Alan Talevi · Pablo A. Quiroga *Editors*

ADME Processes in Pharmaceutical Sciences

Dosage, Design, and Pharmacotherapy

Second Edition

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Chapter 16
Relationship Between Pharmacokinetics and Pharmacogenomics, and Its Impact on Drug Choice and Dose Regimens

Matías F. Martínez and Luis A. Quiñones

16.1 Introduction

16.1.1 Drug Metabolism: Pharmacokinetics and Pharmacodynamics

Definition
Pharmacokinetics can be defined as the relationship between the administered dose and plasma concentration of a drug, which implies the study of different processes, such as absorption, distribution, biotransformation, and elimination. In short, "what the organism does with the drug." Pharmacokinetics determine the concentration of drugs in the recipient subject and, therefore, contribute to the intensity of the response observed. Modifications in pharmacokinetics help explain different responses between people since there may be different physiological situations, such as extreme age and organic failure (renal, hepatic) situations of hypo-hypervolemia. Pharmacokinetics parameters vary among subjects and also depend on the route of administration (Fig. 16.1).

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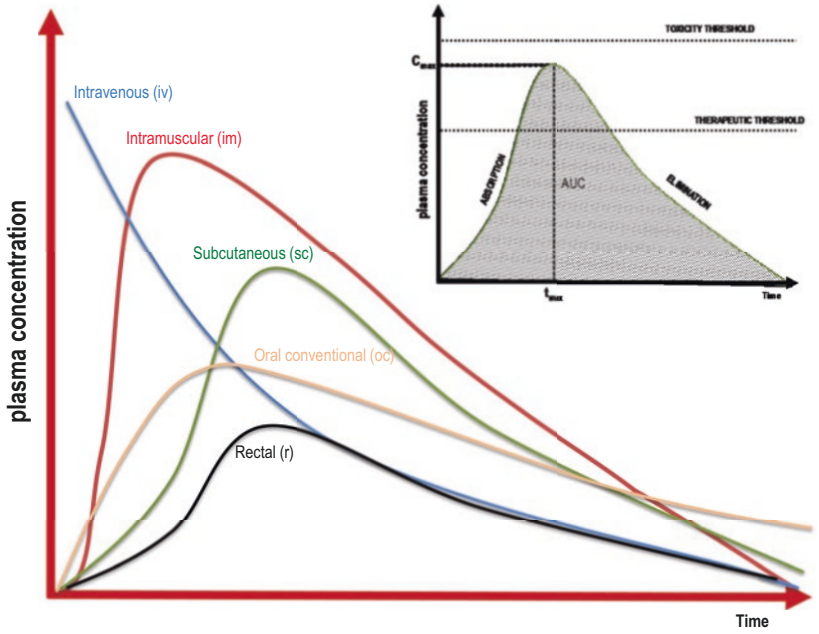

Fig. 16.1 Representative pharmacokinetic curves of different routes of administration. The main pharmacokinetic parameters are shown in the superior right box. AUC, area under the curve; C_{max} maximum serum concentration, *Tmax* time to maximum serum concentration

As shown in Fig. 16.1 (right superior box), the first part of the ascending curve primarily reflects the absorption process. Here, there is an equilibrium between passive and active absorption and the activity of the efflux pumps. When the drug is absorbed into the systemic circulation or undergoes first-pass metabolism, both processes can affect the maximum level the drug can achieve (plasma peak). The elimination or excretion processes dominate the second part of the curve. There, drug metabolism and urinary and fecal excretion are the most relevant processes, decreasing the plasma concentration of the drug.

On the other hand, pharmacodynamics is defined as the relationship between the plasma concentration and the pharmacological effect, in short, "what the drug does in the organism." Although the concentrations of the drugs are commonly determined in the plasma, it is more accurate to evaluate the concentrations in the target tissue, which is currently impossible due to the anatomical location and analytical methods.

Important
Generally, there is a direct relationship between plasma concentration and concentration in the target tissue. The pharmacokinetic-pharmacodynamic models that allow us to establish this union have proven helpful in explaining many clinical observations. Sometimes, the correlation between plasma levels and the therapeutic effect is not good or difficult to predict in some patients. In such cases, genetics can help us better understand the response to a drug.

16.1.1.1 The ADME Processes

Different processes occur, from the moment that the medication is administered until our organism completes the elimination of the drug: absorption, distribution, metabolism, and excretion (abbreviated: ADME). Although some drugs exert their effects without entering the systemic circulation (creams that serve as a barrier, some laxatives, and others), most of them must enter the organism via enteral or parenteral (absorption) routes, be transported by the blood to the target organ, cross lipid barriers/cell walls, and finally reach the cell target. Therefore, the body is a nonhomogeneous container in which the drug is distributed (distribution). Subsequently, the drug molecules are transformed by metabolizing enzymes of Phase I (cytochrome P450-CYP-, epoxide hydrolase, and others) and II (Glutathione S-transferases, UDP Glucuronyl transferases, sulfotransferases, and others) developed to face endogenous and exogenous compounds (metabolism). Thus, depending on the activity of the metabolizing enzyme, the drug level increases or decreases with the consequent impact on the therapeutic response, which also varies genetically or by interaction with other molecules. Finally, urinary or biliary excretion, the main but not the only excretion routes, allow drugs to be excreted (Fig. 16.2) (Brunton et al. 2017).

CYP is the most studied metabolizing system and is responsible for the oxidation/reduction of several endogenous (endobiotic) and exogenous (xenobiotic) compounds. It is distributed in many tissues, and is more abundant in the liver. Based on

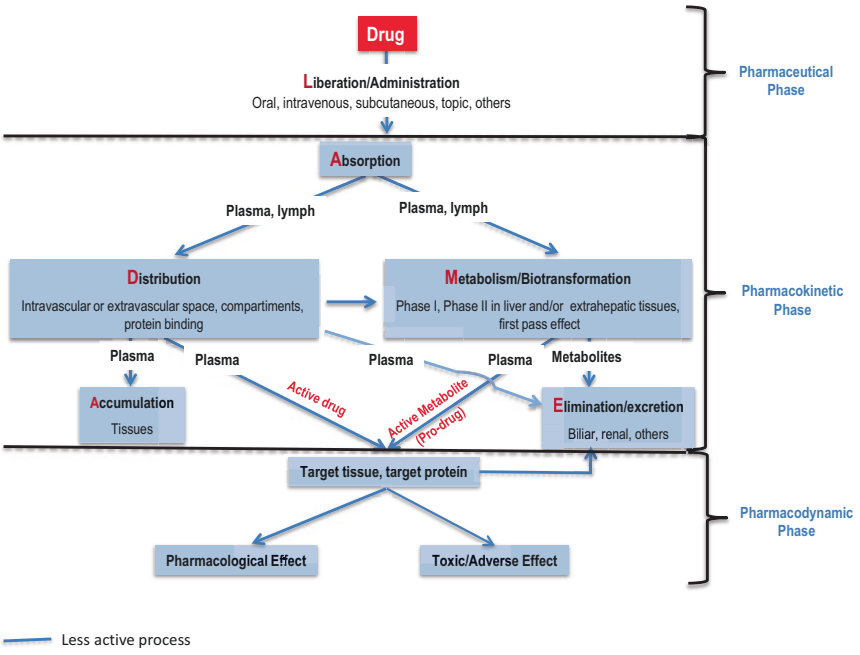

Fig. 16.2 The pharmacokinetic phases in the body

the similarities in their amino acid sequences, CYP members are classified into families, subfamilies, and isoforms (Orellana and Guajardo 2004). In humans, 18 CYP families and 43 subfamilies have been identified. Among these, 57 genes and 59 pseudogenes have been sequenced. The nomenclature and variants of these enzymes are widely agreed upon, and new agreements are constantly being updated (https://drnelson.uthsc.edu).

Several studies have suggested that differences in basal levels of CYP constitute one of the primary sources of inter-individual variability in response to xenobiotics (Lin and Lu 2001; Quiñones et al. 2008; Fujikura et al. 2015).

Glutathione S-transferases (GSTs). Human glutathione-S-transferase is a family of multigenes of soluble dimeric enzymes (Strange et al. 2001), including alpha (α), mu (μ), pi (π), zeta (ζ), sigma (σ), kappa (κ), omega (ω), and theta (τ), with a broad subcellular distribution and partial superposition of specificities. Its principal function is to detoxify pollutants, carcinogens, and mutagens by conjugation with glutathione (GSH). They also protect tissues against reactive oxygen species (ROS) and lipid hydroperoxides during oxidative stress (Hayes and Strange 1995; Gallagher et al. 2006).

16.1.2 Efficacy and Safety of Drugs

Definition
Efficacy is defined as the ability of a drug to produce an effect. This effect is related to its affinity for, and activation of, the receptor to produce a biological response. The degree (proportion of the maximum) to which a drug activates a biological system is known as the intrinsic activity or efficacy.

The efficacy of a drug is the maximum pharmacological effect that can be obtained, above which, although the doses are increased, a more significant effect is not obtained even when the toxicity threshold could be reached, favoring the incidence of adverse reactions. Efficacy is not necessarily related to potency or, therefore, to the dose. The measure of effectiveness is the maximum effect reached; the lower the effect, the less effective the drug is in producing an effect. For instance, opioids are more effective in relieving high-intensity pain than maximum aspirin doses: the maximum effect of the latter is lower than that of opiates against the analgesic effect (Sear 2004).

On the other hand, the safety fo a drug depends on its ability to induce damage in the body, i.e., toxicity. Therefore, any substance, artificial or natural, that harms living beings when in contact with them can be considered toxic. No chemical substance can be considered nontoxic because any substance can produce a toxic effect if a sufficient dose is administered. This is represented in the famous phrase of Paracelsus “The dose makes the poison” (Klaassen and Watkins 2013). In this sense,

adverse drug reactions (ADRs) can be considered a form of toxicity; however, the term toxicity is most often applied to the effects of overdoses (accidental or intentional), the presence of high blood concentrations, or exacerbated pharmacological effects that appear during the correct use of the drug (e.g., when the metabolism of the drug is temporarily inhibited by a disease or the administration of another drug). ADR is a broad term that refers to the unwanted effects of a drug that causes discomfort or damage. It is important to highlight that the term "secondary effect" is an imprecise term frequently used to refer to the undesirable effects of a drug that occur within the therapeutic range. Since all drugs can cause adverse reactions, whenever a medication is prescribed, a risk-benefit analysis must be carried out (evaluate the probability of obtaining benefits against the risk of adverse reactions to the drug) (Brunton et al. 2017).

16.1.3 Drug Variability, Pharmacogenetics, Pharmacogenomics and the Role of Epigenetics

The success of modern medicine is partly due to the availability of more effective pharmacological treatments. It is well known that individuals respond differently to drug therapy and no medication is 100% effective in all patients. Consequently, the margin of response to pharmacological treatment is diverse since individuals can obtain the expected effects, whereas others do not obtain a therapeutic result and may even experience adverse effects (Wilkinson 2005; Xie and Frueh 2005; Zhou et al. 2008).

The existence of inter-individual heterogeneity in the response to drugs, which affects both efficacy and toxicity, can be mediated by altering the pharmacokinetics and pharmacodynamics of drugs. The interaction between genetics and environment shapes these mechanisms of variability. The contribution of each factor varies for each drug (Evans and McLeod 2003; Wijnen et al. 2007).

Definition
In 1959, Friedrich Vogel defined pharmacogenetics as the genetic variation in the incidence of adverse effects in patients (Vogel 1959). In 1962, this definition changed to the study of how genetic variants cause variability in response to drugs (Kalow 1962). Currently, the most accepted definition is "the discipline that allows identifying the genetic basis of the inter-individual differences in the drug response."

Pharmacogenomics studies gene variation related to pharmacokinetics and pharmacodynamics and their relationship with pharmacological response. In other words, pharmacogenomics studies the relationship between whole genetic information and therapeutic responses to drugs (adverse reactions and/or efficacy) (Meyer 2000, 2004).

Important
Pharmacogenetics and pharmacogenomics are tools that explain and predict variability in the ADME processes and their association with clinical success or therapeutic failure due to a lack of efficacy or safety. For instance, both disciplines are essential tools for personalized medicine (Roses 2000) (Fig. 16.3).

On the other hand, pharmacoepigenetics and pharmacoepigenomics are new promising areas impacting pharmacology. Both areas study the epigenetic basis of variations in drug response. Epigenetic changes include DNA methylation, histone modifications, and RNA-mediated silencing. Altering these epigenetic mechanisms leads to inappropriate gene expression and the development of cancer and other epigenetic-mediated diseases (Ingelman-Sundberg and Gomez 2010). DNA methylation is the most studied epigenetic mechanism influencing gene expression. Currently, a major component of pharmacoepigenetics is therapy; thus, some epigenetic drugs, such as inhibitors of DNA methyltransferases and histone deacetylases, have been studied, mainly in cancer treatment (Peedicayil 2008). Pharmacoepigenetics could help to understand why genetics sometimes fails to predict the phenotype of a patient.

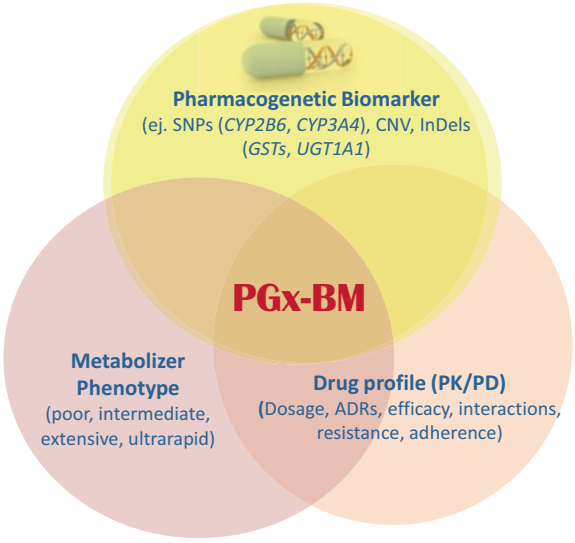

Fig. 16.3 Schematic representation of the relationship among pharmacogenetic biomarkers, metabolizer phenotype and drug response. *PGx-BM* Pharmacogenomic-based medicine, *CYP* Cytochrome P-450, *GSTs* Glutathione S-transferases, *SNPs* Single Nucleotide Polymorphisms; InDels: Insertion/deletions; *CNV* copy number variation, *UGT1A1* uridine diphosphate glucuronosyl transferase 1A1, *ADRs* Adverse drug reactions, *PK/PD* Pharmacokinetics/Pharmacodynamics

16.2 How Can We Explain Response Variability Due to Genetics

Genetic variability can affect every process in normal physiology; therefore, pharmacokinetics and xenobiotic detoxification are no exceptions. A mutation could increase or decrease the protein activity or affect the protein amount due to dysregulated expression or variation in the copy number of genes. By identifying the type of mutation, we can begin to understand the potential effect on the protein and the consequences of the involved processes.

16.2.1 Type of Variations and Their Impact on Protein Function: Polymorphisms

Definition
When a genetic variant that conduces to at least two non-rare phenotypes has a frequency of 1% or greater in a population, it is called polymorphism.

These variations play a crucial role in determining the unique characteristics and traits of the individuals within a species. As shown in Fig. 16.4, there are several types of genetic variation.

Single Nucleotide Polymorphisms (SNPs) SNPs (promounced *snips*) are the most common type of genetic variations. They involve alteration of a single nucleotide base (A, T, C, or G) at a specific position in the DNA sequence. SNPs can influence traits such as eye color, susceptibility to diseases, and drug responses (eg. CYP3A5*3).

Insertions and Deletions (InDels) Indels involve the insertion or deletion of one or more nucleotides in a DNA sequence. These variations can disrupt the reading frame of a gene, leading to changes in protein structure or function (eg. GSTT1 deletion).

Copy Number Variations (CNVs) CNVs are large-scale variations in the number of copies of a particular DNA segment. They can range from a few hundred to several thousand base pairs in length. CNVs can significantly affect gene dosage and expression, contributing to phenotypic diversity and disease susceptibility (eg. CYP2D6 duplication).

Variable Number Tandem Repeats (VNTRs) VNTRs are regions of DNA where a short DNA sequence is repeated in a tandem arrangement, meaning that the sequence is repeated one after another. The number of repeats in a VNTR can vary among individuals, which is why they are called “variable number” tandem repeats

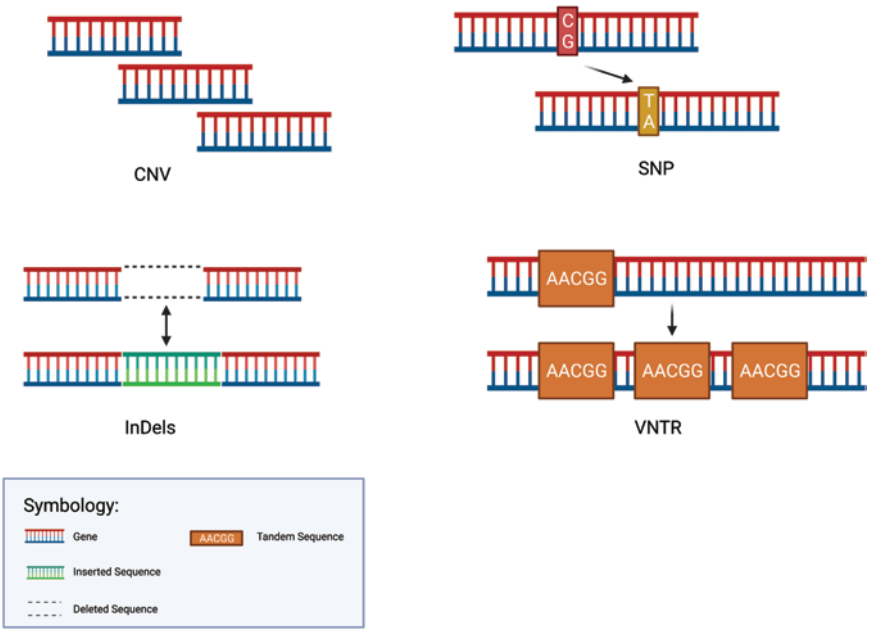

Fig. 16.4 Types of genetic polymorphisms. *CNV* copy number variation, *SNP single nucleotide polymorphism*, *VNTR* variable number in tandem repeats; *InDelsc* insertion-deletions. (From Cerpa et al. 2021)

(UGT1A1*28). These repeats are polymorphic, meaning that they differ in length between individuals due to variations in the number of repeats. VNTRs are commonly used as genetic markers in various applications including forensic DNA analysis, population genetics, and paternity testing.

Moreover, structural variations, that is, alterations in the structure of chromosomes or large segments of DNA, can also be relevant factors. Examples include chromosomal inversions, translocations, and duplications. Structural variations can lead to genetic disorders or contribute to the evolution of a species (Feuk et al. 2006).

The most common type of mutations are SNPs, which is the change of one nucleotide by another in the same position. Depending on the zone where this change occurs, the effect is different, and depending on the change in amino acid codification, the protein structure and function could be affected. A *synonymous substitution* occurs when the base change encodes the same original amino acid; a *missense substitution* is a change in the encoded amino acid, so it changes the protein structure and could affect its functionality; finally, a *nonsense substitution* changes the encoded amino acid for a stop codon, so the produced protein is shorter than the original, affecting its structure and function, or the protein is degraded in a process called nonsense-mediated decay (Fig. 16.5).

Deletions represent other type of mutations. In this case, a variable number of bases are "eliminated" from the original sequence with consequences in the structure and function of the protein. Depending on the deletion size, the transcription process can result in shorter or no protein.

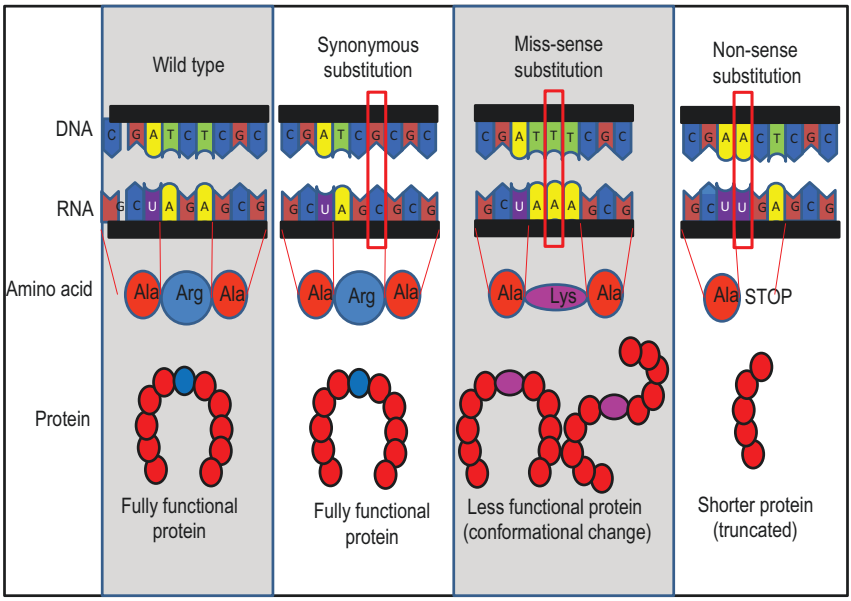

Fig. 16.5 Types of mutation in the coding region of a gene

CNVs are another mutation that can affect pharmacoksnetics. One example is the duplication or multiplication of whole genes, which allows for a higher amount of protein. In contrast, losing one of the two copies of a whole gene results in a lower amount of protein and is sometimes associated with a lower effect.

In Fig. 16.6, we can observe a basic structure of a gene, the untranslated region (3'-UTR and 5′-UTR), the Promotor zone, and enhancer/silencer binding sequence is called the "regulatory sequence." A mutation there modifies the gene expression affecting the amount of transcript and, consequently, the amount of protein. For example, if there is a mutation in an enhancer site that results in a weak binding with the enhancer, there will be lower expression of the genes, decreasing the amount of transcript. Conversely, if this mutation allows a tight binding of the enhancer, the number of transcripts and the amount of protein will increase.

However, the presence of a mutation in the open reading frame region could affect the activity or structure of the protein. When a non-synonymous substitution occurs in the protein-coding region (i.e., exons), the amino acid change could modify the protein's tertiary structure, affecting, for example, the activity, affinity for the substrate, or protein stability. When the substitution is in the intron, principally in the splice-donor or splice-acceptor sites, it could lead to alternative (or novel) splicing or suppress splicing in that intron, producing a larger protein than usual. An intronic mutation could also affect the regulatory system of splicing, affecting the normal functions of proteins due to problems in mRNA maturation.

Pharmacogenetics has mainly focused on SNPs, but every variant could be a factor that explains the pharmacological variability. Several genetic variations are

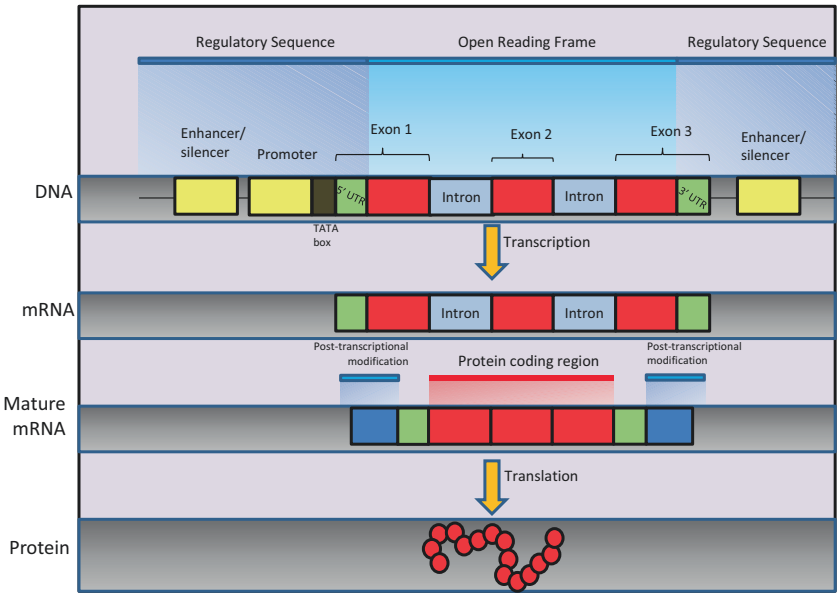

Fig. 16.6 Basic structure of a gene

known. Figure 16.7 shows the different possibilities of genetic variation and their potential phenotypic expression in drug-metabolizing enzymes.

16.2.2 Drug Metabolizing Enzymes

Drug metabolism is the most studied area in pharmacogenetics and pharmacogenomics. Depending on the degree of functionality of the drug metabolism, patients can be categorized into three principal groups:

Those with a lower functionality are classified as "poor metabolizer," these patients could carry a polymorphism (in heterozygote or homozygote form) that decrease the genetic expression of a metabolic enzyme, have a deletion of the gene, or carry a polymorphism that affects the activity of the enzyme (see Fig. 16.7). Those patients with a normal metabolism o without any polymorphism that affect the metabolism are called "extensive metabolizers", "normal metabolizer", or "wild-type subject." Patients with an accelerated or augmented metabolism are called "rapid or ultra-rapid metabolizers." These patients probably carry a variant that increases genetic expression by a mutation at the site of union of a silencer or by carrying an extra copy of the whole gene.

As previously mentioned, in xenobiotic metabolism phase I is related to functionalization reactions and the principal family of enzymes is cytochrome P450

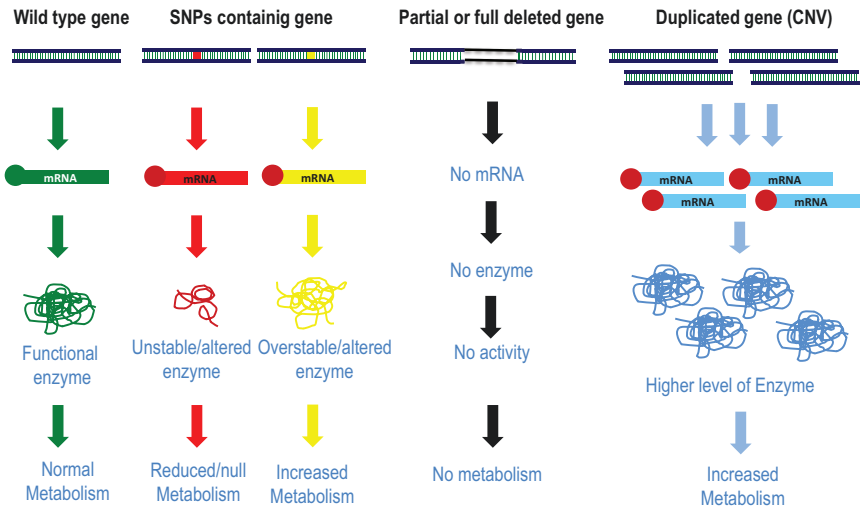

Fig. 16.7 Gene variants in drug-metabolizing enzymes

(CYP450). Phase II is related to conjugation reactions with more hydrophilic molecules that favor elimination.

16.2.2.1 Phase I Enzymes

The main phase I enzymes, CYP450, is a superfamily of metabolic enzymes that catabolize phase I metabolism, generally by introducing or exposing a hydrophilic group in a drug. These enzymes are highly polymorphic, and their relationships with drug responses have been widely described in the scientific literature. Generally, one CYP enzyme metabolizes more than one drug and a drug is metabolized by more than one CYP enzyme.

Besides drug detoxification, CYP enzymes participate in the bio-activation of pro-drugs, and if these enzymes are less functional, the therapeutic effect will be minor due to less transformation to the active drug (Zhou et al. 2009).

Among all CYP families, the most studied are families 1, 2, and 3, all of which are polymorphic. In this respect, polymorphisms are usually named with the number of the enzyme followed by a star and then the number of a variant (for example, *CYP3A4*1B*), but the best way to name them when this corresponds to an SNP is using the "rs number," a unique number assigned by NCBI dbSNP to each single nucleotide polymorphism.

In the CYP450 superfamily, there is a broad spectrum of relevant polymorphisms. There are SNPs related to a higher activity of the enzyme, such as CYP1A1*2C, which is an amino acid change near the active site that has been related to an increase in enzymatic capacity. Other SNPs are related to a higher level of genetic expression, such as CYP1A1*2A, which is a substitution in the 3′-region

of polyadenylation. Moreover, a polymorphism could be related to the suppression of activity, for example, CYP3A4*26, which is a nonsense substitution. Therefore, this protein exhibits no activity (Preissner et al. 2013).

16.2.2.2 Phase II Enzymes

This phase of metabolism is associated with conjugation with hydrophilic molecules, which favors the elimination of drugs. After this stage drugs are generally excreted, and their destiny is the urinary or fecal excretion. In a few cases, after phase II, the drug can gain toxic activity. For example, the nephrotoxicity of hydroquinone and bromobenzene is mediated via quinone-glutathione conjugates.

Glutathione – S – transferase (GST) and uridine 5′-diphosphate-glucuronosyltransferase (UDP – glucuronosyl transferase, or UGT) metabolize approximately half of the drugs. Other relevant phase II enzymes include sulfotransferases (SULTs), N-acetyltransferases (NATs), and thiopurine S-methyltransferase (TPMT).

Gene–related deficiency in phase II enzymes is related to an increased risk of toxicity, and clinical recommendations for drugs metabolized by these enzymes are focused on avoiding adverse effects and improving security in patients (Jancova et al. 2010). For example, UGT is related to bilirubin metabolism, and its deficiency generates Gilbert's syndrome characterized by jaundice, which is also associated with alteration of the hepatic profile after administration of atazanavir (a drug used in HIV patients) due to reduced conjugation and elimination. In addition, TPMT is related to the metabolism of 6-mercaptopurine and thioguanine, and enzymatic deficiency is related to moderate or severe myelosuppression depending on the metabolizer type; poor and intermediate metabolizers have a higher risk than extensive metabolizers.

16.2.3 Drug Transporters

Drug transporters participate in many pharmacokinetic processes, including absorption, distribution, and excretion. Therefore, their dysfunction could compromise the achievement of therapeutic goals, because the drug does not reach optimal plasma levels.

There are two families of transporters: ATP-binding cassettes (ABC) and solute-linking carriers (SLC). These transporters have different functions. They could be efflux or influx transporters; in enterocytes, they affect drug absorption; in renal tubules, transporters can change the urinary elimination; or those transporters expressed in the liver or brain blood barrier could affect the drug distribution to the whole organism, and even the drug available for metabolism.

The ABC Family is a widespread type of transporter in different species, and it participates in active transport owing to a well-conserved nucleotide-binding

domain where ATP is hydrolyzed. ABC transporters are expressed predominantly in the liver, intestine, blood-brain barrier, blood-testis barrier, placenta, and kidneys. ABC proteins transport many endogenous substrates, including inorganic anions, metal ions, peptides, amino acids, sugars, and drugs (Vasiliou et al. 2009). ABCB1 (ATP binding cassette subfamily B member 1) or P-glycoprotein, is a well-studied member of this family. It could expulse some drugs affecting their absorption or arrival to the therapeutic target. ABCB1, a key drug transporter, can be inhibited or overexpressed by drug interactions. Dysfunction of this protein can affect the achievement of therapeutic goals (Fromm 2002).

The SLC Family is very diverse, including the SLC22 family, organic cation transporters (OCTs), organic zwitterion/cation transporters (OCTNs), organic anion transporters (OATs), and others as the human SLC6 family members such as serotonin, norepinephrine, and dopamine transporters (SERT, NET, and DAT, respectively) (Colas et al. 2016).

SLC21A6 or *SLCO1B1* gene (solute carrier organic anion transporter family member 1B1) encodes the transporter OATP1B1. This protein is found in liver cells and transports compounds from the blood into the liver to be cleared from the body. For example, the rs4149056 C allele is related to decreased transporter activityin vitro (Tirona et al. 2001; Kameyama et al. 2005) and reduced clearance of some drugs in vivo (Niemi et al. 2004; Pasanen et al. 2007), and it has been associated with an increased risk of developing myopathy after administration of simvastatin, a drug used for hypercholesterolemia, due to a decreased arrival to the liver (Group 2008).

16.2.4 Other Polymorphic Targets

Other targets of pharmacogenomic studies are related to specific processes. For example, VKORC1 (Vitamin K Epoxide Reductase Complex subunit 1) is the therapeutic target of warfarin. The polymorphism -1639 G > A has been described as a critical factor in dosage modification because it produces less VKORC1 protein, and a patient will need a lower dose of warfarin to reach the same therapeutic target.

The *DPYD* gene, which encodes dihydro pyrimidine dehydrogenase (DPD), is a limiting enzyme in fluoropyrimidine catabolism, and its reduced function is related to a high risk of toxicity due to 5-Fluorouracil, tegafur, and capecitabine, which may drive to neutropenia, nausea, vomiting, severe diarrhea, stomatitis, mucositis, and hand-foot syndrome.

Another case is Glucose 6-phosphate dehydrogenase (G6PD), which is the enzyme that converts glucose-6-phosphate into 6-phosphogluconolactone, the first step of the pentose phosphate pathway. It is essential in erythrocytes because 6-phosphogluconate dehydrogenase is the only available source of NADPH, which is required to protect erythrocytes from oxidative stress. A deficiency in this enzyme results in a high risk of hemolytic anemia due to the high oxidative stress that is sometimes generated by drugs.

Finally, HLA is a major histocompatibility complex (MHC) gene member. HLA molecules are expressed in almost all cells and present peptides to immune cells. Variations in HLA-B levels have been associated with several autoimmune conditions. Several variants of HLA-B have been associated with ADR phenotypes. Patients with the *HLA-B*15:02* genotype have an increased risk of developing Stevens–Johnson syndrome after treatment with carbamazepine (Phillips et al. 2018), whereas *HLA-B*58:01* is associated with an increased risk of severe cutaneous adverse reactions in response to allopurinol (Saito et al. 2016).

16.3 Relationship Between Pharmacogenetics and ADME Processes

Pharmacogenetics can affect every pharmacokinetic process, and this effect can be observed at the drug plasma level. Figure 16.6 shows the plasma concentration versus time curve of three patients who received the same medication p.o. at the same dose. The therapeutic threshold (the minimum level that must be reached to have a pharmacological effect) was not reached by patient A; therefore, this treatment will fail. The toxicity threshold (the minimum level at which there is a probability of adverse effects) was reached by Patient C; thus, the treatment could fail due to a toxic event. The range between the therapeutic and toxicity thresholds is called the therapeutic range. The plasma concentration of drugs should be within this range to achieve clinical goals.

Suppose we have subjects with polymorphic variants exclusively affecting the absorption, distribution, metabolism, or elimination genes. The potential impact on each of these processes is discussed in the nexty subsections.

16.3.1 Absorption

P-gp activity is variable. It can vary due to inducers or inhibitors of this protein or genetic differences in the encoding gene (*MDR1*). A less functional P-gp will increase the absorbed fraction of the drug due to a decrease in its return to the intestinal lumen. In contrast, P-gp overexpression decreases the amount of absorbed drug (Hoffmeyer et al. 2000; Fromm 2002). In Fig. 16.8, we present the pharmacokinetic curves for three patients differing only in the P-gp polymorphism. Patient A carries the MDR1 Ser893 polymorphism, which promotes higher P-gp activity in enterocytes. Patient B has a “wild type” gene, and finally, patient C is homozygous for the SNP C3435T; this variant affects the expression of the gene, so there is a lower amount of P-gp in the cell surface, decreasing the efflux.

Note that patient A did not reach the therapeutic threshold and patient C trespassed the toxicity threshold. The absorption ratio differed for each patient. The

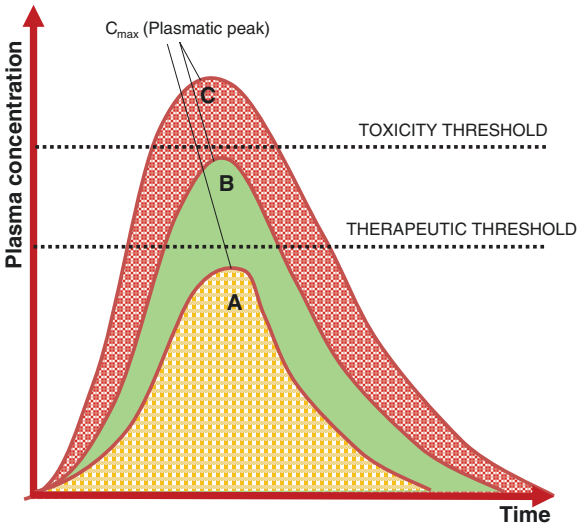

Fig. 16.8 Scheme of the plasma concentration vs. time curve in patients with different genotypes of P-gp, that affect absorption of drugs that are P-gp substrates

velocity was higher in patient C, and the slope of the ascendant phase was higher than that in patients B and A; however, the elimination rate was similar in every patient (parallel curves in the descendant phase).

Another relevant point to highlight is the plasma peak. In patient C, the peak (C_{max}) is higher and earlier than that in the wild-type patient and the patient with under-expression of P-gp.

However, contrary to this hypothetical example, it is well known that several transporters are involved in this absorption process, some of which favor absorption and others favor efflux.

16.3.2 Distribution

Genetic polymorphisms in genes responsible for blood proteins haven't been thoroughly documented. Although there are suggestions about specific vitamin needs associated with certain plasma proteins like haptoglobin and vitamin D binding protein, these variations haven't yet been linked to how our bodies respond to medications. Consider a hypothetical scenario: if a change in the albumin gene affects how drugs bind, reducing their transportation in the bloodstream, it could significantly impact drug effectiveness. For instance, in the case of a drug like warfarin, which binds strongly to proteins, this genetic alteration could mean a smaller dose of the drug is needed for therapeutic effects in individuals with this gene variant. Conversely, the risk of toxicity from this drug might increase, leading to a higher chance of harmful bleeding. Hence, adjusting the dosage for such patients becomes crucial to prevent these adverse effects. Certain transporters in our body play a vital role in how drugs are distributed, affecting their effectiveness. For instance, the

P-glycoprotein (P-gp) present in the blood-brain barrier might limit the passage of active drugs into the central nervous system if overly active. Additionally, other transporters like MRP2 and MRP4 (encoded by *ABCC2* and *ABCC4* genes) found in the liver can also impact drug distribution and metabolism. Specific variations in these genes, like rs12762549 in *ABCC2* and rs11568658 in *ABCC4*, can reduce the activity of these transporters, affecting drug delivery to the liver, altering both how the drug spreads in the body and how it gets metabolized.

16.3.3 Metabolism (Biotransformation)

Drug metabolism is the most studied field of pharmacogenetics and pharmacogenomics. Variations in phase I and phase II enzymes have a very known relationship with therapeutic response, and the effect of rapid or slow metabolism is easily studied through the plasma level of a drug.

At this level, we have two different scenarios: the most common, an active drug in a patient with rapid metabolism, will be eliminated quicker than an extensive metabolizer, making it challenging to reach efficacy; in a patient with poor metabolism, the drug will stay longer in the organism than in a patient with normal metabolism, favoring the incidence of adverse reactions. For example, *CYP2C19* metabolizes voriconazole, an antifungal drug, and different polymorphic variants associated with metabolic variations in the gene that encodes the protein can be identified. *CYP2C19*17* is related to higher metabolism and *CYP2C19*3* is related to poor metabolism. Figure 16.9 shows the plasma levels of voriconazole after an oral dose in the three hypothetical patients. Patient A has the genotype *CYP2C19*17/*17* (rapid metabolizer), patient B has the genotype *CYP2C19*1/*1 (*wild-type genotype), and patient C has the genotype *CYP2C19*3/*3* (poor metabolizer).

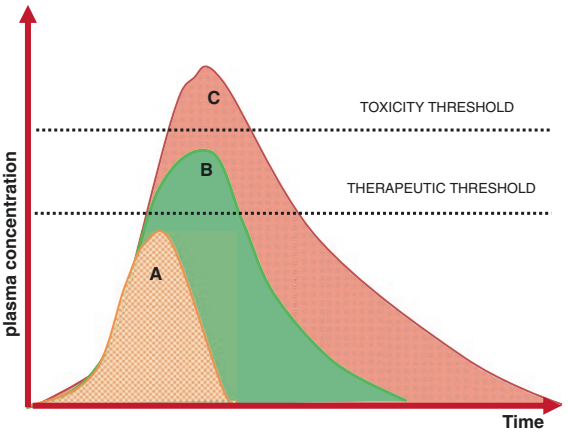

Fig. 16.9 Plasma concentration *versus* time curve in patients with different genotypes of metabolic enzymes

Patient A has a lower level due to an accelerated metabolism. The consequences of not reaching the therapeutic level are progression to severe sepsis and septic shock and, finally, the potential death of the patient. Patient B will likely reach the therapeutic goals with this treatment. Patient C has the highest plasma concentration; this treatment will probably produce toxicity and should be suspended. In the case of voriconazole, one of the most severe reactions is liver damage leading to hepatic failure, which can be avoided with the correct dosage and monitoring. It is also notorious for the differences in the final phase of the curve, where the slope represents the elimination rate, and the half-life can be calculated. Patient C eliminated more slowly than patients B and A, which resulted in a longer half-life.

Another relevant situation to be analyzed is when the administered molecule is not active, but its metabolite is the active drug (prodrugs). Thus, poor metabolism is associated with lower bio-activation and lower or no therapeutic effect. In this respect, Fig. 16.10 represents plasmatic levels for patients who received an oral dose of a pro-drug, for example, codeine, a pro-drug bio-transformed by CYP2D6 to morphine in the liver. Patient B carries the wild-type allele for the CYP2D6 enzyme in a homozygous way, so the patient is an extensive metabolizer of codeine. Patient A carries an extra copy of the *CYP2D6* gene in one allele, so the patient is an ultra-rapid metabolizer of codeine that could be observed in the low plasma level reached, this patient reached a lower peak than the other two patients because when codeine was absorbed was rapidly metabolized, and the downward curve falls quicker. Patient C carries the genotype *CYP2D6*5/*5*; therefore, the patient is a poor metabolizer of codeine, so the elimination is slower than that of A and B and

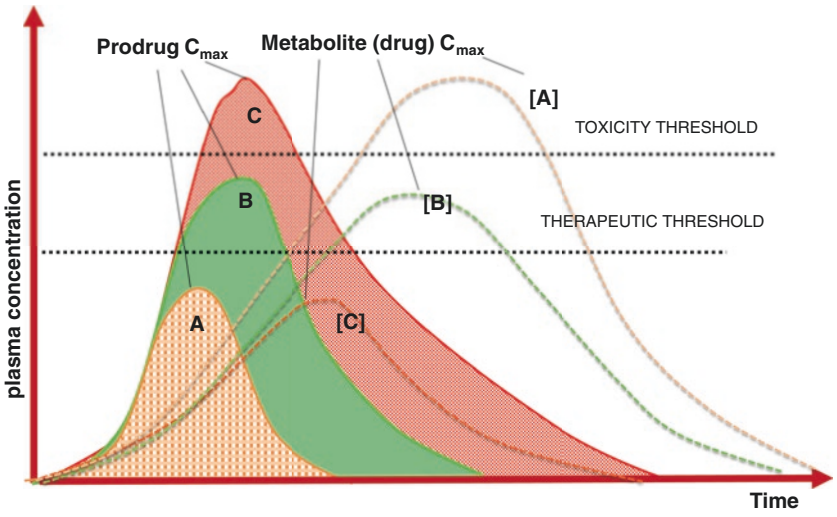

Fig. 16.10 Scheme of the plasma concentration versus time curve for a prodrug administered to patients with different genotypes of metabolic enzymes. C_{max} Maximum serum concentration; square brackets represent metabolite curves

the peak of codeine should be higher than in the other cases. Metabolites (e.g., morphine, represented in square brackets) will appear in plasma after the biotransformation of codeine. Of course, the plasma levels and thresholds are not necessarily on the same scale for the prodrug and its metabolite in this representation.

Note that the absorption phase is similar in all patients, but the elimination phase and peak (C_{max}) differ for the pro-drug and metabolite. However, this is not always true. The metabolite appearance in plasma could be different, and the elimination rate could also be different. In this case, the therapeutic threshold is associated only with the metabolite and the toxic threshold could be related to both compounds. In the case of codeine, no therapeutic activity is related to the prodrug (codeine), but to the "activated" drug, which is the metabolite (morphine). Our example shows that patient B reaches the therapeutic threshold but does not trespass the toxicity level because the regular dosage is effective and safe in wild-type *CYP2D6* carriers. However, in patient A, with a lower level of codeine, the level of morphine was higher due to faster biotransformation, reaching the toxicity threshold (possible adverse reactions). On the other hand, patient C had a higher level of codeine, but the level of morphine was lower because of poor metabolism to transform codeine to morphine; thus, this patient will not have therapeutic success.

Also note that the ascendant curve for metabolites in Fig. 16.10 does not reflect absorption but biotransformation from codeine. Thus, the starting point was not time equal to zero. In patient A, the bio-activation of codeine was faster (higher slope) than that in the other two patients because of an extra copy of the gene that encodes the responsible enzyme.

In another example, tamoxifen and its metabolites (4-hydroxytamoxifen, N-demethyltamoxifen, and endoxifen) all have therapeutic activity, even though endoxifen is suggested to be more active (Fig. 16.11) (Miranda 2016).

16.3.4 Excretion

Genetic variants related to drug excretion are particularly relevant if the drug is not widely metabolized and excretion occurs not by glomerular filtration but by active secretion into the renal tubule. This is the case for β-lactam antibiotics, such as penicillin and cephalosporins, which are poorly metabolized; therefore, their excretion is principally led by the elimination process of the unaltered drug, and those responsible are transporters.

In the proximal renal tubule, three transporters from the family of Organic Anion Transporters (OAT) are implicated in the active secretion of β-lactam drugs: OAT1, encoded by SLC22A6 and located in the basolateral membrane together with OAT3, encoded by *SLC22A8*, and in the apical membrane OAT4, encoded by the *SLC22A11* gene (Fig. 16.12) (Lin et al. 2015).

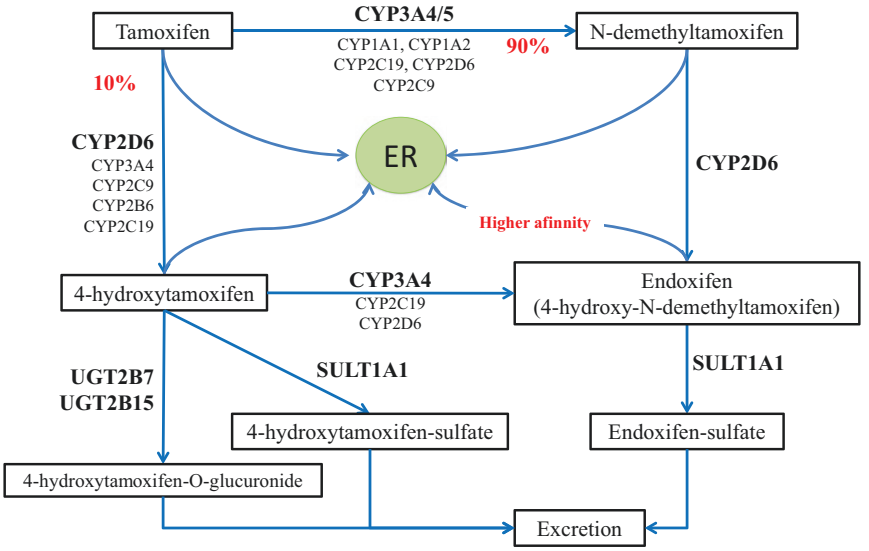

Fig. 16.11 Metabolic pathways of tamoxifen. (adapted from Miranda 2016). *E.R.:* Estrogen Receptor, *CYP:* Cytochrome P450, *UGT: UDP* Glucuronosyltransferase; *SULT:* Sulfotransferase

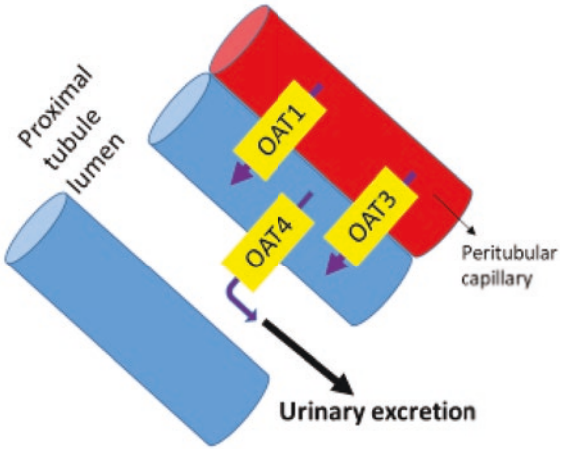

Fig. 16.12 Location of SLC family transporters in proximal kidney tubule. *OAT* Organic anion transporter

It has been described that all these genes have SNPs affecting the normal activity of transporters. The most studied SNPs are rs11568626 (149C > T) in OAT1, rs11568482 in OAT3 (62,763,264 T > A) and rs11231809 (64,302,950 T > A) in OAT4. For patients carrying some of these polymorphic alleles, the elimination process is slower, favoring the incidence of adverse effects. On the other hand, overexpression of genes encoding these excretion transporters will decrease plasma levels, and the therapeutic effect will not be reached.

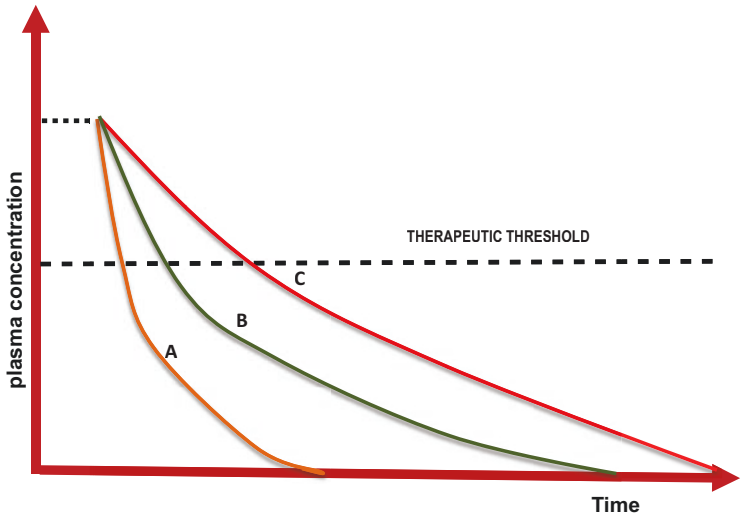

Fig. 16.13 Scheme of the plasma concentration versus time curve of a drug (e.g., cefotaxime) in patients with different genotypes in the OAT3 gene
**In the case of cefotaxime, the therapeutic threshold corresponds to the minimum inhibitory concentration*

For example, cefotaxime, a third generation cephalosporin antibiotic, is eliminated from the body via OAT3. Figure 16.13 shows the plasma level of an intravenous dose of a drug, such as cefotaxime, in patients with different genotypes for rs11568482 in OAT3. Patient C would be homozygous for rs11568482, leading to a reduced clearance (Yee et al. 2013); patient B is heterozygous, also having a reduced clearance but less than C; and patient A is a wild-type genotype.

For cefotaxime, the therapeutic threshold is now called the minimum inhibitory concentration (MIC), which is the minimum plasma level of the drug that inhibits bacterial growth at a therapeutic level. In the case of other antibiotics, such as cephalosporins, the period at which the concentration remains above the MIC (and not only reaching the MIC) is relevant.

Patient A had a faster elimination process due to a faster elimination compared to the other patients. This patient has a wild-type genotype for the variant rs11568482 in the *OAT3* gene, and the infection will probably not be resolved at this dose of cefotaxime. Patient B is a heterozygote for this variant and has a better pharmacokinetic profile, that is, nough time over the MIC is observed, without exceeding the toxic threshold. Patient C, homozygote for the variation, will have good activity against bacteria, but will probably develop adverse effects in the case of cefotaxime, nausea, diarrhea, or kidney deterioration due to slower elimination of the drug.

16.3.5 Drug Toxicity (Safety)

The first historical report of pharmacogenetics was in 510 B.C. when Pythagoras observed that some people, but not all, suffered a potentially fatal reaction after ingestion of fava beans (Pirmohamed 2001). This reaction was later related to a deficiency in glucose-6-phosphate dehydrogenase (G6PD). This deficiency is related to several adverse effects of some drugs, such as rasburicase (a drug used for hyperuricemia), but patients with a G6PD deficiency could have hemolytic anemia, which is potentially fatal (Nguyen and Ness 2014). Another example of this adverse reaction is fluoroquinolone antibiotics, which are contraindicated in patients with G6PD deficiency.

Another good example of using polymorphisms as toxicity markers is the *HLA-B*5701* genotyping. Patients using abacavir, an HIV reverse transcriptase inhibitor, need to be studied for *HLA-B*5701* genotypes because those who carry this variant have a high risk of hypersensitivity reactions, with cutaneous expression and life risk.

16.4 Dosing Modifications and Drug Selection

The Clinical Pharmacogenetic Implementation Consortium (CPIC) is an international organization that is interested in facilitating the use of pharmacogenetics in clinical practice. CPIC has created some clinical guidelines for different gene/drug pairs. These guidelines have indications for drug dosage or selection to improve patient responses.

The consensus CPIC recommendations are presented in Table 16.1. Recommendations are related to dose adjustment, drug selection, and the alternative use of other drugs.

The main phase I enzymes involved in these guidelines are CYP2C9, CYP2C19, CYP2D6, CYP3A5, and CYP4F2. The main phase II enzymes are UGT1A1 and TPMT. Other polymorphic enzymes and proteins involved are DPD, VKORC1, G6PD, HLA-B, IFNL3, and CFTR. The transporters with clinical recommendations are SLCO1B1 and ABCG2 for statins, HTR2A for antidepressants as a pharmacodynamic factor, and SLC6A4 as a pharmacokinetic factor.

In addition to CPIC, other institutions are working in the clinical implementation of pharmacogenetics worldwide. The Dutch Pharmacogenetics Working Group (DPWG) and Canadian Pharmacogenomics Network for Drug Safety (CPNDS) are good examples. DPWG have implemented guidelines for aripiprazole, atomoxetine, clozapine, duloxetine, flecainide, flupenthixol, haloperidol, metoprolol, mirtazapine, olanzapine, oxycodone, propafenone, risperidone, tamoxifen, tolbutamide, tramadol, venlafaxine and zuclopenthixol, every one of them in relation with *CYP2D6* genotype. Esomeprazole and omeprazole with CYP2C19, glibenclamide and tolbutamide with CYP2C9, and irinotecan with UGT1A1. CPNDS has guidelines for daunorubicin and doxorubicin with *RARG*, *SLC8A3*, and *UGT1A6* polymorphisms, and cisplatin with polymorphisms in *TPMT gene* among others.

Table 16.1 CPIC guidelines according to protein, genotypes and drugs (https://cpicpgx.org/guidelines/)

GENES	Genotypes	Phenotypes	DRUGS	GUIDELINES	Additional Genes
CYP2C19	*1/*17, *17/*17	(rapid and ultra-rapid metabolizer)	Clopidogrel	Clinical Pharmacogenetics Implementation Consortium Guideline for CYP2C19 Genotype and Clopidogrel Therapy: 2022 update	
	*1/*2, *1/*3, *2/*17	(intermediate metabolizer)	Dexlansoprazole, esomeprazole, lansoprazole, omeprazole, pantoprazole, rabeprazole.	Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2C19 and Proton Pump Inhibitor Dosing	
	*2/*2, *2/*3, *3/*3	(poor metabolizer)	Voriconazole	Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2C19 and Voriconazole Therapy	
			Amitriptyline, clomipramine, desipramine, doxepin, imipramine, nortriptyline, trimipramine.	Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for CYP2D6 and CYP2C19 Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update	*CYP2D6*
			Citalopram, desvenlafaxine, duloxetine, escitalopram, fluoxetine, fluvoxamine, levomilnacipranmilnacipran, paroxetine, sertraline, venlafaxine, vilazodone, vortioxetine.	Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6, CYP2C19, CYP2B6, SLC6A4, and HTR2A Genotypes and Serotonin Reuptake Inhibitor Antidepressants	*HTR2A, SLC6A4, CYP2B6, CYP2D6*

CYP2D6	(*1/*1)xN,(*1/*2)xN,(*2/*2)xN	(ultra-rapid metabolizer)	Atomoxetine	Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6 Genotype and Atomoxetine	
	*4/*41, *5/*9, *4/*10	(intermediate metabolizer)	Ondansetron, tropisetron	Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6 Genotype and Use of Ondansetron and Tropisetron	
	*4/*4, (*4/*4)xN,*3/*4,*5/*5,*5/*6	(poor metabolizer)	Tamoxifen	Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6 and Tamoxifen Therapy	
			Amitriptyline, clomipramine, desipramine, doxepin, imipramine, nortriptyline, trimipramine.	Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for CYP2D6 and CYP2C19 Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update	*CYP2C19*
			Citalopram, desvenlafaxine, duloxetine, escitalopram, fluoxetine, fluvoxamine, levomilnacipranmilnacipran, paroxetine, sertraline, venlafaxine, vilazodone, vortioxetine.	Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6, CYP2C19, CYP2B6, SLC6A4, and HTR2A Genotypes and Serotonin Reuptake Inhibitor Antidepressants	*HTR2A, SLC6A4, CYP2B6, CYP2C19*
			Alfentanil, buprenorphine, codeine, fentanyl, hydrocodone, hydromorphone, levomethadonemethadone, morphine, naltrexone, oxycodone, remifentanil, sufentanil, tramadol.	Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for CYP2D6, OPRM1, and COMT genotype and select opioid therapy	*COMT, OPRM1*

(continued)

Table 16.1 (continued)

GENES	Genotypes	Phenotypes	DRUGS	GUIDELINES	Additional Genes
CYP2C9	*1/*1	Normal metabolizer	Atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin.	The Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for SLCO1B1, ABCG2, and CYP2C9 and statin-associated musculoskeletal symptoms	*ABCG2, SLCO1B1*
	*1/*3, *1/*2 *2/*2	Intermediate metabolizer	Aceclofenac, celecoxib, diclofenac, flurbiprofen, ibuprofen, indomethacin, lornoxicamlumiracoxib, meloxicam, metamizole, nabumetone, naproxen, piroxicam, tenoxicam.	Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2C9 and NSAID Therapy	
	*3/*3, *2/*3	Poor metabolizer	Fosphenytoin, phenytoin	Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for CYP2C9 and HLA-B Genotype and Phenytoin Dosing	*HLA-B*
	*1/*7, *1/*10, *7/*10	Incierto	Warfarin	Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for Pharmacogenetics-guided Warfarin Dosing	*CYP4F2, VKORC!*

G6PD	B, Sao Borja, IV B/B, B/Sao Borja, B/A, IV/IV	Normal: A person with one X chromosome carrying a nondeficient (class IV) allele.A person carrying two nondeficient (class IV) alleles	Aminosalicylic acid, aspirin, chloramphenicol, chloroquine, chlorpropamide, ciprofloxacin, dabrafenibdapsone, dimercaprol, doxorubicin, furazolidone, gliclazide, glimepiride, glipizide, glyburide, hydroxychloroquine, mafenide, mepacrine, mesalazine, methylene blue, moxifloxacin, nalidixic acid, nicorandil, nitrofural, nitrofurantoin, norfloxacin, ofloxacin, pegloticase, phenazopyridine, primaquine, probenecid, quinine, rasburicase, sodium nitrite, sulfacetamide, sulfadiazine, sulfadimidine, sulfamethoxazole / trimethoprim, sulfanilamide, sulfasalazine, sulfisoxazole, tafenoquine, tolazamide, tolbutamide, toluidine blue, trametinib, vitamin c, vitamin k.	Expanded Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Medication Use in the Context of G6PD Genotype (August 2022)	
	A-, Orissa, Kalyan-Kerala, Mediterranean, Canton, Chatham, II, III A−/A-, A-/Orissa, Orissa/Kalyan-Kerala, Mediterranean/ Mediterranean, Chatham/ Mediterranean, Canton/Viangchan, II/II, II/III, III/III, I/II, I/III	Deficient: A person with one X chromosome carrying a deficient (class II–III) alleleA person carrying two deficient (class II–III) alleles ORone class I allele and one class II or III allele			
	Bangkok, Villeurbanne, I Bangkok/ Bangkok, Bangkok/Villeurbanne, I/I	Deficient with CNSHA: A person with one X chromosome carrying a deficient(class I) alleleA person carrying two deficient (class I) alleles			
	B/Bangkok, B/Mediterranean, B/A-, IV/I, IV/II, IV/III	Variable: A person carrying one nondeficient (class IV) allele andone deficient (class I–III) allele			
	Laibin, Yunan B/Yunan, II/Yunan, III/ Laibin, IV/Yunan	Indeterminate: A person carrying at least one uncertain function allele			

(continued)

Table 16.1 (continued)

GENES	Genotypes	Phenotypes	DRUGS	GUIDELINES	Additional Genes
TPMT	*1/*1	Normal metabolizer	Azathioprine, mercaptopurine, thioguanine	Clinical Pharmacogenetics Implementation Consortium Guidelines for thiopurine dosing based on TPMT and NUDT15 genotypes: 2018 Update	
	*1/*2, *1/*3A, *1/*3B, *1/*3C, *1/*4, *2/*8, *3A/*7	Intermediate metabolizer			
	*3A/*3A, *2/*3A, *3A/*3C, *3C/*4, *2/*3C, *3A/*4	Poor metabolizer			
NUDT15	*1/*1	Normal metabolizer			
	*1/*2, *1/*3, *2/*5, *3/*6	Intermediate metabolizer			
	*2/*2, *2/*3, *3/*3	Poor metabolizer			
CYP2B6	*4/*4, *22/*22, *4/*22	CYP2B6 ultrarapid metabolizer	Efavirenz	Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2B6 and Efavirenz-containing Antiretroviral Therapy	
	*1/*4, *1/*22	CYP2B6 rapid metabolizer			
	*1/*1	CYP2B6 normal metabolizer			
	*1/*6, *1/*18, *4/*6, *4/*18, *6/*22, *18/*22	CYP2B6 intermediate metabolizer			
	*6/*6, *18/*18, *6/*18	CYP2B6 poor metabolizer			
CYP3A5	*1/*1	Extensive metabolizer	Tacrolimus	Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for CYP3A5 genotype and Tacrolimus Dosing	
	*1/*3, *1/*6, *1/*7	Intermediate metabolizer			
	*3/*3, *6/*6, *7/*7, *3/*6, *3/*7, *6/*7	Poor metabolizer			

DPYD	c.[=]; [=], c.[85 T > C];[=], c.[1627A > G];[=]	DPYD normal metabolizer: An individual carrying twonormal function alleles.	Capecitabine, fluorouracil, tegafur	Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Dihydropyrimidine Dehydrogenase Genotype and Fluoropyrimidine Dosing	
	c.[190511G > A];[=], c.[1679 T > G];[=], c.[2846A > T];[=], c.[1129–5923C > G];[=], c.[1129–5923C > G];[1129–5923C > G], c.[2846A > T];[2846A > T]	DPYD intermediate metabolizer: An individual carrying one normal function allele plus one no function allele or one decreased function allele, or an individual carrying two decreased function alleles.			
	c.[190511G > A];[190511G > A], c.[1679 T > G];[1679 T > G], c.[190511G > A];[2846A > T], c.[190511G > A]; [1129-5923C > G]	DPYD poor metabolizer: An individual carrying two nofunction alleles or an individualcarrying one no function plusone decreased function allele			
UGT1A1	*1/*1; *1/*36; *36/*36; rs887829 C/C	Extensive metabolizer	Atazanavir	Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for UGT1A1 and Atazanavir Prescribing	
	*1/*28; *1/*37; *36/*28; *36/*37; rs887829 C/T, *1/*6	Intermediate metabolizer			
	*28/*28; *28/*37; *37/*37; rs887829 T/T (*80/*80), *6/*6	Poor metabolizer			

In Latin America, Pharmacogenetics and Pharmacogenomics are emerging fields, and the main focus of the research is to evaluate ethnic differences in order to apply adapted guidelines to manage personalized pharmacotherapy. Significant differences between countries in awareness and pharmacogenomic testing use are presumed, but not well assessed. The RIBEF (Red Iberoamericana de Farmacogenética y Farmacogenómica) and RELIVAF (Red Latinoamericana de Implementación y Validación de Guías Clínicas Farmacogenómicas) are focused on studying and adapting pharmacogenomic guidelines to the region. Two recent publications collected current efforts to investigate variability in drug response using molecular approaches and the limitations of applying pharmacogenomic tests in clinical centers and hospitals in Latin America (Quiñones et al. 2017; Salas-Hernández et al. 2023).

16.5 Integrative Pharmacogenomic Clinical Cases

Clinical Case 1

A 14-years-old male patient was diagnosed with acute lymphoblastic leukemia and treated with chemotherapy using the BFM protocol.

During his hospital stay, the patient developed fever and septic shock refractory to volume administration. He received vasoactive drugs and broad-spectrum antibiotics. He was then transferred to the critical patient unit.

Aspergillus spp. was identified in a bronchoalveolar sample. Therefore, the clinical team administered 200 mg of voriconazole twice daily. A plasmatic level was taken three days later, and the result was 13.24 mcg/mL (expected range 1.5–5 g/mL). Therefore, the dose was reduced to 100 mg, twice daily. Two days later, an alteration in hepatic enzymes was detected, with an increase in conjugated Bilirubin, Aspartate Aminotransferase (AST/GOT), and Gamma-Glutamyl Transferase (GGT).

Pharmacogenetic tests were performed, and the results were as follows.

CYP2C19 rs4244285 (*2): *2/*2
CYP2C19 rs4986893 (*3) *3/*3
CYP2C19 rs12248560 (*17): 1/*17

The patient carried two homozygous variants, leading to poor metabolism (*2 and *3) due to impaired activity. The other studied variant (*17) could increase gene expression, but all together conferred a poor metabolizer genotype to the patient.

The CPIC guidelines for *CYP2C19* gene and voriconazole therapy indicate, “Choose an alternative agent that is not dependent on CYP2C19 metabolism as primary therapy in lieu of voriconazole.”

The clinical team changed voriconazole to liposomal amphotericin B, and hepatic enzymes returned to normal levels.

Clinical Case 2

A 68-years-old female patient with antecedents of hypercholesterolemia, Acute Myocardial Infarction, depression, and epilepsy was treated with rosuvastatin, aspirin, warfarin, clopidogrel, sertraline, and phenytoin, each of them at usual doses, with unsatisfactory results. To improve the pharmacological therapy, the clinical team required a genotyping test.

The following gene variation panel was proposed for analyses according to the medication:

Rosuvastatin: *SLCO1B1* and *ABCG2*
Sertraline: *CYP2C19* and
Clopidogrel: *CYP2C19*
Warfarin: *CYP2C9* and *VKORC1*
Phenytoin: *CYP2C9* and *HLA-B* haplotypes

The result of the genotyping procedure was:

- *SLCO1B1*: *15/*15
- *ABCG2* (rs2231142): C/A
- *CYP2C19*: *2/*2
- *CYP2B6*:*1/*1
- *CYP2C9*: *1/*3
- *HLA-B 15:02*: noncarrier
- *VKORC1*: −1639 GG

The *SLCO1B1* *1/*15 genotype indicated that the transporter had a poor function in rosuvastatin transport. Additionally, the heterozygous genotype of *ABCG2* indicates impaired transporter function; therefore, the recommendation was to prescribe a starting dose lower than 20 mg. An alternative should be considered if a higher dose was required to control pathology.

*CYP2C19*2/*2* genotype corresponds to a poor metabolizer; therefore, the dose of sertraline must be decreased by at least 50%. Since CYP2B6 corresponded to an extensive metabolizer, the dose adjustment should only be conducted in relation to the *CYP2C19* genotype.

Considering the poor metabolism conferred by the *2/*2 genotype of *CYP2C19*, clopidogrel will not be effective because it is a prodrug bioactivated by CYP2C19. This recommendation should include a change in the drug. Prasugrel was a good treatment option in this case. In both cases, monitoring is necessary to ensure therapeutic results.

The *CYP2C9*1/*3* genotype indicates an intermediate metabolizer phenotype, and together with *VKORC1–1639 GG* (wild-type genotype), this patient probably required a lower dose than usual. Warfarin dosage must be based on validated published pharmacogenetic algorithms considering other non-genetic factors.

Similarly, considering the intermediate metabolizer phenotype of CYP2C9 and the non-carrier *HLA-B15:01* genotype, the use of phenytoin does not increase the risk of hypersensitivity reactions. However, this dose should be reduced, starting with a dose 25% lower than usual, and adjusted according to the response to improve the therapeutic result.

Clinical Case 3

A young Chilean man (24 years old), 1.60 m in height and 54 kg in weight, experienced torsion of his right ankle. He visited the traumatologist, who diagnosed a sprain and indicated immobilization and NSAIDs. Several days after the visit, he started experiencing pain in the popliteal zone near the right knee. Thus, he consulted another traumatologist, who suspected deep venous thrombosis (DVT) and requested eco-Doppler analysis to confirm DVT. The patient was referred to an internist who prescribed warfarin 5 mg daily and control 15 days after. He also indicated not to consume green vegetables. To control his treatment, he decided to evaluate his coagulation on the fourth day; advised by a physician, the INR value was 5.6. Therefore, the doctor decided to decrease the weekly dose to half a tablet from Monday to Friday and one tablet on Saturday and Sunday. After one week, the INR value was 3.9. Therefore, the new dose adjustment was half a tablet from Monday to Saturday, and one tablet on Sunday. After this adjustment, INR was between 2.0 and 3.0, as recommended.

Genotypic analysis was performed after the therapeutic start genetic test. The result was:

– *VKORC1–1639*: G/A (Heterozygous)
– *CYP2C9*2: C/C* (Homozygous Wild Type)
– *CYP2C9*3: C/C* (Homozygous variant)

The warfarin dosage for a patient's genetics differs considering the previous antecedents. There are two tools to choose the correct warfarin dose: the table in the warfarin product insert approved by the U.S. Food and Drug Administration (FDA) (Table 16.2) and the online warfarin dosage algorithm (www.wafarindosing.org).

Table 16.2 shows that the patient was heterozygous for the VKORC1–1639 polymorphism. Thus, we must follow the second row until the *CYP2C9* genotype (*CYP2C9*1/*3*) is reached; accordingly, the recommended daily dose for this patient is between 3 and 4 mg of warfarin. This method does not consider other factors such as age, sex, height, or weight.

The online algorithm considers genetic and not genetic factors (such as demographic, pharmacology, and smoking) to calculate the dose. If we include these factors in the field, the daily recommended dose is 2.3 mg warfarin to achieve an INR of 2.0. This dose is very close to the accurate, stable dose. If the algorithm had been used from the beginning, the patient would not have been at risk of hemorrhage, with INR values >3.0.

Table 16.2 Recommended daily warfarin doses (mg/day) to achieve a therapeutic INR based on *CYP2C9* and *VKORC1* genotype

VKORC1: −1639G > A	*CYP2C9* *1/*1	*CYP2C9* *1/*2	*CYP2C9* *1/*3	*CYP2C9* *2/*2	*CYP2C9* *2/*3	*CYP2C9* *3/*3
GG	5–7	5–7	3–4	3–4	3–4	0.5–2
GA	5–7	3–4	3–4	3–4	0.5–2	0.5–2
AA	3–4	3–4	0.5–2	0.5–2	0.5–2	0.5–2

*Reproduced from www.wafarindosing.org. *CYP* Cytochrome P-450, *VKORC1* vitamin K epoxide reductase complex subunit 1

Clinical Case 4

A clinical case of a Chilean 10-year-old patient, diagnosed with dilated cardiomyopathy, who received standard doses of acenocoumarol and reached INR values greater than 10, forcing treatment to be suspended and restarted more than once. Expected and stable INR levels were achieved after more than 30 days of treatment, unexpectedly, with half the recommended dose for the patient's age.

It was decided to carry out a pharmacogenomic retrospective study that included five single nucleotide genetic polymorphisms (SNPs) with different degrees of association with dose/response to antivitamin K (AVK) drugs: rs2108622 (*CYP4F2 gene*), rs9923231, rs7294 (*VKORC1* gene), rs1799853 and rs1057910 (*CYP2C9* gene). The patient had the following genotype profile (Table 16.3).

National and international evidence shows that this genetic profile, i.e. homozygous for rs9923231 (*VKORC1*) and heterozygous for rs2108622 (*CYP4F2*), is strongly associated with AVK lower doses requirement. (https://www.pharmgkb.org).

The pharmacogenetic analysis confirmed that the patient, with low expression of the therapeutic target of Vitamin K epoxide reductase (VKA), required a lower predictable dose than established according to clinical protocols and literature, as recommended by the FDA and PharmGKB® for coumarin drugs. A priori genotypic analysis of the patient would have allowed the therapeutic range to be reached in shorter times than those observed, avoiding potential risks of bleeding. This finding demonstrates the importance of pharmacogenetic analysis in treatments with high variability and narrow therapeutic ranges.

Reported in Cavieres et al. 2021.

Table 16.3 Results of the genotypes studied for the patient, expected phenotype, and dosing recommendation for acenocoumarol based on the evidence

Gene (SNP)	*VKORC1 (rs9923231)*	*VKORC1 (rs7294)*	*CYP2C9 (rs1799853)*	*CYP2C9 (rs1057910)*	*CYP4F2 (rs2108622)*
Genotype	A/A Variant homozygote	C/C Wild type	C/C Wild type	A/A Wild type	C/T Heterozygote
Phenotype	High risk of bleeding (low enzyme levels)	Low risk of bleeding	Low risk of bleeding	Low risk of bleeding	Low to moderate risk of thrombosis (intermediate metabolism of vitamin K to hidroxy-vitamin K)
Recomendation	Lower dose than standard treatment	Standard dose	Standard dose	Standard dose	Standard dose
Level of evidence[a]	High	Low	Low	Moderate	Moderate

VKORC1 vitamin K epoxide reductase complex subunit 1, *wt* wild type, *ACC* acenocoumarol
[a]From https://www.pharmgkb.org

16.6 Conclusions

The pharmacokinetics of drugs can vary among patients due to genetic factors, affecting each stage of the absorption, distribution, metabolism, and excretion (ADME) processes, leading to variable therapeutic responses.

The use of pharmacogenetic and pharmacogenomic tools can significantly enhance the clinical outcomes in terms of drug efficacy and safety. These tools should be considered at the beginning of patient treatment.

Therefore, it is important to consider polymorphisms related to ADME when selecting drugs. Other factors, such as HLA-B, which is associated with the immune response, should also be considered to improve efficacy and prevent adverse reactions to certain medications. Also, some genetic variants in therapeutic target could modify the expectec response.

Genetic factors should be considered in conjunction with clinical and demographic factors when making informed decisions regarding medication selection and dosage.

As pharmacogenetics is an evolving discipline, further studies are necessary to expand our understanding of the relationship between genetic variants and pharmacological responses, especially in diverse populations. Clinical trials incorporating genotyping will be instrumental in facilitating the integration of pharmacogenetic principles into routine clinical practice at healthcare facilities.

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