovered.

At that time, however, the key answer to why there is individual variability in metabolism had not yet been answered, which only came to light after the rediscovery of Mendel's study about hereditary around the turn of the 20th century. A well-known example to illustrate this was the influence of the genetic findings of William Bateson on studies of Sir Archibald Garrod about alkaptonuria and phenylketonuria presented in the book entitled "*Inborn Errors of Metabolism*" (Garrod 1909). In fact, the results founded by Garrod were a milestone to explanation of metabolic variability from a genetic perspective. Thereafter, some forerunners separately described series of observations that preceded the conceptualization of the Pharmacogenetics (PGx). In 1932, Arthur Fox found a remarkable variation in the ability of some individuals to

taste a foreign chemical phenylthiocarbamide (PTC) (the 'taste blindness') (Fox 1932). Interestingly, this finding was unexpectedly discovered when some of the PTC molecules escaped in to the air and Fox's co-worker C.R. Noller noticed a bitter taste, while Fox could not taste it. Intrigued by Fox's findings about bitter taste, L.H. Snyder published an important study (especially for the relevant quantity of participants) confirming the Fox's observation that some people perceive the bitter taste of PTC, while others do not. In addition, Snyder found that such non-tasting is a recessive genetic trait, and today it is one of the best-known Mendelian traits in human populations (Snyder 1932).

In a similar fashion, animal studies at that time also supported the genetic contribution in drug metabolism variability. Sawin and Glick (1943) in the study entitled "*Atropinesterase, a Genetically Determined Enzyme in the Rabbit*" demonstrated a genetically determined outcome in rabbits after ingestion of the belladonna leaves (Sawin & Glick 1943).

In the middle of 20th century, evidences necessary to support the transformation of the scattered "pharmacological heritability" in a new science finally appeared. First, Hettie Hughes described a relation between the level of isoniazid (an anti-tuberculosis drug) acetylation and occurrence of peripheral neuritis (Hughes et al. 1954), which absolutely was a landmark step for future demystification of the "one size fits all" system of drug prescribing. Another milestone in PGx was an independent investigation about death of patients caused by a generally safe, local anesthetic drug procaine (Kalow 1962). Further experiments enabled the authors to suggest that a genetically determined alteration in the enzyme structure may cause an abnormal and lethal low cholinesterase activity. The atypical enzyme does not hydrolyze the anesthetic with efficacy, resulting in a prolonged period of high levels of the drug in the blood and increased toxicity (Kalow 2005). Simultaneously, Alf Alving and co-workers observed that African-American soldiers presented an increased risk to develop acute haemolytic crises after primaquine (an antimalarial drug) administration, when compared with Caucasian ones (Clayman et al. 1952). As shown later, this sensitivity is caused by a genetically determined deficiency of glucose 6-phosphate dehydrogenase (G6PD), which alters erythrocyte metabolism (Alving et al. 1956). Thus, it took approximately 2,400 years to explain the Pythagoras observation about favism from a molecular perspective. It is now believed that defect in the *G6PD* gene is related with fava-induced hemolytic anemia in some individuals of Mediterranean descent.

Finally, opening a new era of pharmacological investigation, Arno Motulsky in 1957 published a masterpiece paper entitled "*Drug reactions, enzymes and biochemical genetics*", highlighting the genetic basis of "how hereditary gene-controlled enzymatic factors determine why, with identical exposure, certain individuals become 'sick', whereas others are not affected" (Motulsky 1957). The works of Kalow and Motulsky were (and still are) an unequivocal scientific catalyzer for understanding of the genetic influence in drug metabolism. Friedrich Vogel, a German Pharmacologist in 1959 was the first to coin the term 'Pharmacogenetics' (PGx) for the emergent new area of scientific discoveries, that unifies different conceptions on pharmacotherapy and xenobiotic-induced disease risk (Vogel 1959).

Despite the terms *Pharmacogenetics* and *Pharmacogenomics* are used interchangeably, most authors prefer to use PGx when inherited differences in drug response are being evaluated. On the other hand, Pharmacogenomics is usually used to study general aspects of drug response involving genomic technologies to determine a drug profile or even a new drug. Although is common association between PGx and drug metabolism variation, many others

inherited differences in drug response are investigated by PGx, such as polymorphisms in genes that encode molecules transporters (Vaalburg et al. 2005) and drug targets (Johnson & Liggett 2011; Maggo et al. 2011). For practical purposes, preference will be given to the use of the term Pharmacogenetics throughout this chapter.

2. Metabolizers subpopulations, a brief review

Since physiological responses associated with a particular drug have been linked to biochemical attributes in the body of the recipient, several studies have attempted to elucidate which factors modify the clinical response to a greater or lesser extent. There is now a general understanding that variability in the function of **drug-metabolizing enzymes (DME)** is responsible for many differences in the disposition and clinical consequences of drugs. Although it is a central issue to PGx, in clinical practice most decisions about a medicine prescription are mainly based on the classic factors responsible for drug variability, including co-existing disease (especially those that affect drug distribution, absorption or elimination), body mass, diet, alcohol intake, interaction with others drugs and mechanisms to improve patient compliance. In fact, all of these have been demonstrated to directly affect the indicated dose of the drug. However, they only partly explain why most major drugs are effective in only 25 to 60 percent of patients. Furthermore, taking into account patients with same physical and demographic characteristics, why does a standard dose is toxic to some patient but not to others? Why not all patients demonstrate the expected efficacy in drug treatment trials? Undoubtedly, these and many others questions opened the door for a new era of the personalized medicine and treatment perspectives (Nair 2010).

It is well-know that drug levels can be raised by increasing the dose or by more frequent administration in a non-responder patient. Conversely, if a higher plasmatic drug level with a standard dose administration is expected (in a patient with cirrhosis or malnutrition, for example), increasing the time of administration or suspending the dose may be a reasonable attitude. Although advances in medical technology and potential predictive models have improved the choice of dose, they are not yet sufficient to prevent high level of morbidity and mortality caused by **adverse drug reactions (ADR)**, as shown in the clinical practice (Wu et al. 2010). Thus, it is believed that the study of how genetic variation interface with drug metabolism, especially in genes codifying DMEs, may also lead to improve drug safety.

A variety of factors affecting the expression and activity of DMEs are classified into three major groups: **genetic factors**, **non-genetic host factors** (such as diseases, age, stress, obesity, physical exercise, etc.) and **environmental factors** (environmental pollutants, occupational chemicals, drugs, etc.). Recent studies clearly indicate that interindividual variation in drug metabolism is one of the most important causes of drug response differences. In general, common pharmacokinetic profile is a lighthouse for most prescribers in clinical practice. Figure 1A exemplify a simplified model of a drug biotransformation route. Most pharmaceuticals compounds or molecules (M1 in figure 1) when administrated orally are lipid-soluble enough to be reabsorbed (in the kidneys) and eliminated slowly in small amounts in an unchanged form in urine. Therefore, drug biotransformation by enzymes (represented by E1) has a key role in the control of plasmatic drug concentration. It should be remembered that the metabolites (M2) might also exert pharmacological effect (which will be discussed later). In addition, low activity of the

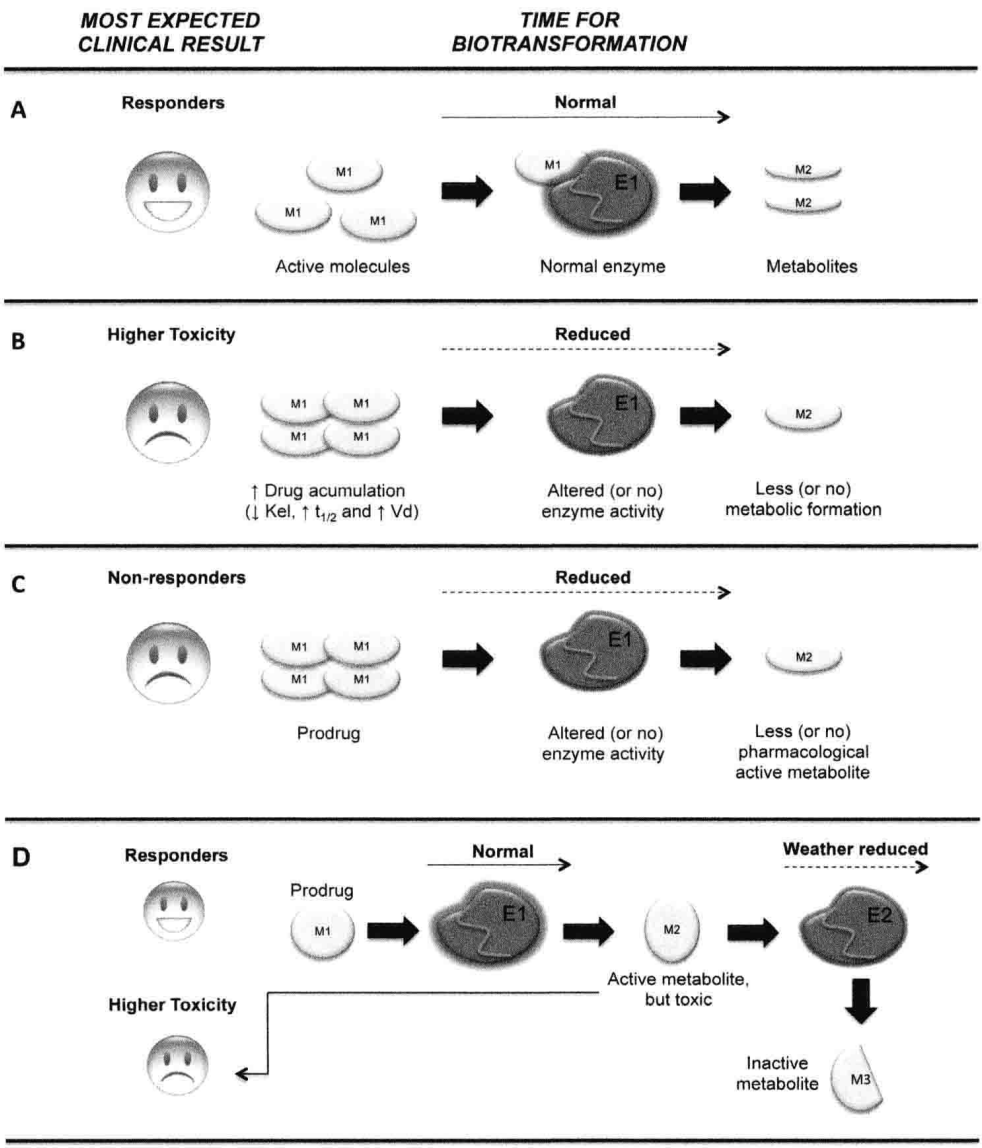

Fig. 1. Overview of the expected clinical result and its relation with activity of drug metabolizing enzymes. M1: pharmaceuticals compounds; E1: phase I biotransformation; M2 and M3: metabolites; E2: phase II biotransformation

metabolic step might cause accumulation of the drug and/or its metabolites in the body if the medicine continues to be taken (Figure 1B). As discussed earlier, genetic mutations in coding and noncoding regions may be involved in such inborn altered enzymatic activity (Ingelman-Sundberg 2001). Some relevant examples come from polymorphisms in CYPs (cytochrome P450) genes, which may result in absence of protein synthesis (*2A6*4*, *2D6*5*),

no enzyme activity (*2A6*2, 2C19*2, 2C19*3, 2D6*4*), altered substrate specificity (*2C9*3*), reduced affinity for substrate (*2D6*17, 3A4*2*), decreased stability (*2D6*10*) or even increased enzyme activity (2D6*2xn) (Tang et al. 2005). It is important to note that such genetically determined enzyme variation may directly interfere in the drug concentration at the target tissue, and though the pharmacological effect may be observed, the risk of toxicity will also be higher in "poor metabolizers" since it might accumulate to possibly harmful levels. Reduction in drug biotransformation, as observed in drug-drug interactions, will also result in altered expected values for the constant of elimination (Ke), half-life of the drug ($t^{1}/_{2}$), volume of distribution (Vd), area under the curve (AUC) and others common useful pharmacokinetic parameters used in therapeutic drug monitoring and adjustment. Based on these reasons, PGx approaches may contribute to the enhancement of clinical outcomes by providing a more effective match between patient and drug dose or type, and consequently reducing the probability of an adverse drug reaction.

Since the effect of inherited variation (genotype) on enzymatic activity isresult of changes in DNA sequence (will be discussed in more details later), it is plausible that there are distinct subgroups of subjects who have different metabolic capabilities (**phenotype**). IIndeed, epidemiologic studies have revealed at least two sub-populations of individuals based on drug metabolizing profile, classified as either "rapid", or "slow" metabolizers. Importantly to note that each metabolic group (rapid or slow) has advantages and disadvantages, and potential outcomes have been related to the type of drug studied. For example, administration of a prodrug may have higher therapeutic efficacy in a rapid than in slow metabolizer phenotype, as the metabolization of such drug is necessary to make it active. In addition, drug biotransformation is fundamental to generate an active-molecule (M2) from a less (or not) active form (M1) (Figure 1C). Despite controversies that exist in the literature about the real impact of pharmacogenetics on clinical practice (Padol et al. 2006), studies have reported different therapeutic response in patients treated with proton pump inhibitors (Tanigawara et al. 1999; Furuta et al. 2001; Klotz 2006). These examples illustrate how important PGx is, on a case-by-case basis.

Furthermore, it is evident that PGx approaches cited here are simplified assumptions of metabolism. Actually, many drugs are sequentially metabolized (Figure 1D) by parallel pathways or a broad range of enzymes to other intermediary metabolites. For practical purposes, two main classes of reactions are considered in the biotransformation of drugs. Exclusively for readability, some basic generalities of each phase reactions will be introduced below from a PGx perspective.

3. Lessons from phase I and II reactions

As discussed elsewhere in this book, the "**phase I**" metabolizing enzymes (or "nonsynthetic reactions") can convert drugs in reactive electrophilic metabolites by oxidation, hydrolysis, cyclization, reduction, and decyclization. The major and the most common phase I enzyme involved in drug metabolism are the microsomal cytochrome P450 (CYP) superfamily. CYPs mediate monooxygenase reactions that generate polar metabolites that may be readily excreted in the urine. Major CYP isoforms responsible for biotransformation of drugs include CYP3A4, CYP2D6, CYP2C9, CYP2C19, CYP1A2 and CYP2E1. However, CYP2D6, a member of this family, has been a true landmark in phase I reactions and also a common target of study in PGx.

Following the scientific vision of Evans and Sjöqvist concerning the inheritability of metabolism profile, Alexanderson continued the refining of the pharmacogenetic studies using twin models. Metabolism of some drugs such as nortriptiline (tricyclic antidepressant) were demonstrated to be under genetic control (Alexanderson et al. 1969). Later, Robert Smith (Mahgoub et al. 1977) and Michel Eichelbaum (Eichelbaum et al. 1979) and their co-workers independently attributed variability in debrisoquine/sparteine oxidation to feasible genetic polymorphisms in debrisoquine hydroxylase or sparteine oxidase (now known as CIP2D6, the same metabolizing enzyme of nortriptiline). In these studies, they suggested that at least two phenotypic subpopulations could be distinguished as "poor" and "extensive" metabolizers. This association between genotype and phenotype was explored only almost ten years after, when the gene encoding CYP2D6 was identified (Gonzalez et al. 1988). Nowadays, it is well recognized that *CYP2D6* polymorphisms may result in four phenotypes according to enzyme activity: **poor metabolizers** (PMs); **intermediate metabolizers** (IMs); **extensive metabolizers** (EMs); and **ultrarapid metabolizers** (UMs). The EM phenotype, considered as "reference", is the most frequent in worldwide populations. PMs inherit two deficient *CYP2D6* alleles, which result in a significant slower CYP2D6 metabolism rate (characterized by increase of the plasma drug levels) (Figure 2). Individuals carrying only one defective *CYP2D6* allele are considered IMs, the "functional" phenotype. Since IMs still have some CYP2D6 metabolic activity, pharmacological responses in those patients are considered marginally better than those observed in PM phenotype.

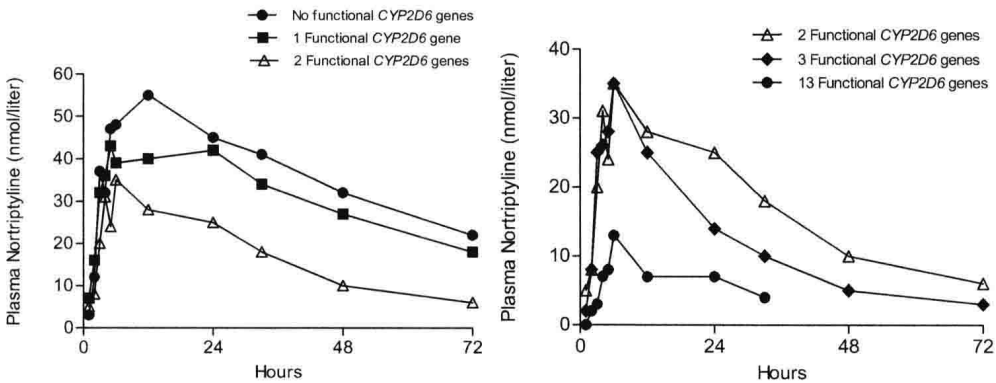

Fig. 2. Effect of functional *CYP2D6* genes in mean plasma concentrations of nortriptyline after a 25-mg oral dose administration. (Dalen et al. 1998).

The UM phenotype results from a gene duplication or even multiduplications. Individuals UMs tend to metabolize drugs at an ultrarapid rate (Ingelman-Sundberg et al. 2007). The relevance of such genetic variation in the biotransformation of drugs is very impressive. First, at least one fifth of all drugs used in clinical practice (or their active metabolites) share a pathway in CYP2D6 route. Among them, include those used to treat heart disease, depression and schizophrenia, for example (Ingelman-Sundberg & Sim 2010; Lohoff & Ferraro 2010). Second, phenotype status directly affects clinical response. Analgesic effects of some prodrugs, such as tramadol, codeine and oxycodone are CYP2D6-dependent, and PMs present low analgesic efficacy (Poulsen et al. 1996; Stamer et al. 2003; Stamer & Stuber 2007; Zwisler et al. 2009). On the other hand, loss of therapeutic efficacy at standard doses

can also be observed in UMs since the drug metabolization occurs at a fast rate (Davis & Homsi 2001). Finally, UM may also present either improved therapeutic efficacy or more frequently severe adverse effects, due to a higher rate of toxic metabolites formation (Kirchheiner et al. 2008; Elkalioubie et al. 2011).

An interesting point is that many chemicals become more toxic (even carcinogenic) only when they are converted to a reactive form by phase 1 enzyme (represented by M2 in Figure 1D). Thus, subsequent biotransformation pathway has a critical role in protecting cells from damage by promoting elimination of such potentially dangerous compounds. In this context, many phase I products are not rapidly eliminated and they may undergo a subsequent reaction, known as **phase II** (represented by E2 in Figure 1D). Phase II reactions are characterized by incorporation of an endogenous substrate (for this reason are called "conjugation reactions") such as glutathione (GSH), sulfate, glycine, or glucuronic acid within specific sites in the target containing mainly carboxyl (-COOH), hydroxyl (-OH), amino ($-NH_2$), and sulfhydryl (-SH) groups to form a highly polar conjugate (represented by M3 in the figure 1D). As phase I, most phase II reactions generally produces more water-soluble metabolites, increasing the rate of their excretion from the body. However, it is important to notice that the conjugation of reactive compounds by phase 2 metabolizing enzymes will not necessarily convert them into inactive compounds before elimination. Actually, "phase I" and "phase II" terminologies have been more related to a historical classification rather than a biologically based one, since phase II reactions can occur alone, or even precede phase I reactions. In general, more complex routes are involved in drug metabolism though some pathways are preferentially used.

It is worthwhile to mention some clinical considerations with regard to recent advances seen in PGx. First, although genotyping may be useful in predicting a drug response or toxicological risk, classical factors related with variability in drug response (age, organic status, patient compliance and others) must also be considered at every stage of the therapeutic individualization (Vetti et al. 2010). Second, it is widely accepted that genetic variability in DMEs are also directly correlated with susceptibility to unexpected outcomes, such as suicide (Penas-Lledo et al. 2011), cancer (Di Pietro et al. 2010) and other complex diseases (Ma et al. 2011). In others words, PGx approaches are not limited to drug response.

Knowledge of the relevance of phase II enzymes for PGx precedes the CYP2D6 findings. The final touch of this association was done by Price Evans in an elegant and well-designed research on the Finish in 1950`s (Evans et al. 1960). Although his studies about variation of isoniazid metabolism had more impact on public health, Evans advanced the ideas of Hughes and McCusick about the influence of Mendelian inheritance on drug metabolism. In this regard, his findings allowed introduction of the 'fast' and 'slow' metabolizers nomenclature, and which finally provided evidences that genetic variation in drug metabolism could be shown using random families. Subsequent studies demonstrated that the common trimodal profile in plasma isoniazid levels as a result of genetically determined forms of hepatic **N-acetyltransferase (NAT)**. Particularly NAT2 (EC 2.3.1.5), catalyzes not only N-acetylation, but following N-hydroxylation also catalyzes subsequent O-acetylation and N,O-acetylation. NAT2 is a crucial enzyme to convert some environmental carcinogens such as polycyclic aromatic hydrocarbons (PAHs), aromatic amines (AAs), heterocyclic amines (HAs) and nitrosamines (NAs) in more water-soluble metabolite, avoiding accumulation of potentially dangerous metabolites (Hein et al. 2000).

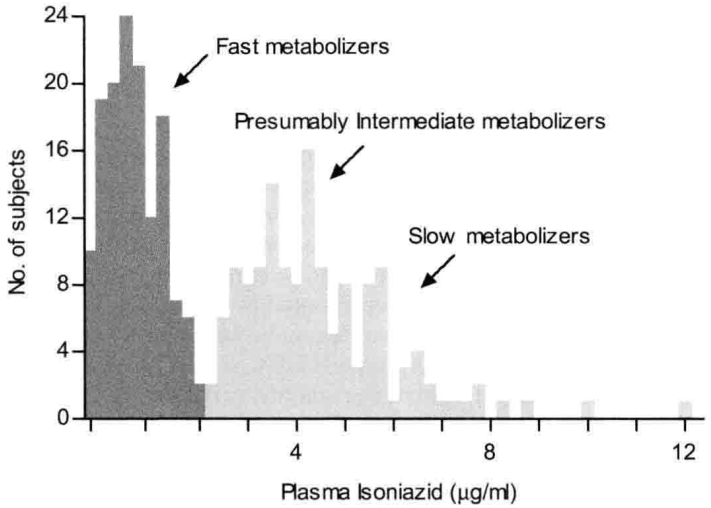

Fig. 3. Polymodal distribution of plasmatic concentrations after an oral dose of isoniazid in 267 subjects (Price-Evans 1962).

Other important phase II enzymes are **Glutathione S-transferases** (**GSTs**; EC 2.5.1.18), which constitute a superfamily of ubiquitous and multifunctional enzymes. As NAT2, GSTs play a key role in cellular detoxification, protecting macromolecules from attack by reactive electrophiles, including environmental carcinogens, reactive oxygen species and chemotherapeutic agents (Ginsberg et al. 2009). One common feature of all GSTs is their ability to catalyze the nucleophilic addition of the tripeptide glutathione (GSH; γ-Glu-Cys-Gly) to a wide variety of exogenous and endogenous chemicals with electrophilic functional groups, thereby neutralizing such sites, and similar with NAT2, rendering the products more water-soluble, facilitating their elimination from the cell. Besides NATs and GSTs, other enzymes are also important in phase II metabolism, such as UDP-glucuronosyl transferases (UGTs), sulfotransferases (SULTs), methyltransferases (as TPMT) and acyltransferases (as GNPAT).

Assumptions between functional variability in DMEs and heritable genetic polymorphisms have allowed recent studies to evaluate, for example, why exposition to a particular toxic substance does not result in the same degree of risk for all individuals. This approach called **toxicogenetics** is considered another arm of PGx. Additionally, toxicological perspectives provide opportunities to evaluate the interindividual variability in susceptibility to a number of disorders such as cancer (Orphanides & Kimber 2003; Di Pietro et al. 2010). As discussed later, it is inevitable that this knowledge would bring out endless debates about ethical questions.

4. Genetic variability in drug response

At this point, it is clear that variability in drug response depends on the complex interplay between multiple factors (including age, organ function, concomitant therapy, drug interactions, and the nature of the disease) and genetic background. Now, we will focus on