Pharmacogenes

Scientific Background

A truncated version of the book “Pharmacogenes: Scientific Background and Clinical Applications”

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Pharmacogenes: Scientific Background

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With love to Shirley, Jenna, and Devin Kisor
David Kisor

To my love and lifelong inspiration, Shannon,

Sarah and Erin Kane

Michael Kane

With love to Annie, Eliza, Lydia, and Laura Talbot

Jeffery Talbot

With love to Heather, Lainey, and Lydia Bright

David Bright

With love to Aimee, Emily, and Ryan Sprague

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Section I

Pharmacogenomics, Pharmacokinetics, Pharmacodynamics, and Precision Medicine

Section I presents, in a narrative manner, genetics related to evolution. The structure of the genome and its regulation are discussed in terms of the underlying cell biology. Variations in the genome are connected to precision medicine, describing specific types of polymorphisms and their relationship to phenotypes and drug response.

CHAPTER One

Introduction to Precision Medicine

Learning Objectives

Upon completion of this chapter, the student will be able to:

1. Recognize basic mechanisms of the expression of genetic information as traits—from the deoxyribonucleic acid (DNA) sequence to transcribed ribonucleic acid (RNA), to translated proteins, to phenotype.

2. Differentiate among the major types of genetic variation, including nonsynonymous, synonymous, nonsense single nucleotide polymorphisms (SNPs), and insertion/deletion (indel) polymorphisms in both genotypic and phenotypic terms.

3. Provide specific examples that establish the relationship between altered drug disposition (absorption, distribution, metabolism, and excretion; ADME) and polymorphic cytochrome P450 enzymes.

4. Describe how polymorphic genetic variation can be utilized to predict individualized responses to drug therapy.

Key Terms	Definition
allele(s)	Alternate sequences or versions of the same gene inherited from each parent.
biomarker (genomic)	A measurable DNA and/or RNA characteristic that is an indicator of normal biologic processes, pathogenic processes, and/or response to therapeutic or other interventions.
codon	Three adjacent nucleotide bases that ultimately encodes a specific amino acid.
exon	A nucleotide sequence that codes information for protein synthesis.
gene	Regions of the genome (DNA) that contain the instructions to make proteins.
genome	The entire DNA of an organism.
genotype	The specific set of alleles inherited at a locus on a given gene.
haplotype	A series of polymorphisms that are inherited together.
heterozygous	Possessing two different alleles for the same trait.
histone	A protein around which DNA coils to form chromatin, thus “packaging” DNA.
homozygous	Possessing identical alleles for the same trait.
indel	Insertion or deletion of DNA either as single nucleotides or spanning regions of DNA involving many nucleotides.
intron	A nucleotide sequence in DNA that does not code information for protein synthesis and is removed before translation of messenger RNA.
monogenic trait	Characteristics derived from a single gene.
multigenic trait	Characteristics derived from multiple genes.
mutation	A change in DNA sequence between individuals.
nucleoside/nucleotide	One of the structural components, or building blocks, of DNA, including adenine (A), cytosine (C), guanine (G), and thymine (T), and of RNA, including adenine (A), cytosine (C), guanine (G), and uracil (U)./Nucleoside linked to a phosphate group.
precision medicine	The use of patient-specific information and biomarkers to make more informed choices regarding the optimal therapeutic treatment regimen for a given patient. "an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person."
pharmacodynamics (PD)	The relationship between drug exposure and pharmacologic response.
pharmacogenetics (PGt)	The study of a gene involved in response to a drug. Pharmacogenomics will be used as the preferred term, which encompasses pharmacogenetics.
pharmacogenomics (PGx)	The study of many genes, in some cases the entire genome, involved in response to a drug.
pharmacokinetics (PK)	The relationship of time and drug absorption, distribution, metabolism, and excretion.
phenotype	An individual’s expression of a physical trait or physiologic function due to genetic makeup and environmental and other factors.
polymorphism	A mutation in DNA in a given population that may be observed at greater than 1% frequency.
reference sequence number (refSNP, rs#, rs)	A unique and consistent identifier of a given single nucleotide polymorphism (SNP).
single nucleotide polymorphism (SNP)	A variant DNA sequence in which a single nucleotide has been replaced by another base.
topoisomerase	A class of enzymes that alter the supercoiling of double-stranded DNA.
wild-type	The typical or normally occurring genotype of an organism.
xenobiotics	Substances (often drugs) introduced into the body but not produced by it.

Introduction

In its simplest terms, **precision medicine** is the use of patient-specific information and **biomarkers**, including genes to make more informed choices regarding the optimal therapeutic treatment regimen for that patient, rather than reliance on population-based therapeutic trends. **Pharmacogenomics (PGx)** is the aspect of precision medicine whereby patient-specific genomic biomarkers are used to choose the optimal "first-line" drug and/or dose for the patient, with the goal of assuring drug efficacy in the patient while minimizing or avoiding the risk of an adverse drug reaction. The successful implementation of pharmacogenomics in the clinic is dependent upon a number of different processes and data, including *a priori* knowledge about a specific **allele** in the genome and its linkage to altered **pharmacokinetics (PK)** and/or **pharmacodynamics (PD)**(compared to the statistical norm in the population), the ability to accurately test a patient for the presence of a specific allele in his or her genome, and the ability to offer the patient more effective alternatives than would be typically offered to a patient in the statistical norm of the population. Key to this process is the utilization of prior discoveries and clinical findings (e.g., data) regarding a specific genomic allele relevant to the pharmacokinetics and/or pharmacodynamics of the prescribed or intended drug, and then *predicting* how the patient will respond to the drug. Finally, the utilization of pharmacogenomics and precision medicine must add value to healthcare. In other words, the costs associated with the implementation and utilization of pharmacogenomics and precision medicine must be ethically and economically justified by reducing the negative (adverse) effects and costs associated with adverse drug reactions as well as the assurance of more effective drug therapy outcomes for the healthcare consumer population.

Here, we distinguish the term *pharmacogenetics* from the now more commonly used ***pharmacogenomics*** **(PGx)**. In its purest sense, pharmacogenetics refers to the study of a gene involved in response to a drug, whereas pharmacogenomics (PGx) refers to the study of all genes in the genome involved in

response to a drug.[1] However, the vernacular that has emerged in recent years often uses the term *pharmacogenomics* to reference the entirety of the science and the methods that study the interface of genomics, genetics, and drugs used in clinical therapeutics.

Living Systems and the Genome

The adult human body contains trillions of different cells, each performing different functions to sustain life. Some of these are muscle cells, some make up our skin, some are blood cells, some form bone, some are brain or liver cells, and so on. Each of these cells has developed within a specific tissue in the body to perform a specific function. For example, our red blood cells are capable of transporting molecular oxygen from our lungs to organs and tissues, and then transporting carbon dioxide back to the lungs to be removed from the body. This unique cellular capability is due to the presence of a specific protein in the red blood cell called hemoglobin. More specifically, what we commonly refer to as hemoglobin is actually a multimolecular structure that contains a heterocyclic organic molecule called heme, which is bound to an atom of iron, as well as two specific globular proteins. These globular proteins are alpha-globulin and beta-globulin, which are each derived from a specific gene in our genome. Thus, the genes are used by the cellular machinery as a "blueprint" or "instruction set" on how to make these proteins. Hence, **genes** are the regions of our genome that contain the instructions to make proteins, and proteins are the functional components of living systems. In simpler terms, if the genes are the "blueprints" for life, then proteins are the "bricks and mortar" of living systems. Proteins are the inherited, functional components of living organisms—inherited because they are derived from our genome, which we inherit from our parents and ancestors. Interestingly, less than 2% of our genome is actually used as template genes to make proteins.[2] We will discuss aspects of proteins later; let us first take a closer look at our genome.

The **genome** of an organism is the instruction set for that organism, or, more specifically, the instruction set for the

development of its cells and tissues, as well as the maintenance of these cells and tissues throughout the life of the organism. The functional molecule that makes up our genome is DNA (deoxyribonucleic acid). Our genome is made up of four different DNA **nucleoside** bases (adenine-A, cytosine-C, guanine-G, and thymine-T), which are somewhat equivalent to a written language with four different letters. The human genome contains about three billion nucleotide bases (or letters; A, C, G, T) in the genome, and essentially each cell (that contains a nucleus) contains a copy of the entire genome. To appreciate the size of the human genome, consider that, if our genome was printed in paperback novel form, it would contain over four million pages. By no means is the human genome considered large within the spectrum of living organisms on earth. The onion (yes, the one you eat) has a genome that is more than six times the size of the human genome, and it has been estimated that certain lilies (flowering plants) have a genome that is 30 times bigger than our own.[3,4] We will not be discussing the complexities of plant genomes in this textbook, as scientists are only beginning to understand the different complex genomes in living organisms.

If we take the perspective of our genome representing information, then we must recognize that each cell has the instructions (genes) for all the proteins that the organism can ever make, even though the cell may not use all this information. In other words, each cell (that has a nucleus) has all of the chromosomes of the genome, and therefore all the genes that we have inherited from our parents, yet each cell only uses a subset of these genes to make the proteins it needs to thrive and carry out its various functions. To conceptualize this phenomenon, imagine that each person in your college or organization was a cell, and each person has a computer that contains all the programs needed to make the entire organization run successfully. A person working in the accounting office would use the computer programs (i.e., genes) that are used to manage the organization's resources and inventory but would not use the programs used for personnel management (even though those programs are stored on the computer). Similarly, a muscle cell uses the genes to make the proteins used for mechanical

contraction, but not the genes that make the proteins for detecting light that are used in the retinal cells of the eye.

Genetic Evolution and the Evolution of Genetics

Sexual reproduction is the fundamental process for enabling genetic diversity and the propagation of life on earth. It involves the passing of genetic information from viable parent organisms to their offspring. Thus, the offspring inherit the genetic information that allowed their parent organisms to thrive and survive in the environment. Furthermore, it allows two different successful organisms (the biological mother and father) with different genomic content to create variations of their respective genomes in their offspring, thereby creating genetically varied offspring. The life of these offspring represents a test of the content of their genome, and reaching sexual maturity and successfully reproducing reinforces the rigor of their genomic content (i.e., the viability of the organism was sufficient to endure its environment), which is passed on to subsequent generations. This is the basis of natural selection, or "survival of the fittest," from the perspective of the inherited genome. The genetic variability among the population (of a given species) appears extremely important for the evolutionary success of the species because it allows the species to adapt to changes in its environment over generations by reinforcing the traits that confer viability. In other words, as changes in the environment emerge and exert selective pressure on the species, members of the population that harbor the genetic content (that encode physical or behavioral traits) to overcome these environmental changes survive and shape the genetic content and physical traits of subsequent generations. This process is a fundamental tenet of evolution on earth.

We are all familiar with the genetic diversity and variation in the human population, as evidenced by obvious physical traits, such as eye color, hair color, and so on. These physical traits are the result of inherited genes from our biological parents, and dictate aspects of our physical appearance. This genetic variation extends into many aspects of our genome and cell biology that

are not always as obvious, such as those that affect behavior or aspects of cellular biology. These variations in the human population are very important to the perpetuation of our species. As our regional and planetary environments change over long periods of time, those individuals that are best suited to survive in the changing environment will thrive and continue to bear offspring. The makeup of our genome, and therefore our physiology, is the result of millions of years of evolution under the selective pressures of our environment as well as competition for survival. In other words, the physical and behavioral traits that provided our ancestors with a competitive advantage allowed those individuals to thrive and bear offspring, whereas individuals who lacked a given competitive advantage were much less likely to thrive and bear offspring. Therefore, the individuals that harbored advantageous traits passed their genes onto their offspring, and after thousands of generations of human evolution, the content of our genome is the result of this evolutionary process. Thus, our genome contains the genes that conferred the beneficial traits needed for our ancestors to thrive.

These traits may be very subtle yet important, such as the ability to digest lactose into adulthood and therefore derive sustenance from the milk of the beasts of burden that our ancestors domesticated over the last 10,000 years. Our ancestors who experienced this evolutionary adaptation could better survive periods of famine and drought, and this trait was retained in our recent evolution. It should become obvious that the changes in our genetic makeup that resulted in specific competitive advantages would be passed down through generations, whereas changes in our genome that did not serve a beneficial purpose, or in many cases even reduced the viability of an individual, are not seen in the modern human genome. Hence, we are the modern beneficiaries of this genetic "arms race" of inherited traits that has been ongoing for millions of years.

When we consider the past influences that have shaped our biology and the content of our genome, we can begin to

understand why exposure to certain chemicals and compounds that we may ingest pose a threat to our survival whereas other substances are safe. For example, certain mushrooms (e.g., the death cap mushroom) and frogs (e.g., the poison dart frog of South America) synthesize compounds that are toxic to organisms that would otherwise consume them as a nutrient source. The death cap mushroom synthesizes a compound called amanitin, and the poisonous dart frog synthesizes epibatidine (among other alkaloids). It is well known that these compounds are highly toxic to humans and many other organisms.[5]

No evidence suggests that there are variations in the sensitivity to these poisons among humans, and thus we all avoid consuming these mushrooms and frogs, an adaptation that benefits the mushroom and the frog. Because these organisms, and their poisons, have existed in nature for millions of years alongside our ancestors, it is not necessary to consider variations in the toxicity of the poisonous compounds across the modern human population. Building upon our evolutionary theory, we can imagine a fictionalized paradigm of selective pressure 50,000 years ago where these mushrooms were abundant and only a subpopulation of humans harbored the ability to detoxify the poison in the mushroom and therefore safely consume the mushroom as a nutrient source. If this were the case and the mushrooms were an abundant source of nutrients, the humans that could safely consume the mushrooms would thrive, whereas the humans that were sensitive to the toxin would be less likely to thrive. In this fictitious example, it is likely that all humans living today would harbor the ability to detoxify and safely consume the poison simply due to selective pressure on our ancestors. In genomic terms, modern humans would harbor a gene in their genome that encoded an enzyme capable of breaking down the poison. Although this is not true for the poisons mentioned in this example, it is true for many other substances in nature that our ancestors encountered.

The example above is presented to demonstrate a fundamental difference between naturally occurring substances

and modern pharmaceutical products. When we look at modern pharmaceutical compounds, we see a much more varied response among humans to both the safety and efficacy of these compounds, even though the process of drug development attempts to provide drugs that are efficacious and safe for the entire population. One reason for the varied responses to drugs is that these pharmaceutical compounds did *not* exist in nature and were not available for consumption by our ancestors. Therefore, no evolutionary selective pressures have been experienced in humans with respect to exposure to these pharmaceutical compounds, and the outcome of evolutionary selective pressure has not been manifested in the genome through thousands of generations. Thus, we expect a much more varied response in the population to these modern medicinal chemicals, compared to naturally occurring substances.

The development of safe and effective medicinal compounds is a challenge because there can be a spectrum of responses in the population regarding the safety and efficacy of a drug and this can complicate the management of pharmacy and therapeutics in our modern healthcare system. In other words, due to the methods used to assess and approve new drug entities, modern drug approval requires that it be safe and effective in a large majority of the population. Thus, drugs under development that have shown large variation as to their safety and/or efficacy have not gained marketing approval. It should be obvious that if the genetic basis for variations within the population to the safety and/or efficacy of a drug are studied and understood, then a drug that is effective in a known subpopulation could be approved, if that subpopulation can be identified through genomic testing. In fact, this movement in pharmacotherapy will result in safer and more effective drug use within subpopulations in our society and enable healthcare professionals to use genomic screening to predict how a patient will respond to a specific drug and therefore inform healthcare professionals as to which drug and/or dose is optimal for the patient. This is a principal tenet for the adoption of precision medicine.

We have discussed how selective pressure and evolution

have shaped the content of our genome, now let us look at our genome from a completely different perspective: How have advances in modern healthcare and disease management or, more specifically, extending the length of human life, inadvertently revealed (or invented) a new type of genetic predisposition to disease?

At some time in our recent history, pre-modern humans lived together in groups composed primarily of three generations (i.e., children, parents, and grandparents). In a simple version of anthropological theory, the parental generation (in the physical prime of their life) worked to search for resources, gather food, and defend the group, while the elders helped oversee the young of the group. It is important to note that larger groups of individuals consume, and therefore require, more resources (e.g., food, water) than smaller groups. Thus, larger groups of early humans were at a disadvantage in times of limited resources (e.g., drought, famine), compared to smaller groups. Therefore, it was not beneficial for pre-modern humans to have a long lifespan, as this would result in large groups that were at a competitive disadvantage; for this reason, human life expectancy was much shorter than it is today.

Australopithecines appear to have had an average life expectancy of only 15 to 20 years and survived for about 300,000 generations, ending about two million years ago. More recently, early agriculturalists and nomadic pre-modern humans had an average life span of about 25 years and survived only about 500 generations. These ancestral life spans suggest that age-related declines in function after the age of 25 were due to the forces of natural selection. In modern times, the last 200 years of human history (about 10 generations), the average life span has increased from 43 to 75 years of age.[6]

If we consider that the vast majority of our ancestors only had life expectancies of younger than 45 years, then certainly their genomic content and function was to maintain optimal health until this age, with no evolutionary advantage to

extending life span. The increase in life expectancy between pre-modern and post-modern humans is nearly instantaneous as compared to the much longer timelines associated with pre-modern human evolution. For the purposes of illuminating this perspective, it can be assumed that our genome is essentially identical to our pre-modern ancestors' of 5,000 years ago.

In other words, the modern human genome has evolved to support an individual's life until they reach about 45 years old (this may even be a generous estimate), even if living in a modern society. Or, more accurately, any health problems that have a basis in genetics would not have been passed down from our ancestors if the health problem manifested itself early in life (i.e., before about 35 years). However, if the genetic-based health problem manifests itself after the age of 45 years, it would not have exerted selective pressure against individuals that harbored this genetic allele. Therefore, it would not have negatively influenced the survival of our pre-modern ancestors, and it would be expected to be present in our genome today. In other words, extending human life beyond the age of 50 years "reveals" new diseases in the human population, and the management of these age-related disorders becomes more dependent on modern healthcare methods, practices, and technology as we age. From this perspective, if it is determined that an individual has a genetic predisposition for a disease or disorder with an expected onset at 60 years of age, it is not a failure of human evolutionary processes but simply an artifact of extending human life.

Many examples of age-related disorders with genetic underpinnings can be found in humans, and it is certain that more discoveries will be made linking specific genetic markers with age-related disorders. An example of an age-related disorder with a known genetic link is Huntington's disease. Huntington's disease involves an inherited genetic defect where an expansion of a three-nucleotide repeat (CAG) in the protein-coding region of a specific gene (named Huntington) causes the protein to self-aggregate. The deleterious effects (symptoms) of this genetic defect are usually first manifested at about 40 years

of age. Thus, there were no selective pressures to eliminate this genetic defect from the population in pre-modern humans because it was not a genetic defect until our life expectancy increased beyond 40 years. This can be said for essentially all genetically linked diseases in humans older than 40 years of age.

As we consider age-related diseases, the ability to utilize genomic screening in the clinic is very important in identifying people who are predisposed to a specific age-dependent disorder. Ideally, utilizing genomic screening in this paradigm allows the patient ample time to take measures to reduce or eliminate the risk of the disorder, such as changes in diet, exercise, prophylactic medicines, and so on. In this case, it is important to note that although there is currently no "cure" for Huntington's disease, the disease can be diagnosed using genetic screening methods prior to the appearance of any disease symptoms. Thus, the use of genetic screening methods for disease risk should be carried out with adequate genetic counseling because (1) the results of genetic screening must be interpreted correctly and (2) there can be significant psychological ramifications associated with the results of genetic screening for the patient and family.

Genome Structure and Gene Regulation

Less than 2% of the human genome is made up of gene sequences that encode proteins, and these genes are distributed throughout the 23 chromosomes and mitochondrial DNA of the human genome. The remaining 98% of the genome exists between gene sequences (i.e., intergenic DNA sequence) and contains many important regions that are key elements to DNA replication and DNA regulatory machinery. For example, polymorphic variations in intergenic DNA sequence may influence DNA tertiary structure directly or alter binding sites of DNA regulatory machinery, including **histones** and **topoisomerases**, which exert profound influence on overall gene regulation, cellular signaling, and homeostatic responses to environmental stresses. Indeed, recent studies implicate mechanisms of DNA–histone binding in the potential underlying pathophysiology of mood disorders and drug addiction while

pointing to potential therapeutic targets for novel antidepressant and antipsychotic therapies.[7] One insight that is gained when considering the size of plant and animal genomes, and the relatively small fraction of these genomes that actually encode proteins (i.e., genes), is that the retention of large noncoding regions in the genome over millions of years of generations does not appear to consume excessive cellular resources that place the organism at a disadvantage to survival and/or there is an evolutionary advantage to retaining these large noncoding regions, even those regions that do not appear to be critical for DNA replication. Note that the relevance of intergenic DNA sequence to pharmacogenomics is emerging. One potential example of these effects is that of the O^6-methylguanine DNA methyltransferase (*MGMT*) enzyme that repairs DNA damage induced by alkylating chemotherapeutic drugs such as temozolomide. Evidence suggests that hypermethylation of DNA regions upstream of MGMT suppress its expression in some types of B lymphoma cells, causing increased susceptibility to the cytotoxic effects of anticancer medications used in treatment.[8] However, more research and linkage studies must be carried out to fully understand how specific allelic variations in these regions will be utilized to alter drug dose and/or drug choice in clinical practice.

The human genome is made up of approximately 25,000 distinct genes, each capable of coding a unique protein, and it is these proteins that enable our cells to carry out the many different molecular, enzymatic, and mechanical processes that enable life. Because most drugs interact with proteins, pharmacogenomics deals primarily with genetic variations that affect gene regulation (i.e., DNA sequence variations that alter how much of each protein is being synthesized in the cell) and protein function or activity (i.e., DNA sequence variations in the gene that alter the amino acid sequence of the protein). Pharmacogenomics involves an understanding of how individual genetic differences in a population are the cause of variable responses to a specific dose of a drug in a population. In order to effectively examine the interactions between pharmacokinetics, pharmacodynamics, and genetics, we must first understand how genes are regulated in the cell and how the gene sequence (coding sequence) defines the

primary sequence of a protein.

The simplest description of a gene's structure can be divided into (1) a regulatory region, where the cellular machinery exerts its effect on if, and how much, the gene will be "activated" or used by the cell and (2) the coding region, where the DNA sequence directly correlates with the protein sequence (see **Figure 1-1**).

The regulatory region contains specific DNA sequences and motifs where transcription factors and other regulatory elements bind, thereby promoting or preventing the transcription of the gene. During gene transcription, ribonucleic acid (RNA) polymerase binds within the regulatory region and then moves along the coding sequence to create a direct copy of the gene sequence. This RNA copy will undergo further processing before leaving the nucleus of the cell, ultimately coupling with the ribosome to synthesize the protein from the gene. In **Figure 1-2**, the details of eukaryotic transcription are described.

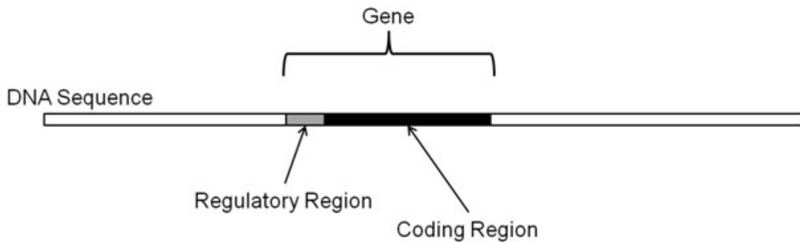


Figure 1-1 Simplified gene structure. The structure of a gene can be divided into the regulatory region, which is responsive to cellular machinery controlling its expression, and the coding region, where the DNA sequence directly correlates with the protein sequence.

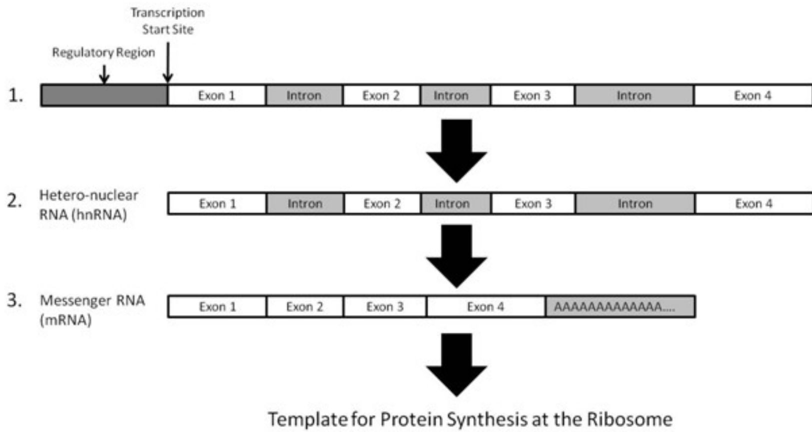


Figure 1-2 Eukaryotic transcription and translation. Genetic information derived from the DNA sequence is converted to functional proteins when: beginning at the transcription start site, the double-stranded DNA sequence is directly copied into a single-stranded heteronuclear RNA (hnRNA); messenger RNA (mRNA) is formed when introns are spliced or removed from the hnRNA and a poly-A tail is added; and mRNA is translated into a protein via protein synthesis at the ribosome.

As shown in the figure, the double-stranded DNA sequence (1) is directly copied into a single-stranded RNA sequence known as heteronuclear *RNA* (hnRNA), beginning at the transcription start site. Heteronuclear RNA is then (2) processed by removing the **intron** regions, a process termed splicing, and (3) a poly-A tail is added, resulting in messenger RNA (mRNA), which moves from the nucleus to the ribosome for protein synthesis.

Note that the removal of the intron sequence in the hnRNA results in the concatenation of the **exon** sequences in the mRNA, which represents the coding sequence for the protein. At the ribosome, the genetic coding sequence (nucleic acids) is converted to the protein sequence (amino acids). Each and every

amino acid in a protein is coded by three nucleic acids, called a **codon** (see **Figure 1-3** for a codon key). For example, the nucleic acid codon “AUG” encodes for the amino acid methionine in a protein sequence. Note that the thymine (T) in DNA is replaced by uracil (U) in RNA. In addition to the codons that encode specific amino acids, three codons (UAA, UAG,

		Second Position				
First Position		U	C	A	G	Third Position
	U	UUU - Phe	UCU - Ser	UAU - Tyr	UGU - Cys	U
		UUC - Phe	UCC - Ser	UAC - Tyr	UGC - Cys	C
		UUA - Leu	UCA - Ser	UAA - STOP	UGA - STOP	A
		UUG - Leu	UCG - Ser	UAG - STOP	UGG - Trp	G
	C	CUU - Leu	CCU - Pro	CAU - His	CGU - Arg	U
		CUC - Leu	CCC - Pro	CAC - His	CGC - Arg	C
		CUA - Leu	CCA - Pro	CAA - Gln	CGA - Arg	A
		CUG - Leu	CCG - Pro	CAG - Gln	CGG - Arg	G
	A	AUU - Ile	ACU - Thr	AAU - Asn	AGU - Ser	U
		AUC - Ile	ACC - Thr	AAC - Asn	AGC - Ser	C
		AUA - Ile	ACA - Thr	AAA - Lys	AGA - Arg	A
		AUG - Met	ACG - Thr	AAG - Lys	AGG - Arg	G
	G	GUU - Val	GCU - Ala	GAU - Asp	GGU - Gly	U
		GUC - Val	GCC - Ala	GAC - Asp	GGC - Gly	C
		GUA - Val	GCA - Ala	GAA - Glu	GGA - Gly	A
		GUG - Val	GCG - Ala	GAG - Glu	GGG - Gly	G

Ala	Alanine	Gly	Clysine	Pro	Proline
Arg	Arginine	His	Histidine	Ser	Serine
Asn	Asparagine	Ile	Isoleucine	Thr	Threonine
Asp	Aspartic Acid	Leu	Leucine	Trp	Tryptophan
Cys	Cysteine	Lys	Lysine	Tyr	Tyrosine
Gln	Glutamine	Met	Methionine	Val	Valine
Glu	Glutamic Acid	Phe	Phenylalanine		

Figure 1-3 The genetic code. In the expression of genetic information, the codon key describes the code for each amino acid in a protein based on three nucleic acids, termed a “codon.”

and UGA) encode a “stop” command, thereby stopping the growth of the protein at that point in the sequence.

Cell Biology and the Human Genome

In living systems, the cell is the basic unit of life. Each cell that contains a nucleus contains the entire genome of the organism, although the cell only utilizes a subset of genes to enable its viability and function within the organism. Within the nucleus of human cells are 23 pairs of chromosomes (46 chromosomes total). Chromosomes were originally discovered over 100 years ago using basic dyes and state-of-the-art microscopes (at that time), thus the name chromosome simply means “colored body” as a description of how they were first observed in the nucleus of the cell. One pair of chromosomes is associated with gender and is commonly referred to as the sex chromosomes. Females have two “X” sex chromosomes, whereas males have an “X” and a “Y” sex chromosome.

In simple terms, chromosomes are essentially unbroken polymers of double-stranded DNA. They often are associated with histone proteins that enable an efficient “packaging” of the DNA prior to cell division. The state of DNA in the cell correlates with the different phases of cell division (see **Figure 1-4**). It should be obvious that when a cell divides into two daughter cells, each cell must have a copy of the genome to remain viable. The cell goes through four phases to replicate itself, which includes replication of its genomic content. In the G1 phase*,* the activity of the cell is largely dedicated to growth and maintenance of the functions of the cell. As a cell prepares to undergo mitotic division, it enters the S phase, during which the entirety of the DNA (chromosomes) in the cell is duplicated (i.e., DNA synthesis = “S” phase), resulting in two copies of each chromosome. Completion of the DNA (chromosome) duplication leads to the G2 phase*,* and the chromosomes are

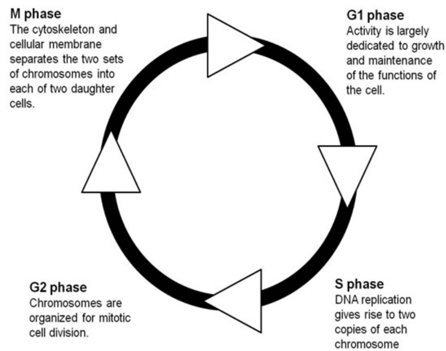


Figure 1-4 The four phases of the cell cycle.

organized in preparation for mitotic cell division. During the M phase of cell division, the cellular membrane separates the two sets of chromosomes into each of two daughter cells, and each daughter cell reenters the G1 phase, each with a complete copy of the genome within the chromosomes of its nucleus.

In addition to the chromosomal DNA found in the nucleus of the cell, a relatively small amount of DNA is found in the mitochondria. Mitochondria harbor about 16 kilobases of DNA (called mtDNA) in a circular form. In humans, the mtDNA contains 37 genes that encode proteins utilized by the mitochondria for energy production and protein synthesis. The mtDNA undergoes its own replication as mitochondria replicate within the cells of our body. Importantly, mtDNA is maternally inherited because the large female gamete (egg cell) contains hundreds of thousands of mitochondria, whereas the mtDNA in the much smaller male gamete (sperm cell) is not incorporated during fertilization of the egg. Thus, the fertilized egg only contains maternal mtDNA and is therefore used in genetic research for mapping maternal inheritance across generations.

Cells exist in the human body that do not harbor a nucleus

and therefore lack a copy of the human genome. The red blood cells (RBCs, also known as erythrocytes) and platelets (also called thrombocytes) of the blood are derived from parent cells in the long bones of our bodies. Red blood cells are involved in oxygen transport in the blood and are derived from a process called erythropoiesis involving progenitor cells (e.g., proerythroblasts, polychromatic erythroblasts), whereas platelets are involved in blood clotting and are derived from megakaryocytes. These non-nucleated cells lack nuclear DNA, yet they harbor genetic information in the form of RNA, allowing the cells to synthesize proteins. The DNA that is obtained from a blood sample is actually derived from the white blood cells, which make up about 1% of blood volume in healthy adults.[9]

This is important when we consider using DNA genotyping to support advances in healthcare. If we use genotyping to screen for a neurological disease, we do not need to sample the human brain directly because almost all other cells in our body contain the complete genome. Therefore, we can carry out genotyping using cells that are easily obtainable (e.g., white blood cells, from a buccal swab to gather the cells from inside the mouth), thereby providing a noninvasive, nondestructive method for gaining access to our genomic information.

Categorically, genetic testing in humans is routinely carried out in four distinct areas: paternity and/or maternity, DNA forensics, disease predisposition, and pharmacogenomics (see **Table 1-1**). Paternity/maternity testing is used to establish a biological relationship between a parent and an offspring, whereas DNA forensics can determine the origin and/or identity of a biological sample. In both of these areas, the genomic

Table 1-1 Categories of Human Genetic Testing

	Paternity or Maternity Testing	**DNA Forensics**	**Disease Predisposition**	**Pharmacogenomics**
Utility	Determine biological parent.	Determine identity of crime scene DNA sample.	Determine cause of, or predisposition for, disease or disorder, or if the patient is a carrier for an inherited disease.	Predict optimal drug and/or dose for specific patient.
Sample source	Buccal swab	Varied	Buccal swab, saliva, or blood sample	Buccal swab, saliva, or blood sample
Target	Short tandem repeats (STR)	Short tandem repeats (STR)	Allelic variations linked to disease/disorder	Genes for drug metabolism enzymes, drug transporters, and drug receptors
Rapid testing turnaround required	Infrequently	Infrequently	No	Yes

biomarkers commonly tested are called short tandem repeats, or STRs, which are short repeated sequences of DNA. Another growing area of human genomics involves testing for specific genomic biomarkers associated with disease, where the genetic cause for a disease or disorder is established as a diagnostic tool or used to determine the risk of developing the disease.

Pharmacogenomics, however, points to important distinctions among these areas of genetic testing. Each has shown tremendous utility and societal value. Yet, in order to derive the full clinical potential of genetic testing in pharmacogenomics, information regarding genetic variation as it relates to the disposition and effect of medications must be immediately available to caregivers. Thus, the value of pharmacogenomics is more likely to be dependent on technologies and information systems/ procedures that allow for rapid testing and provide clinicians with more real-time access to a patient's individual genetic data.

Genetic Variation and Precision Medicine

The essence of precision medicine is individual genetic

variation. The most obvious and perhaps most basic examples of individual genetic variation are observed outwardly. Readily apparent physical traits, such as skin tone, eye color, hair color, height, and even shoe size, are all dictated by genes that vary, in some cases dramatically, between individuals. In this sense, the gene–trait interface could be described in modern, colloquial terms as "designing" an avatar in a video game. Each player is offered choices that determine the appearance of the avatar. Analogous to a genetic menu of sorts, one can scroll through screens of options, ranging from body type to facial structure, where nuances such as the thickness of the eyebrows, shape of the nose, and distance between the eyes are presented. These choices allow an avatar to assume a uniqueness that, although immensely oversimplified, can be extrapolated to represent genetic variation and the direct relationship between genetic identity and physical traits (see **Figure 1-5**). Yet, as we move from electronic simplification to genetic reality, the avatar analogy quickly fades—the vast complexity of the human genome provides for a much deeper level of variation between individuals.

Analysis of the human genome following publication of its first complete sequence in 2003 only begins to describe this complexity. As described earlier, each human germ-line cell contains approximately three billion nucleotide base pairs of DNA comprising around 25,000 genes, and among this immense store of genetic code there is tremendous intraspecies homogeneity, a fact underscored by the discovery that all humans share roughly 99.9% of the DNA sequence.
Such uniformity makes perfect sense. Genes encode for proteins for which functions are nearly always precisely limited by their tertiary and quaternary structure, which dictates efficiency of enzymatic and/or biological processes. One dramatic example is actin, a type of cytoskeletal scaffold that owns the title of being the most abundant protein in nearly all human cells, comprising anywhere from 10–20% of total cell protein. In fact, the typical hepatocyte contains an estimated 500 million actin molecules, giving the cytosol a gel-like rather than fluid consistency.[10] As is implied by their abundance, actin proteins are essential for a variety of biological functions, such as structural integrity, cell

shape, cell motility, chromosome morphology, and muscle contraction, as well as a host of intracellular events, including gene transcription and translation. Thus, it is of little surprise that the six human genes encoding for the three actin isoforms (α, β, and γ) are among the most highly conserved in the entire genome, being second only to the histone family of DNA-binding proteins. In fact, the DNA sequence of human actin is over 80% identical to that found in yeast, with a near 96% amino acid homology.[11]

This incredible degree of interspecies homogeneity means that biological activity in eukaryotes is extremely sensitive to changes in the DNA sequence. Indeed, entire clusters of genes exist with a sole recognized function of minimizing DNA mutations during cell -division. One such family of proteins is known as the mismatch repair genes.[12] Also highly conserved, this family of nine unique proteins "proofread" newly replicated daughter strands of DNA for relatively common errors in base incorporation by DNA polymerases, errors that would otherwise result in nearly one mutation for every 1,000 base pairs replicated. Instead, mismatch repair enzymes identify "mismatched" bases, excise them from the newly replicated daughter strand, and finally reinsert the correct deoxyribonucleotide base. This effectively reduces the average mutation rate by six orders of magnitude, or to less than one base change per billion bases.

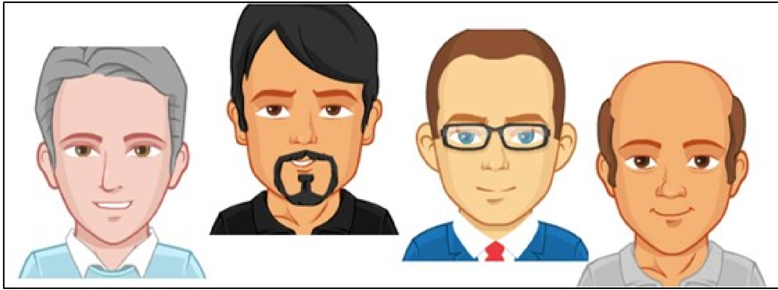


Figure 1-5 Avatars representing four of the authors of this text (generated by www.pickaface.net). *Source*: Courtesy of Fredy Sujono from www.pickaface.net.

The importance of mutation-reducing enzymatic activity is obvious. DNA sequence fidelity transmitted from parent cell to daughter and from parent organism to offspring allows for continuity of gene sequence, which provides for continuity of inherited traits. Moreover, evolutionary pressures of selection work toward maintaining individuals with as little genetic diversity as possible, at least with respect to the many thousands of genes, like those for actin, whose activity is required for sustainable life.

In spite of these Herculean cellular efforts and the constant evolutionary pressures that favor DNA fidelity across generations, genetic variation persists. Small changes in genetic code continue to arise, and these often more subtle mutations, known as polymorphisms, give rise to a deeper, and in some ways more defining, characteristic of genetic variation among individuals.

Polymorphic Genetic Variation

In the most basic sense, changes in the genetic code are observed as differences in DNA sequence called **mutations**. These changes in sequence may or may not produce observable differences in traits either in an individual or in its offspring. Mutations that occur in genomic DNA between individuals gives rise to genetic variation—that one person's DNA sequence differs from another at specific bases. Some mutations are more common than others in a population. When a particular mutation occurs in at least 1% of individuals in the population, it is -commonly referred to as a **polymorphism**, which is derived literally from the Greek word meaning "many forms." For example, if at a given location in the genome 4% of individuals contain adenine (A) but the other 96% contain a cytosine (C), the A represents a polymorphism. In this way, the term polymorphism is used to help describe the prevalence of a specific genetic variation between individuals within a population.

Variants are incredibly common. Individuals differ in their DNA on average by one base pair for every 100 to 300 base pairs throughout the genome, although their frequency can be much greater within a given gene. It has been estimated that as many as 9 to 10 million polymorphisms may reside in the human genome, yet it is highly unlikely that any one individual will carry all possible polymorphic variations.[13] However, because of their frequency, polymorphisms are particularly useful in describing genetic differences between individuals, especially differences that define discrete subpopulations within the population as a whole.

The manifestation of variation in the genetic code can be dramatic. One such example is found in the human α-actin gene. Familial hypertrophic cardiomyopathy (FHC) is an autosomal dominant congenital disease that leads to compromised cardiac function (syncope, angina, arrhythmias, and heart failure) and is the leading cause of sudden death in young people.[14] At least nine different mutations in α-actin have been directly linked to FHC, including a guanine to thymine (G→T) mutation at base 253 of exon 5 in the actin gene. This change, where the **wild-**

type or typical sequence found in "normal" individuals is altered, results in the substitution of the amino acid serine for alanine at position 295 within the actin protein and is denoted as Ala295Ser. The simple G→T variation results in an actin molecule whose binding affinity for β-myosin is diminished, which reduces the strength of cardiac muscle contraction and can -contribute to -potentially fatal hypertrophy of the left ventricle (see **Figure 1-6**).[15]

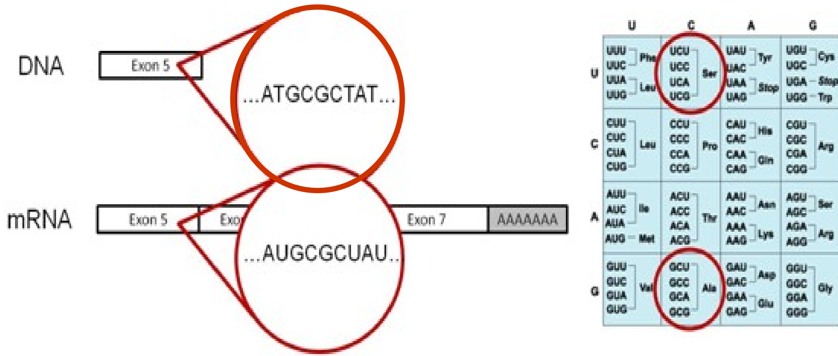
Understandably, potentially serious physiological consequences that can be expressed at a young age make the actin Ala295Ser variation less likely to be transmitted generationally. However, a far greater degree of genetic variation is interspersed throughout the genome. Remember that nearly 99% of the genome is contained within regions of DNA considered noncoding or intergenic that do not directly encode for protein. Thus, the vast majority of variations are likely to be neither harmful nor beneficial *per se*. Yet, there is a growing appreciation for the potential role of polymorphisms in directly causing, or indirectly associating with, characteristics and traits that vary between groups within a population, especially as it pertains to individual responses to drugs.

In general, polymorphisms can be categorized into two main types: **single nucleotide polymorphisms**, commonly referred to as **SNPs** (pronounced "snips"), and insertions or deletions, commonly referred to as **indels**, with each category further differentiated into subcategories based on the nature, location, and effect of the polymorphism.

The most common type of polymorphism in pharmacogenomics is the SNP (see **Figure 1-7**). Single nucleotide polymorphisms are polymorphisms that occur at a single nucleotide where any one of the four bases of DNA (A, C, G, and T) may be substituted for another. An estimated 90% of all genetic variation in the human genome is thought to be derived from SNPs. Interestingly, the substitution of C → T constitutes roughly two out of every three SNPs.[13] Single nucleotide polymorphisms can be located in either coding or noncoding regions of DNA. Recall that coding regions contained within the genes make up

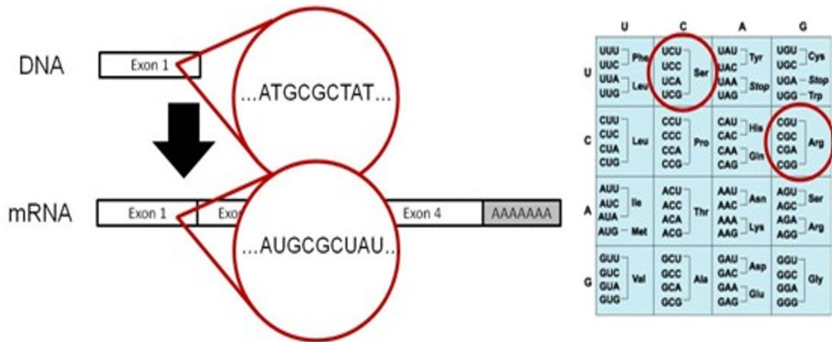
less than 2% of the total DNA in the genome. As a result of the relative paucity of bases that make up this region, SNPs in coding regions occur less frequently than SNPs in noncoding regions but have a far greater potential to influence the phenotype of an individual. In this sense, the old colloquialism “location, location, location” certainly applies to SNPs.

Single nucleotide polymorphisms with the most direct genetic influence are located within the coding region of DNA. These polymorphisms are classified as either synonymous (also



	Wild-type Sequence	Genetic Variation
mRNA	...ACC—**GCC**—UAU...	...ACC—**UCC**—UAU...
Protein	...Thr—**Ala**—Tyr...	...Thr—**Ser**—Tyr...
Protein Activity	Normal binding to β-myosin	Reduced β-myosin binding
Clinical Outcome	Normal cardiac muscle contraction	Decreased cardiac muscle contraction and clinical symptoms of left ventricular hypertrophy

Figure 1-6 Variation in the human cardiac α-actin gene associated with familial hypertrophic cardiomyopathy (FHC). A guanine to thymine (G→T) mutation in exon 5 of the human cardiac α-actin gene results in variation in the mRNA codon sequence and subsequent mistranslation of serine at amino acid position 295 rather than alanine. The resulting actin molecule exhibits reduced binding affinity for β-myosin, resulting in diminished cardiac muscle contraction and clinical symptoms associated with hypertrophy of the left ventricle.



	Wild-type	Synonymous SNP	Non-synonymous SNP
mRNA codon sequence	...AUG—**CGC**—UAU...	...AUG—**CGA**—UAU...	**...AUG-AGC-UAU...**
Protein sequence	...Met—**Arg**—Tyr...	...Met—**Arg**—Tyr...	...Met—**Ser**—Tyr...

Figure 1-7 Synonymous and nonsynonymous SNPs. Synonymous, or sense, SNPs are changes to a single nucleotide that alter the mRNA codon sequence without changes to the translated protein. In this hypothetical example, a cytosine to adenine (C → A) polymorphism changes the codon from CGC to CGA, but both codons are translated to arginine. In contrast, nonsynonymous, or missense, SNPs are changes to a single nucleotide that result in altered mRNA codon sequence and subsequent mistranslation of the protein. In this case, a cytosine to adenine (C → A) polymorphism results in the translation of a serine rather than arginine.

called sense mutations), which result in translation of the same amino acid, or nonsynonymous (also called missense mutations), which result in translation of a different amino acid. Another type of coding SNP can be classified as a nonsense mutation in that the polymorphism results in the inappropriate insertion of a stop codon in the growing mRNA, ultimately leading to a truncated protein product. In these ways, SNPs may cause important differences in gene function and/or expression. For example, mRNA transcripts used for translation can be directly altered by SNPs, leading to compromised transcript stability or altered RNA splicing. Likewise, coding nonsynonymous or nonsense SNPs may influence protein structure, stability, substrate affinities, and so on.

Apolipoprotein E (ApoE), a gene associated with Alzheimer's disease, can serve as an example of the effects of nonsynonymous SNPs located in the coding region of a gene.[16] Apolipoprotein E is a member of a family of proteins whose function is to bind to and assist in the transport of lipids in the circulatory system and is the predominant lipoprotein in the brain. Two SNPs, both thymine to cytosine (T→C) substitutions, are located within ApoE that result in the translation of more basic arginine residues at amino acid positions 112 and 158 instead of neutral cysteines. These changes, when found together, are known as the ApoE ε4 allele and transform ApoE into an isoform that exhibits increased binding affinity to amyloid β, a small protein involved in the pathology of Alzheimer's disease.[17] Apolipoprotein ε4 is found in high abundance in neurofibrillary tangles characteristic of Alzheimer's disease.[17] In fact, the SNPs associated with the ApoE ε4 allele, which occur in 5% of the population, are now considered to be the single greatest genetic risk factor for the development of Alzheimer's disease, which is the leading cause of senile dementia in the elderly and effects nearly 25 million adults worldwide.

Importantly, the influence of SNPs is not limited to those found directly in coding regions. At least one important function of noncoding DNA is to regulate the expression of mRNA transcripts. Thus, noncoding polymorphisms located in

regulatory regions, including promoters, areas of DNA that respond to cellular machinery that control gene expression, introns, and the boundary between exons and introns, lead to

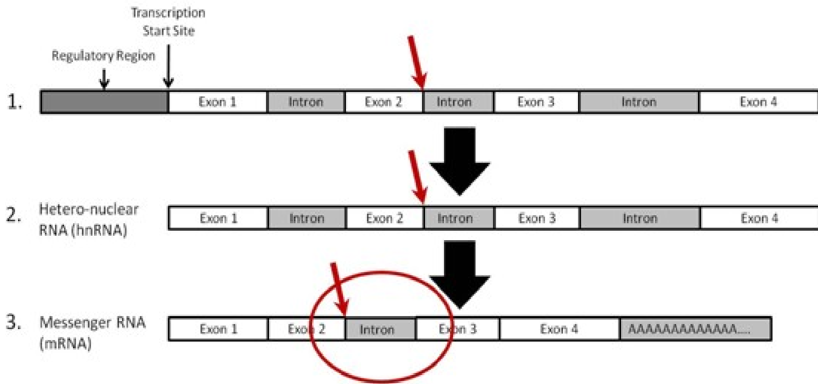


Figure 1-8 The potential impact of noncoding SNPs. SNPs located in noncoding regions of DNA, such as promoters, introns, and the boundary between exons and introns, can result in altered splicing and/or expression of mRNA transcripts. In this example, the SNP located at the proximal intronic boundary between exons 2 and 3 (indicated by arrows) alters the DNA sequence recognized by splicing machinery within the cell, eliminating the splice site. The resulting mRNA transcript erroneously retains the intron, leading to translation of the intron sequence into an altered protein product.

potential changes in transcription factor binding, mRNA transcript stability, or RNA splicing (see **Figure 1-8**).[18]

It is worth noting, not without irony, that there is considerable variation in the nomenclature used to describe genetic variation. Frequently, the same polymorphisms are described by different names in various basic science and clinical sources in the literature. For instance, a hypothetical single-base variation from adenine to thymine could be designated as A→T, A/T, A>T, or even A123T or 123A>T to denote base position within the gene. Making matters even more confusing, early studies of polymorphisms did not benefit from the standardized

DNA sequence databases that exist today, such as the National Center for Biotechnology Information, or NCBI (www.ncbi.nlm.nih.gov). Rather, investigators studying identical regions of DNA frequently used sequences or fragments of DNA with different starting points relative to the actual genomic sequence. Thus, studies of our hypothetical polymorphism at position 123 could appear in the literature as A123T in one study and A323T in another if the sequence used in the latter began 200 bases upstream relative to that used in the former. Although it will likely take some time for standardized nomenclature to take hold in the literature, recent efforts have produced several proposals for a systematic methodology of SNP nomenclature.

One prominent example is from the Human Genome Variation Society.[19] Its recommendations for the naming of human sequence variation promote a basic system focusing on first naming the gene of interest followed by designating the level of sequence variation: at the level of DNA, located in either coding regions designated as “c,” genomic or noncoding regions as “g,” or mitochondrial regions as “m.” This nomenclature is not to be confused with the molecular biological term complementary DNA, which also is designated cDNA, and is likewise derived from reverse transcribing messenger RNA, or mRNA, so that only exons are included in the sequence. Thus, a coding reference sequence represents only DNA information contained in processed mRNA, whereas gDNA sequences represent DNA information identical to how it exists in the genome, containing DNA from introns, exons, and intergenic regions. Ribonucleic acid and protein sequence variation are respectively designated by “r.” or “p.” Actual variation in a sequence is described by listing first the reference or wild-type sequence/base followed by the sequence variation. Thus, applying this nomenclature system, the α-actin variation already described would be named c.253G>T to indicate the variation of sequence at position 253 in the coding reference sequence in the α-actin gene where the reference base guanine has been replaced by the variant thymine. This naming system could further be

applied to describe the resulting change in terms of base substitution at the protein level using p.295S>A or p.295Ser>Ala

where the serine at amino acid position 295 in the α-actin protein is changed to alanine.

Another SNP nomenclature system that is widely used is the **reference sequence number**, or the **ref SNP**, **rs#**, or **rs**. Developed for use in the Single Nucleotide Polymorphism Database (dbSNP) hosted by the NCBI, this system is designed to reference genetic variation such as SNPs according to more precise locations within the genome rather than the arbitrary and varying segments of DNA frequently used in individual studies.[20] This is akin to providing each SNP with an exact chromosomal street address, where possible, that is used to define the SNP. For instance, rs113513162 is the specific, consistent identifier in dbSNP for the c.253G>T actin mutation in exon 5 of the ACTC gene located on chromosome 15. Efforts such as these that normalize the nomenclature and referencing of variation in the genetic code have proven valuable in decreasing the incidence of ambiguous or misleading literature references to SNPs.

Genetic variation can also be described at the whole-gene level. Perhaps the most relevant example for the purposes of this text is that of the human cytochrome (CYP) P450 genes for which gene-wide variation is defined by well-accepted nomenclature.[21] In this system, the superfamily designation of "CYP" precedes that given for family (indicated by number), subfamily (indicated by letter), and individual subfamily member (again indicated by number). Importantly, allelic differences are defined by a number or a number and a letter following an asterisk (*) designation. It is important to note that in this nomenclature system the "*1" designation most commonly refers to the wild-type gene, whereas integers of "2" or greater denote polymorphic alleles typically numbered in order of their discovery and validation. For some genes, the nomenclature also includes the designation of "*1A" as the wild-type and "*1B," "*1C," "*1D," and so on as variants.

All told, this system allows for genotypic variation, in some cases involving multiple SNPs, to be described in phenotypic terms by referencing differences in an allele rather than a nucleotide. For example, CYP2C9 is a primary metabolizing

enzyme of drugs, including the antiseizure medication phenytoin, the anticoagulant warfarin, and many nonsteroidal anti-inflammatory drugs, such as naproxen. A SNP that occurs within the *CYP2C9* gene resulting in a cytosine to thymine (C→T; rs1799853) conversion leads to decreased enzymatic function. This allelic polymorphism is designated by *CYP2C9**2 and is used to denote individuals susceptible to elevated drug levels following administration of typical doses of these medications (see **Figure 1-9**).

The other major category of polymorphism is indels. This -genetic variation involves the insertion or deletion of DNA either as single -nucleotides or as two or more nucleotides, in some cases -spanning regions of DNA encompassing an entire gene. One of the best-characterized forms of indels is the duplication of the cytochrome P450 drug metabolizing enzyme CYP2D6, where individuals have been found to possess as many as 13 copies of the gene. In contrast, GSTT1, a gene encoding for the glutathione-conjugating enzyme glutathione S-transferase -theta-1, is entirely deleted in some individuals, which sometimes leads to reduced metabolism of **xenobiotics**, particularly those with electrophillic and hydrophobic properties.[22] In this case, the existence of alternative metabolic pathways for some compounds means that the phenotypic effect of this gene deletion may not be observed.

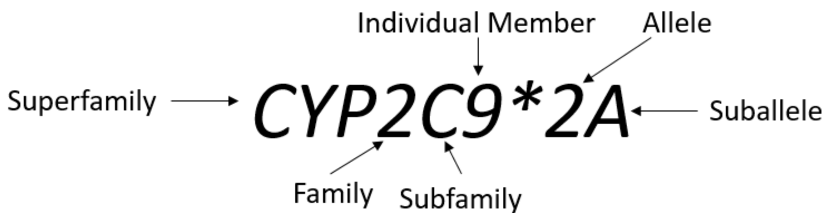


Figure 1-9 Nomenclature for the cytochrome P-450 (CYP) alleles. The established nomenclature system for alleles of the cytochrome P-450 (CYP) superfamily designates "CYP" followed by family number, subfamily letter, and individual subfamily number. Allelic differences are defined by number or a number and letter following an asterisk (*).

A classic example of the pharmacogenomic consequences

of polymorphic variation can be found in a family member of the cytochrome P450 enzymes, CYP2D6. Located on chromosome 22, CYP2D6 is a primary mechanism for the metabolism of nearly 100 drugs, including many antidepressants, such as fluoxetine; many neuroleptics, such as haloperidol; beta blockers, such as propanolol; and analgesics, such as codeine. Individuals carrying the wild-type alleles for *CYP2D6* (*CYP2D6**1) are phenotypically considered extensive/normal metabolizers (NM) in that substrates at CYP2D6, such as the drugs aforementioned, are metabolized efficiently.[23] If one were to compare CYP2D6 metabolic activity to the volume dial on a stereo, the *CYP2D6**1 allele would be analogous to a normal setting (see **Figure 1-10**).

Most of the clinically relevant *CYP2D6* SNPs identified thus far result in diminished enzymatic activity associated with poor metabolizer (PM) or intermediate metabolizer (IM) phenotypes. For example, the *CYP2D6**4 allele containing the 1846G>A polymorphism is a splicing defect in *CYP2D6* that results in a truncated, nonfunctional protein product.[24] It is among the most common *CYP2D6* SNPs found in Caucasian populations accounting for a significant percentage of mutant alleles. Another example of the PM phenotype is the *CYP2D6**10 allele containing the 100C>T SNP, which results in diminished enzymatic activity via enhanced protein degradation.[25] It is the most common CYP-related polymorphism found in Asian populations (nearly 50% of individuals), whereas the *CYP2D6**4 allele is seen at a much lower frequency in this group. Among individuals of African ethnicity, the *CYP2D6**17 allele containing the 1023C>T polymorphism is most common, resulting in a deficiency of hydrolase activity due to reduced substrate-binding affinity.[26]

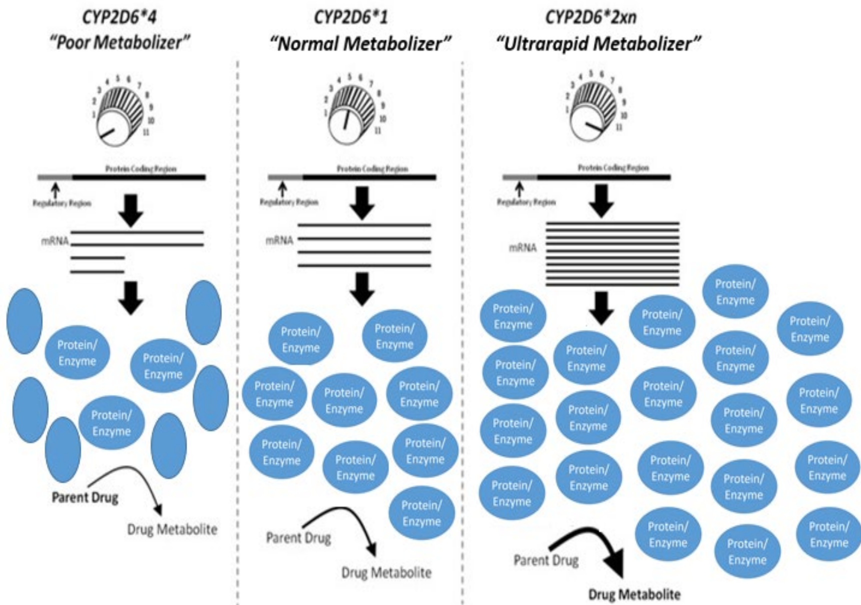


Figure 1-10 Genotypic and phenotypic differences in CYP2D6 mediated metabolism. Individuals carrying the wild-type alleles for *CYP2D6* (*CYP2D6**1) are phenotypically considered "extensive/normal metabolizers (NM)". Carriers of the *CYP2D6**4 allele containing the 1846G>A polymorphism produce a truncated, nonfunctional protein product and therefore exhibit a "poor metabolizer" phenotype. The *CYP2D6**2xN allele indicates repetition of a 42-kilobase DNA fragment, resulting in *CYP2D6* duplication that is phenotypically expressed as an "ultrarapid metabolizer" phenotype.

Evidence also suggests indel polymorphic expression of *CYP2D6*. Repetition of a 42-kilobase DNA fragment containing *CYP2D6**2 results in *CYP2D6* duplication that is phenotypically expressed as an ultrarapid metabolizer (UM) phenotype.[27] In fact, as many as 13 copies of the enzyme have been identified in one individual's genome. Interestingly, this phenomenon is thought to have arisen from selective pressures associated with specific geographic regions. The incidence of *CYP2D6* duplication has been reported with a frequency of less than 2% in Asians and less than 5% in Western Europeans but as much as 16% in Ethiopians.[28,29] Thus, the frequency of individuals possessing *CYP2D6* duplication suggests a geographical gradient, possibly

resulting from dietary pressures where, historically speaking, the detoxification capacity afforded by *CYP2D6* duplication may have been essential for African diets relative to more European-based diets.

Fascinating though they may be from purely anthropological and genetic viewpoints, these observations have profound clinical implications. First, for each of these groups, individuals possessing PM *CYP2D6* polymorphisms may require reduced dosing of substrate drugs in order to avoid toxicities associated with decreased drug metabolism, which in many cases can be severe or even fatal. In contrast, individuals possessing *CYP2D6* UM polymorphisms may require the polar opposite therapeutic course, that of increased rather than decreased dosing, in order to avoid symptoms associated with drug inefficacy. Second, these examples highlight the potential for pharmacogenomics to provide a mechanistic basis as to why individuals belonging to specific ethnic groups may respond very differently to standard drug therapy and eventually may provide a means for precision dosing of those medications in advance. At the same time, these findings should provide ample caution against making assumptions based on ethnic background when treating individual patients. Remember, precision medicine deals with using individual genetic information to support clinical decision making for optimal patient care. Ideally, increased prevalence of a "pharmacogenomically" relevant SNP in an ethnic population affords valuable consideration, but not a conclusion, at least not without genetic data specific to the individual patient.

Consider the therapeutic challenge of treating a patient with a needed medication whose primary metabolism occurs via CYP2D6, all while facing the unknown possibility that the patient's metabolic capacity could range anywhere from PM status to UM status. With this perspective, it is hardly surprising that according to the U.S. Food and Drug Administration nearly one million adverse drug reactions are reported each year in the United States, half of which lead to serious patient outcomes such as hospitalization or disability and almost 100,000 directly result in death.[30] Clearly, not all of these adverse events are attributable to pharmacogenomic influences. Many are

undoubtedly the result of human error, such as administering the incorrect dose contrary to a correctly prescribed regimen. However, it ought to give one pause to realize that many adverse events are -attributable not to human error, but to errors in humans. Or, in other words, adverse events arise not just when incorrect medications and/or doses are administered, but also when they are correctly prescribed and administered to individuals whose pharmacogenomic profiles may contraindicate such therapy.

In spite of these examples of dramatic phenotypes of polymorphic variation between individuals, it is more common that a pharmacogenomic trait cannot be clearly associated with a single SNP or indel. In this case, haplotypes can sometimes be used to associate **genotype** with phenotype. In true genetic terms, a **haplotype** refers to regions of DNA, such as a combination of alleles, that are inherited but that may or may not determine phenotype traits. Haplotypes are relatively common. It has been estimated that most genes contain between 2 and 53 haplotypes, with an average of 14. A haplotype has been frequently used to describe groups of SNPs that are inherited together. Haplotypes themselves may not have a direct effect on drug response, but their proximity to an unidentified causative mutation may allow them to act as a marker for a particular drug response.

One example of the use of haplotypes in predicting individual drug responses is found in the β_2 adrenergic receptor (β_2AR).[31] Twelve haplotypes have been identified in the 5′ untranslated region (UTR) and in the coding region of the *ADRB2* gene that encodes for the β_2AR receptor. Several of these haplotypes have been associated with a greater than two-fold increase in response to the β_2AR agonist albuterol, which is the prototypical agent in the class of sympathomimetic drugs used as first-line bronchodilators in treating symptoms of both asthma and chronic obstructive pulmonary disease (COPD).Importantly, no individual SNPs located within the haplotypes were able to be causatively linked to improved β_2AR-mediated bronchodilation. Thus, both SNPs and haplotypes can be used to map genetic changes that are associated with an

individualized drug response.

The examples provided thus far show a direct link between genotype and phenotype—between the specific genetic makeup of an individual and the response of the individual to a drug. However, establishing an association between a genetic polymorphism and a specific drug response is more complicated when multiple polymorphisms within a gene and/or multiple genes are involved.

This is most easily discussed when considering traits that are **monogenic**, or those derived from a single gene. For example, each individual inherits two alleles of *CYP2C9* (one from mom and one from dad). Therefore, the overall activity of CYP2C9 results from the combined contribution of both alleles. By definition, most individuals inherit two wild-type copies of *CYP2C9*, which means that most of us exhibit “normal” metabolic activity of the enzyme. However, what if an individual inherits the wild-type *CYP2C9* allele from one parent but the *CYP2C9**2 polymorphism from the other parent? In this case, the individual would be considered **heterozygous** for *CYP2C9* (written *CYP2C9**1/*2) in that he or she possesses two different alleles for the same gene, one fully functional and the other with compromised enzymatic activity. If both alleles were to contain the *CYP2C9**2 polymorphism, the individual would be considered **homozygous** (*CYP2C9**2/*2), resulting in greatly diminished metabolism by the CYP2C9 enzyme. Thus, one would expect to see a graded loss of metabolism across individuals who are wild-type (*CYP2C9**1/*1), heterozygous (*CYP2C9**1/*2), and homozygous (*CYP2C9**2/*2) for the *CYP2C9**2 polymorphism. This is referred to as a gene-dose response relationship (see **Figure 1-11**).

In this simplistic example, the phenotype of CYP2C9 activity can be explained by the direct relationship between trait and genotype. However, what if we expand our analysis to consider not just CYP2C9 activity but the overall response to a drug metabolized by the enzyme?

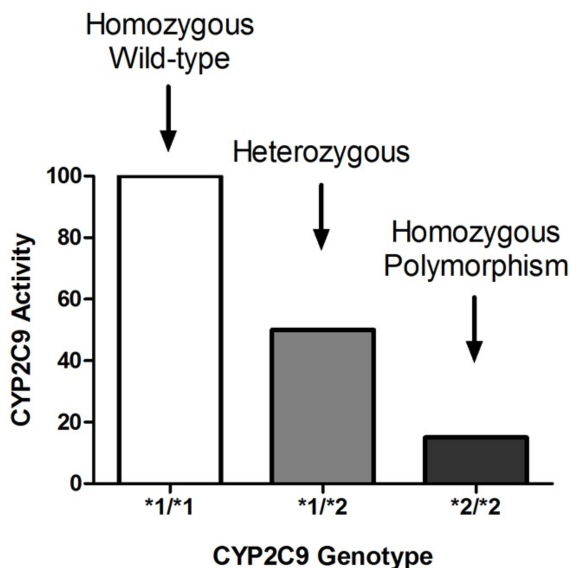


Figure 1-11 Allelic expression of *CYP2C9**2 polymorphisms as an example of a monogenic trait. CYP2C9 activity results from the combined contribution of both alleles. Most individuals carry two alleles of the wild-type *CYP2C9**1 and therefore exhibit full metabolic activity of the enzyme. Individuals who inherit one wild-type *CYP2C9**1 allele and one polymorphic *CYP2C9**2 allele are considered heterozygous for *CYP2C9* and exhibit diminished enzymatic activity. If both alleles are the *CYP2C9**2 polymorphism, the individual would be considered homozygous for the polymorphism, resulting in greatly diminished metabolism via CYP2C9.

As an anticoagulant, warfarin has been used extensively to prevent thromboembolism but is limited in use by a narrow therapeutic index. Inadequate drug therapy increases the likelihood of potentially fatal thrombolytic events, whereas toxicity may result in life--threatening hemorrhaging. The anticoagulant effects of -warfarin are mediated by inhibition of vitamin K epoxide reductase complex subunit 1 (VKORC1), a key factor in the clotting process.[32,33] Thus, the warfarin response is dependent on the function of its drug target, VKORC1, and its metabolizing enzyme, CYP2C9. Importantly, polymorphisms have been identified in *VKORC1*, including a guanine to adenine conversion (−1639G>A; rs9923231) that increases an individual's sensitivity to warfarin.[34,35] This means there exist -subpopulations of patients that carry the *CYP2C9**2

polymorphism, the –1639G>A *VKORC1* polymorphism, or both. Moreover, each individual will be either heterozygous or homozygous for each polymorphism, with each polymorphism potentially altering the anticoagulant response to warfarin.

This more intricate scenario describes a **multigenic trait** where the phenotypic expression of the trait (in this case the -anticoagulant response to warfarin) is dependent upon the function of several genes rather than just one. As complicated as this may appear, this gene–gene interaction still greatly oversimplifies the actual clinical -condition. Consider that the warfarin response is influenced by not just two genes (gene–drug interactions) but rather the confluence of many more factors, such as age, weight, and sex, which are further compounded by other environmental variables (gene-–environment interactions), such as concurrent drug therapy, and behavioral choices, such as smoking or diet. Merely attempting to approximate such complexity helps to highlight the complicated relationship that can exist between a drug response and genotype.

Review Questions

1. Pharmacogenomics is the study of the relationship between genetic variation and drug response.
   a. True
   b. False

2. Genetic variation in the nucleotide sequence of DNA necessarily results in changes in amino acid sequence and protein functionality.
   a. True
   b. False

3. In describing genetic variation, mutations and polymorphisms can be differentiated by which of the following?
   a. Frequency of the variation
   b. Functional effects of the variation
   c. Location of the variation within the genome
   d. Mutations and polymorphisms are indistinguishable

4. A noncoding synonymous single nucleotide polymorphism is most likely to induce a change in which of the following?
   a. Enzyme–substrate affinity
   b. Receptor–ligand binding
   c. RNA splicing
   d. Transcription

5. Which of the following metabolic enzymes is associated with both poor metabolizer (PM) and ultrarapid metabolizer (UM) phenotypes?
   a. NADP
   b. CYP2D6
   c. TPMT
   d. VKORC1

6. Which of the following *CYP2D6* polymorphisms is an example of an indel?
   a. *CYP2D6**4
   b. *CYP2D6**2
   c. *CYP2D6**10
   d. *CYP2D6**17

7. Which of the following is the best description of a haplotype?
   a. A common mutation in DNA in a given population observed at greater than 1% frequency.
   b. An observable characteristic or trait.
   c. A series of polymorphisms that are inherited together.
   d. Possessing two different alleles for the same trait.

8. Polymorphisms such as those found in *CYP2C9* result in heterozygous individuals who often display intermediate enzyme activity and wild-type and homozygous individuals who display either fully functional or nonfunctional enzyme activity, respectively. This trimodal phenotype is indicative of which of the following?
   a. A monogenic trait
   b. A multigenic trait
   c. Neither A nor B
   d. It is not possible to tell.

9. A patient who recently started taking the antipsychotic medication haloperidol presents with dry mouth, restlessness, spasms of the neck muscles, and weight gain, all of which are adverse effects associated with haloperidol toxicity. Based on your knowledge of the pharmacogenomic influence of CYP polymorphisms, you speculate that this patient is:
   a. homozygous for the *CYP2D6**4 allele.
   b. homozygous for the *CYP2D6**2xn allele.
   c. homozygous for the *VKCOR1* (AA) allele.
   d. homozygous for the *CYP2C9**1 allele.

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Section II

Pharmacogenomics Related to Pharmacokinetics and Pharmacodynamics

Section II presents the interface between pharmacogenomics and pharmacokinetics, and pharmacogenomics and pharmacodynamics as underlying concepts influencing a drug's concentration-time profile and concentration-effect relationship(s). This section relates genetic influences on pharmacokinetics and pharmacodynamics in a conceptual and mathematical sense.

CHAPTER Two

Pharmacogenomics and Pharmacokinetics

Learning Objectives

Upon completion of this chapter, the student will be able to:

1. Recognize the influence of genetic polymorphisms on the absorption, distribution, metabolism, and excretion of drugs.

2. Differentiate, based on genetic polymorphisms, cytochrome P450 poor metabolizers, intermediate metabolizers, extensive/normal metabolizers, and ultrarapid metabolizers relative to the absorption, distribution, meta-bolism, and excretion of drugs.

3. Explain how a specific genetic polymorphism would affect the design of a patient's drug dosing regimen.

4. Differentiate between influx and efflux transporters relative to tissue location and influence on the absorption, distribution, metabolism, and excretion of drugs.

5. Propose alterations to a patient's dosing regimen based on the pharmacogenomic influence on absorption, distribution, metabolism, and excretion.

The student should demonstrate an understanding of how drug metabolizing enzymes and drug transporters are influenced by genetic variation. Genes producing proteins that are drug metabolizing enzymes and drug transporters are called pharmacogenes. The student should also understand that variation in these proteins results in variation in pharmacokinetics, which can influence how a person absorbs, distributes, metabolizes, and excretes a given drug, all in the context of the patient's response to the drug.

Key Terms	Definitions
absorption rate constant (k_a; time^{-1})	The rate constant representing the first-order absorption of a drug from an extravascular site (e.g., the gastrointestinal tract).
area under the curve (AUC; amt/vol · time)	A measure of drug exposure as the integrated area under the plasma drug concentration versus time curve from time zero to infinity.
bioavailability (F)	The rate and extent of drug absorption; the fraction of the dose reaching systemic circulation unchanged.
clearance (CL; vol/time)	The volume of biologic fluid from which drug is removed per unit time.
cytochrome P450 (CYP)	A superfamily of oxidative metabolic enzymes.
efflux transporter	A protein that moves drug out of cells/tissues.
elimination rate constant (k_e; time^{-1})	The rate constant representing the first-order elimination of drug.
extensive metabolizer (EM) – old term; normal metabolizer (NM; see below)	An individual with two “normal-function” alleles relative to a drug metabolizing enzyme.
genotype	The specific set of alleles inherited at a locus on a given gene.
intermediate metabolizer (IM)	In general, an individual with one “loss-of-function” allele and one “normal–function” allele relative to a drug metabolizing enzyme.
loading dose (D_L; amt)	The initial dose of a drug administered with the intent of producing a near steady-state average concentration.
maximum concentration (C_{max}; amt/vol)	The highest concentration of drug in biologic fluid following drug administration during a dosing interval.
normal metabolizer (NM)	An individual with two “normal-function” alleles relative to a drug metabolizing enzyme.
pharmacodynamics (PD)	The relationship between drug exposure and pharmacologic response.
pharmacogenomics (PGx)	The study of many genes, in some cases the entire genome, involved in response to a drug.
pharmacokinetics (PK)	The relationship of time and drug absorption, distribution, metabolism, and excretion.
phase 1 metabolism	Drug metabolizing processes involving oxidation, reduction, or hydrolysis.
phase 2 metabolism	Conjugative drug metabolic processes.
phenotype	An individual’s expression of a physical trait or physiologic function due to genetic makeup and environmental and other factors.
poor metabolizer (PM)	In general, an individual with two “reduced–function” or “loss-of-function” alleles relative to a drug-metabolizing enzyme.
prodrug	A drug that requires conversion to an active form.
rapid metabolizer (RM)	An individual with two functional alleles, typically a normal and increased function allele.
tau (s; time)	The dosing interval.
T_{max} (time)	The time of occurrence of the maximum concentration of drug.
ultrarapid metabolizer (UM)	An individual with a “gain-of-function” allele (e.g., overexpression of a metabolic enzyme).
uptake (influx) transporter	A protein that moves drug into cells/tissues.
volume of distribution (V, Vd, V_1, V_{ss}; vol)	A proportionality constant relating the amount of drug in the body to the drug concentration.

Key Equations	**Description**
$AUC = \frac{Dose}{CL}$	Area under the concentration versus time curve, being directly proportional to the dose and inversely proportional to the clearance (CL).
$C_{ave}^{ss} = \frac{F \cdot Dose}{CL \cdot \tau}$	The average steady-state drug concentration being directly related to the bioavailability and the dose and inversely related to the clearance and the dosing interval.
$D_L = C \cdot Vd$	The loading dose related to a desired concentration and the volume of distribution.
$D_M = C_{ss} \cdot CL$	The maintenance dose related to the desired steady-state concentration and the clearance.
$\mathrm{F} = \frac{AUC_{po}}{AUC_{iv}} \cdot \frac{Dose_{iv}}{Dose_{po}} = \frac{(\frac{AUC}{Dose})_{po}}{(\frac{AUC}{Dose})_{iv}}$	Absolute bioavailability relating the extent of absorption of an extravascular dose to the intravenous dose.
$\mathrm{F} = \mathrm{f_a} \cdot \mathrm{ff_p}$	Bioavailability related to the fraction of the dose absorbed and the fraction of the dose that escapes first-pass metabolism.
$\mathrm{F} = (\mathrm{ff} \cdot \mathrm{fg}) \cdot \mathrm{ff_p}$	Bioavailability as above with the fraction of the dose absorbed expanded to include the fraction of the dose that avoids gastrointestinal lumen -metabolism/degradation and the fraction that avoids gastrointestinal wall metabolism and/or efflux.
$\tau = \frac{\ln(\frac{C\max}{C\min})}{ke}$	Tau, the dosing interval, as a function of the In quotient of C_{max} and C_{min} and inversely proportional to the elimination rate constant, k_e.
$t½ = \frac{0.693 \cdot Vd}{CL} = \frac{0.693}{ke}$	The half-life, being directly related to the volume of distribution and inversely related to the clearance; inversely related to the elimination rate constant, k_e.
↑, ↓	The number of arrows indicates the relative difference in the magnitude of the change.

Introduction

Pharmacokinetics (PK) is the study of the time course of drug absorption, distribution, metabolism, and excretion (ADME), describing how the body handles a given drug. Thus, these processes determine the plasma concentration versus time profile of a given drug. The pharmacologic effect(s) of a given drug are related to that drug interacting with biologic receptors. As it is not possible to easily measure the drug concentration at the site of the receptors, plasma concentrations are related to the effect(s) based on the assumption that there is equilibration between the drug concentration in plasma and that at the site of the receptors. The study of the relationship between the plasma concentrations of a drug and the observed pharmacologic effects is referred to as **pharmacodynamics (PD)**, and it describes how the drug affects the body. The common variable relating pharmacokinetics and pharmacodynamics is the drug concentration; this relationship is depicted in **Figure 2-1**.

The pharmacokinetics of a given drug "drives" the pharmacodynamics of that drug in such a way that the drug concentration in the plasma will be in equilibrium with the drug concentration at the receptor site, and responses to the drug, whether therapeutic or toxic, will be a consequence of the drug concentration. The variability in the response to a given drug is due, in part, to the variability in the pharmacokinetics of the drug, although pharmacodynamic variability is typically greater than pharmacokinetic variability. The variability in the

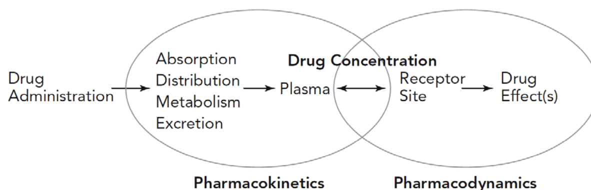


Figure 2-1 The relationship between pharmacokinetics and pharmacodynamics, with the "linking variable" being the drug concentration. As pharmacokinetics determines the plasma concentration versus time profile of the drug, the concentration at the receptor site (i.e., site of action), in equilibrium with the drug in the plasma, elicits the pharmacologic effect(s), which is pharmacodynamics.

pharmacokinetics and pharmacodynamics can be explained, in part, by **pharmacogenomics (PGx)**.

The clinical application of pharmacokinetics is aimed at optimizing drug therapy by designing a **loading dose** (where appropriate) and an initial maintenance regimen, including the maintenance dose and dosing interval, to keep the drug concentration within the desired therapeutic range. This is followed by dosage adjustment based on drug concentration determination for drugs that have a narrow therapeutic range. (i.e., the drug concentrations eliciting a therapeutic effect are close to or overlap those that elicit an adverse effect).

The design of a loading dose is based on the individual's **volume of distribution (Vd)**, which can be influenced, in part, by drug movement into tissues via transporters that are under genetic regulation. Here, a greater Vd will require a higher loading dose to achieve a desired drug concentration in the patient. A lesser Vd in a patient would require a lower loading dose. The maintenance dose of a given drug is determined using the drug's **clearance (CL)**. For some drugs, the CL is determined by drug metabolism via specific drug enzymes that also are under genetic regulation. With a greater CL, a higher maintenance dose is required; conversely, a patient with a lower CL would require a lower maintenance dose. The half-life ($t_{1/2}$) of a drug determines its dosing interval. Here, the Vd and CL influence the $t_{1/2}$. With a larger volume of distribution, the drug has to "travel" farther to the eliminating organ for removal from the body. If the CL is held constant, the increased Vd results in a longer $t_{1/2}$ and the drug will remain in the body longer, meaning that the dosing interval, the time to the next dose, will be longer. For a drug that is eliminated from the body by metabolic routes, an increase in CL is related to increased metabolism. This results in a shorter $t_{1/2}$. With a decrease in metabolism, the CL decreases and the $t_{1/2}$ increases. The relationships among Vd, CL, and $t_{1/2}$ are presented in the Key Equations list. Numerous factors influence these relationships, including the patient's genetic constitution. Many of these relationships are discussed further in this chapter.

A number of pharmacokinetic/pharmacodynamic resources describe the mathematical detail of drug-concentration, concentration-effect relationships. The equations in this chapter are presented only to provide a conceptual “framework” of altered pharmacokinetics, here related to PGx.

Absorption and Bioavailability

Oral drug absorption is the process by which a drug moves from the gastrointestinal lumen, crosses biologic membranes, and reaches systemic circulation. With oral administration, the drug travels down the esophagus to the stomach and then to the small intestine. Although some drug can be absorbed from the stomach, it is the small intestine that is the main site of drug absorption. The small intestine’s large surface area, permeable membranes, and capillary blood flow create a favorable environment for drug absorption.[1,2]

In order for a drug to be absorbed, it must first be in solution. With oral administration, dissolution of the dosage form, such as a tablet or capsule, results in the drug being in solution in the gastrointestinal lumen, thus creating a concentration gradient of drug across the membranes of the small intestine. This creates a favorable situation for drug absorption, especially via passive diffusion. While passive diffusion is a major mechanism of drug absorption, other absorption mechanisms include active transport, facilitated diffusion (facilitated transport), pinocytosis, and ionic diffusion.[3] When considering mechanisms of absorption, variabi-lity in drug absorption has been related to drug transporters, both **uptake (influx) transporters** and **efflux transporters**, which are controlled by the patient’s genetic constitution.[4,5]

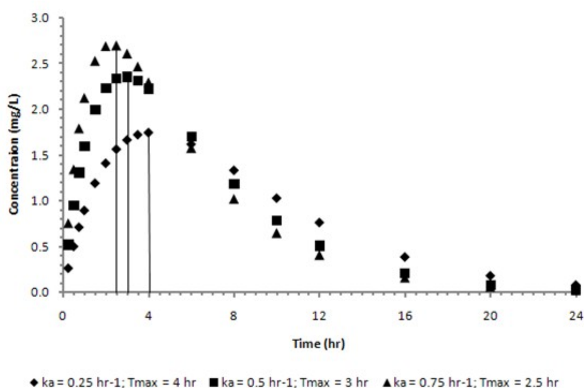
Oral drug absorption is characterized by the drug’s bioavailabi-lity, which has clinical relevance. **Bioavailability (F)** can be defined as the rate and extent to which a drug (the active ingredient) is absorbed from a drug product and reaches the general systemic circulation unchanged, being made

available to the site of action; that is, once a drug reaches systemic circulation it can be "delivered" to the site of action.[6] As per-oral (po; oral) dosing is the most common route of drug administration, it is the absorption of the drug from the gastrointestinal tract that is of interest and defines oral bioavailability, which will be discussed here. Upon oral drug administration, the dosage form (e.g., tablet, capsule) moves down the esophagus to the stomach. Although some of the drug may be released from its dosage form and absorbed from the stomach, it is the large surface area of the small intestine, with villi and microvilli that is the main site of drug absorption. Once drug molecules move across the gastrointestinal wall via various mechanisms, they are carried to the liver, via hepatic portal vein blood flow, where they may be metabolized. Drug that passes through the liver and reaches systemic circulation is considered to be bioavailable.

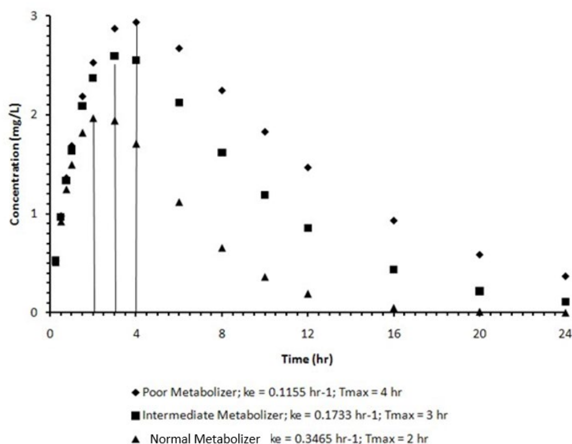
The rate of drug absorption is one component of its defined bioavailability. For most clinical purposes, the rate of drug absorption is adequately expressed by the parameter **T_{max}**. This parameter represents the time of occurrence of the maximum drug concentration following extravascular (e.g., oral) dosing of a drug and is determined by the **absorption rate constant (k_a)** and the **elimination rate constant (k_e)**. The k_a is a rate constant representing the first-order absorption rate of a given drug. The k_e is the rate constant representing the first-order elimination rate of the drug. **Figure 2-2a** presents the concentration versus time profiles of a given drug following oral administration where only the absorption rate constant is altered. **Figure 2-2b** presents the concentration versus time profiles of a given drug following oral administration where only the elimination rate constant is altered for three metabolizer "types" (i.e., poor metabolizer, intermediate metabolizer, extensive/normal metabolizer).

Genetic–Kinetic Connection: T_{max}
An individual may have the genetic constitution that results in the production of an enzyme that is efficient in metabolizing a given drug. In this case, the patient is considered to be an **extensive/normal metabolizer (EM/NM)** of that drug, and the k_e for the drug in this patient is increased relative to that of an **intermediate metabolizer (IM)** or a **poor metabolizer (PM)**. Because the k_e is increased in this individual, indicating that the drug is eliminated faster, the T_{max} will occur sooner. Here, the highest concentration of the drug in this individual will occur sooner rather than later (see Figure 2-2b).

The extent of drug absorption is defined by two parameters: the **maximum concentration (C_{max})** and the area under the drug concentration versus time curve (AUC_{po}). As a component of bioavailability, the values of these parameters for an orally administered drug are compared to those of the same dose of the drug administered intravenously. Equation 1 describes the calculation of a drug's absolute bioavailability, comparing the **area under the curve (AUC)** obtained



(a)



(b)

Figure 2-2 (a) The time of the occurrence of the maximum concentration of a drug (T_{max}) is dependent, in part, on the absorption rate constant (k_a). With the elimination rate constant (k_e) fixed, as the k_a increases, the sooner the drug reaches its maximum concentration. The T_{max} is one of the parameters used to describe a drug's bioavailability. (b) The time of the occurrence of the maximum concentration of a drug (T_{max}) is dependent, in part, on the elimination rate constant (k_e). With the absorption rate constant (k_a) fixed, an increased k_e represents increased drug elimination with the maximum concentration being observed earlier (T_{max} occurring sooner). Here, with the examples of a poor metabolizer, intermediate metabolizer, and an extensive/normal metabolizer. T_{max} is one of the parameters used to describe a drug's bioavailability.

following oral dosing to that obtained after intravenous dosing:

$$F = \frac{AUC_{po}}{AUC_{iv}} \cdot \frac{Dose_{iv}}{Dose_{po}} = \frac{\left(\frac{AUC}{Dose}\right)_{po}}{\left(\frac{AUC}{Dose}\right)_{iv}} \qquad \text{(eq. 1)}$$

Drug administered via the intravenous route is placed directly into systemic circulation, with 100% of the dose reaching systemic circulation, something considered to be "absolute." The ratio of the dose normalized AUC_{po} to the dose normalized AUC_{iv} provides the fraction of the oral dose of the drug that reaches systemic circulation and is termed the absolute bioavailability of the drug and is considered the oral bioavailability.

Genetic–Kinetic Connection: C_{max} and AUC
An individual may have the genetic constitution that results in the production of an enzyme that is inefficient with respect to drug metabolism. In this case, the patient is considered to be a poor metabolizer of that drug, and the C_{max} and AUC for the drug in this patient is increased relative to an intermediate metabolizer or an extensive/normal metabolizer. Such an individual may be at risk of experiencing toxicity, because the drug concentrations will be relatively high (see **Figure 2-3**).

A number of drugs must be "bioactivated" before being able to exert their effects and are administered in the form of a **prodrug**.[7] The bioavailability related to a prodrug points to the active drug reaching systemic circulation. The active drug is formed by metabolic conversion of the "parent" compound. With oral dosing, as the drug moves along the gastrointestinal tract and reaches the small intestine it is presented to and absorbed through

the gut wall and then travels to the liver via portal blood flow. Metabolic conversion can take place in the gut wall and/or the liver, with the active drug then reaching systemic circulation. With efficient conversion of the prodrug to the active compound in the gut wall and/or the liver, the active compound will be bioavailable. In the case of inefficient metabolic conversion of a prodrug, more of the parent compound will reach systemic circulation because it will not have been converted to the active compound. **Figure 2-4** shows the concentration versus time profiles for the parent compound and the active compound in an extensive/normal metabolizer and a poor metabolizer.

The bioavailability of a drug is the fraction of the dose that reaches systemic circulation unchanged and is “made available” to the site of action. Conceptually, this fraction is a product of the fraction of the dose of the drug absorbed (*fa*) and the fraction of

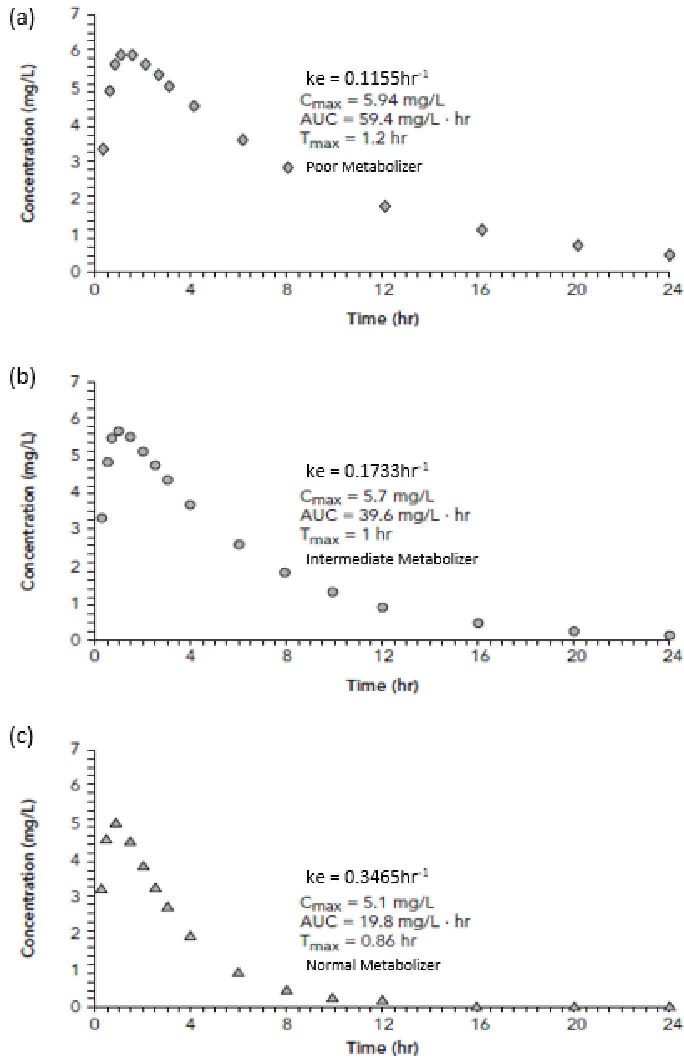


Figure 2-3 As the elimination rate constant (k_e) increases the C_{max} and AUC are lower and the T_{max} occurs earlier. Panel (a) shows the concentration versus time data for a drug that reaches systemic circulation when the k_e is 0.1155 hr^{-1}. The relatively low elimination rate constant may be seen in a poor metabolizer and result in higher drug concentrations. Panels (b) and (c) show the concentration versus time data when the k_e is increased to 0.1733 hr^{-1} and 0.3465 hr^{-1}, respectively, as may be seen in an intermediate metabolizer and an extensive/normal metabolizer.

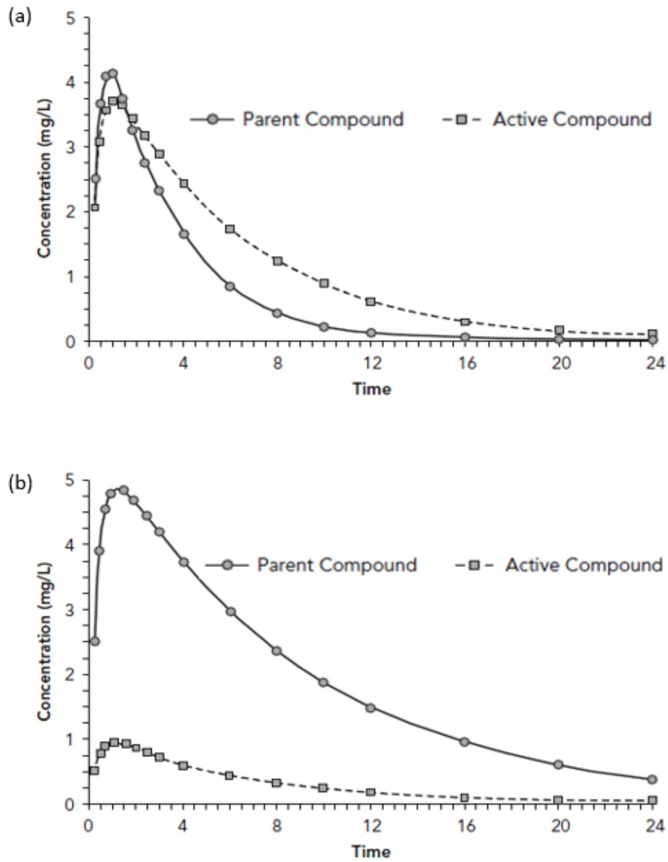


Figure 2-4 Concentration vs. time profile of parent compound (prodrug) and active compound in an extensive/normal metabolizer (EM/NM; panel a) and a poor metabolizer (PM; panel B).

of the dose of the drug that escapes hepatic first-pass metabolism (*ffp;* first pass through the liver; Equation 2):

$$F = fa \cdot ffp \qquad \text{(eq. 2)}$$

The fraction of the dose absorbed can further be defined as the fraction of the dose of the drug that is available for absorption (i.e., that which is neither metabolized/degraded in the gastrointestinal lumen nor eliminated in the feces; *ff*) and the fraction of the dose of the drug that avoids gastrointestinal wall metabolism and/or efflux (*fg*). Equation 3 defines the bioavailability of a drug as:

$$F = (ff \cdot fg) \cdot ffp \qquad \text{(eq. 3)}$$

Gastrointestinal Wall Influx and Efflux Transporters

Drug molecules that are available for absorption may be "taken up" into intestinal epithelial cells and made available to portal blood flow by influx transporters that serve as a mechanism of drug absorption.[8] Along with other mechanisms of absorption, facilitated transport is recognized as a contributing factor to the bioavailability of some compounds. For instance, a number of organic anion transporting polypeptides (OATP) act as influx transporters.[9] **Table 2-1** lists examples of influx (and efflux) transporters found in the intestinal epithelia that impact drug absorption, thus influencing the bioavailability of drugs that are substrates for such transporters.

Table 2-1 Examples of Gastroitestinal Transporter Genes, Transporters, and Drug Substrates.

Gene	Transporter/Type	Example Substrates
SLC01A2	OATP/influx	OATP1: enalapril; OATP2: digoxin, thyroxine, pravastatin; OATP1/P2: fexofenadine
SLC15A1	PEPT1/influx	β-lactam antibiotics, ACE inhibitors
SLC10A2	ASBT/influx	Benzothiazepine, dimeric bile acid derivatives
SLC16A1	MCT1/influx	Salicylic acid, nicotinic acid
ABCC2	MRP2/efflux	Tamoxifen
ABCG2	BCRP/efflux	Methotrexate, mitoxantrone
ABCB1	P-gp/efflux	Lansoprazole

Genetic–Kinetic Connection: Influx Transporters, F, and $C_{ss,ave}$

An individual may have the genetic constitution that results in the overproduction of a protein that acts to move drug from within the gastrointestinal lumen into the epithelial cells (i.e., an influx transporter). If the drug avoids efflux and/or gastrointestinal epithelial metabolism and escapes first-pass metabolism, the bioavailability will increase for that given drug:

$$\uparrow F = (ff \cdot \uparrow fg) \cdot ffp$$

The increased bioavailability will result in a higher drug concentration (Equation 4):

$$\uparrow C_{ave}^{ss} = \frac{\uparrow F \cdot Dose}{CL \cdot \tau} \qquad \text{(eq. 4)}$$

Here, the individual may be at risk of toxicity as the resultant drug concentration may be too high.

Drug molecules available for absorption may not traverse the gastrointestinal wall because efflux transporters move drug back into the gastrointestinal lumen.[4,5,9,10] These efflux transporters are proteins embedded in the cell membrane that remove drug from the cells. Although these transporters are found on many different cell membranes, the discussion here will focus on the gastrointestinal epithelium.

A number of efflux transporters can impact the bioavailability of a given drug. Two superfamilies of efflux transporters have been studied extensively. These include the adenosine triphosphate (ATP) binding cassette transporters (ABC transporters), which include P-glycoprotein (P-gp), among others, and the solute carrier transporters (SLC transporters).[5,11,12]

As drug in solution crosses the intestinal epithelium, efflux transporters move the drug back to the gastrointestinal lumen. Here, the fraction of the drug that avoids gastrointestinal wall efflux (*fg*) decreases, and thus bioavailability (F) is decreased. The resultant concentration of the drug in the blood also would be decreased:

$$\downarrow C_{ave}^{ss} = \frac{\downarrow F \cdot Dose}{CL \cdot \tau}$$

Genetic–Kinetic Connection: Efflux Transporters, F, and $C_{ss,ave}$

An individual may have the genetic constitution that results in the overexpression (overproduction) of a protein that acts to move drug from within gastrointestinal epithelium cells back into the gastrointestinal lumen (i.e., an efflux transporter; e.g., P-glycoprotein). In this case, less of the given drug in this patient avoids efflux (*fg*), and F is decreased:

$$\downarrow F = (ff \cdot \downarrow fg) \cdot ffp$$

The decreased bioavailability will result in a lower drug concentration:

$$\downarrow C_{ave}^{ss} = \frac{\downarrow F \cdot Dose}{CL \cdot \tau}$$

Here, the individual may be at risk of treatment failure because the drug concentrations will be relatively low (i.e., subtherapeutic) (see **Figure 2-5**). The dose of the drug may need to be increased or an alternative drug may need to be used.

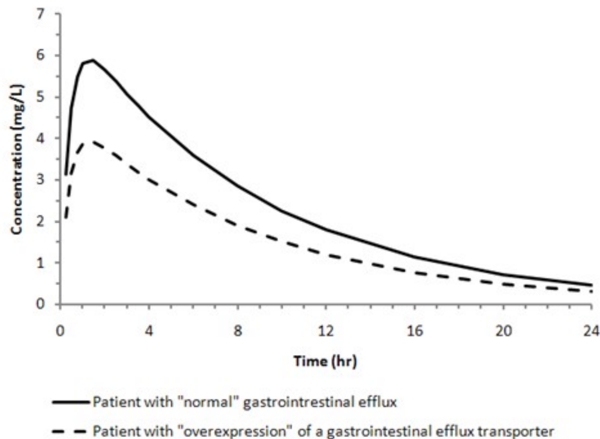


Figure 2-5 Overexpression of an efflux transporter in the gastrointestinal tract results in a decrease in the fraction of the dose that avoids efflux, thus decreasing bioavailability and drug concentration.

Efflux transporters in the gastrointestinal tract can play a major role in the bioavailability of drugs that require transport across the gut wall. Succinctly, with all other processes remaining constant relative to the pharmacokinetics of a drug, "overexpression," or increased activity of gastrointestinal efflux transporters, results in decreased bioavailability and lower systemic drug concentrations, whereas "underexpression," or decreased activity of gastrointestinal efflux transporters, results in increased bioavailability and higher systemic drug concentrations.

Gastrointestinal Wall Metabolism

As drug in solution in the gastrointestinal lumen makes its way into the gastrointestinal epithelium, it may be subject to metabolism by enzymes in the epithelium. Drug metabolized by gastrointestinal wall enzymes does not reach systemic circulation, and thus results in decreased bioavailability; that is, the fraction of the dose avoiding gastrointestinal wall metabolism (*fg*) decreases, as does the fraction of the dose that reaches circulation (Equation 3). This, too, will affect the drug concentration. Although there is large interindividual variability in the content of gastrointestinal wall cytochrome P450 isozymes, the average percent content of CYP3A, CYP2C9, CYP2C19, CYP2J2, and CYP2D6 in the gastrointestinal tract is 82%, 14%, 2%, 1.4%, and 0.7%, respectively.[13]

Poor metabolizers would be expected to have more drug avoid gut wall metabolism. Conversely, extensive/normal and ultrarapid metabolizers would be expected to have less drug avoid gut wall metabolism. Not only will less drug reach the portal vein to be carried to the liver, but hepatic metabolism will further affect the amount of drug that reaches systemic circulation. In the case of individuals who are extensive/normal or ultrarapid metabolizers, too little of the drug may be available systemically to be effective, and other therapeutic modalities may be required. **Figure 2-6** shows the relative differences in the concentration versus time data for a poor metabolizer, an intermediate metabolizer, an extensive/normal metabolizer (wild-type), and an ultrarapid

metabolizer. Note that the identification of a “rapid metabolizer” phenotype (not shown) results in a curve falling between the normal metabolizer and ultrarapid metabolizer. With ultrarapid metabolizers, an alternative therapy may be required, because the drug concentration may not achieve therapeutic levels.

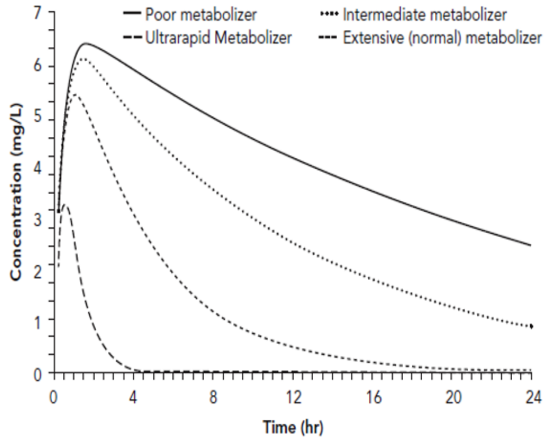


Figure 2-6 Gastrointestinal wall metabolism influences the bioavailability of a given drug. Compared to the extensive/normal (wild type) metabolizer (EM/NM), the poor metabolizer (PM) exhibits a concentration versus time profile with a T_{max} that occurs later and a C_{max} and AUC that are higher. The intermediate metabolizer (IM) falls between the PM and the EM/NM. The EM/NM and UM have a T_{max} that occurs earlier, and a C_{max} and AUC that are lower, relative to the IM and PM. The bioavailability for a given drug in each individual may be different, due, in part, to genetic (single nucleotide polymorphism) differences between the individuals.

Genetic–Kinetic Connection: Gut Wall Metabolism

An individual may have the genetic constitution that results in CYP2C19 ultrarapid metabolism, (e.g., the *17/*17 genotype). In this case, following per-oral administration of a CYP2C19 substrate drug, less of the drug avoids gastrointestinal wall metabolism (*fg*), and F is decreased:

$$\downarrow F = (ff \cdot \downarrow fg) \cdot ffp$$

The decreased bioavailability will result in a lower drug concentration:

$$\downarrow C_{ave}^{ss} = \frac{\downarrow F \cdot Dose}{CL \cdot \tau}$$

Here, the individual may be at risk of treatment failure because the drug concentrations will be relatively low. The dose of the drug may need to be increased, or an alternative drug may need to be used.

Hepatic First-Pass Metabolism

Following oral dosing, drug that is available for absorption and that avoids gastrointestinal efflux and gut wall metabolism is carried via hepatic portal blood flow to the liver, where it may be subject to hepatic metabolism, thus undergoing first-pass metabolism. Drug that escapes hepatic metabolism and reaches systemic circulation is said to be bioavailable.

The same potential differences exist for drug metabolism in the liver as were described for gut wall metabolism. Drug that does make it to the liver may be efficiently metabolized in a patient who is an extensive/normal metabolizer or an ultrarapid metabolizer, leaving little drug reaching systemic circulation. Conversely, the patient may be a poor metabolizer with inefficient hepatic metabolism, thus allowing a higher fraction of the drug to reach systemic circulation, resulting in relatively higher bioavailability. The percentage content of cytochrome P450s in the liver has been reported to be 40%, 25%, 18%, 9%, 6%, 2%, and <1%, for CYP3A, CYP2C, CYP1A2, CYP2E1, CYP2A6, CYP2D6, and CYP2B6, respectively.[14]

Genetic–Kinetic Connection: Hepatic First-Pass Metabolism

An individual may have the genetic constitution that results in the under expression of a drug-metabolizing enzyme (e.g., CYP2C19). In this case, more of the given drug in this patient avoids hepatic first-pass metabolism (*ffp*), and F is increased:

$$\uparrow F = (ff \cdot fg) \cdot \uparrow ffp$$

The increased bioavailability will result in a higher drug concentration:

$$\uparrow C^{ss}_{ave} = \frac{\uparrow F \cdot Dose}{CL \cdot \tau}$$

Here, the individual may be at risk of toxicity because the drug concentration will be relatively high. The dose of the drug may need to be decreased, or an alternative drug may need to be used.

Gastrointestinal Wall Efflux, Metabolism, and Hepatic First-Pass Metabolism

The genetic constitution of an individual will influence each of the variables that determine bioavailability. For instance, a

patient may overexpress the efflux transporter protein P-gp while also being an ultrarapid metabolizer who overexpresses CYP2C19. If a drug is subject to efflux by P-gp and is a metabolic substrate for CYP2C19, the bioavailability of that drug would be expected to be quite low because the fraction avoiding efflux, escaping gut wall metabolism, and escaping hepatic first-pass metabolism would be low:

$$\downarrow\downarrow\downarrow F = (ff \cdot \downarrow\downarrow fg) \cdot \downarrow ffp$$

A drug “handled” in this way by the body may not be suitable for oral administration and may need to be administered by a route that avoids gastrointestinal efflux, gastrointestinal wall metabolism, and first-pass metabolism, such as the intravenous or sublingual route, or an alternative drug may need to be used. **Figure 2-7** shows the potential consequences for a drug molecule relative to oral absorption and bioavailability. Recognize that all the potential processes of a given drug’s absorption are influenced by the patient’s genetic constitution.

Distribution and Volume of Distribution

Influx and efflux transporters are found in many tissues and play a role in the distribution of drugs throughout the body. As discussed previously, transporters in the gastrointestinal epithelium can influence drug absorption and bioavailability. However, these transporters do not influence the distribution of a drug because distribution occurs after the drug reaches systemic circulation. The volume of distribution (Vd) is the proportionality constant relating the amount of drug in the body to the drug concentration.

As traditionally described, the volume of distribution is a primary independent pharmacokinetic parameter that influences the half-life (Equation 5) and is used in the calculation of a drug’s loading dose (Equation 6):[15]

$$t½ = \frac{0.693 \cdot Vd}{CL} \qquad \text{(eq. 5)}$$

$$D_L = C \cdot Vd \qquad \text{(eq. 6)}$$

Alterations in a drug's volume of distribution can effect the drug's plasma concentration and its efficacy and/or the likelihood of producing toxicity. Rearrangement of Equation 6 shows the implications of an altered volume of distribution relative to the drug concentration:

$$C = \frac{D_L}{Vd} \qquad \text{(eq. 6a)}$$

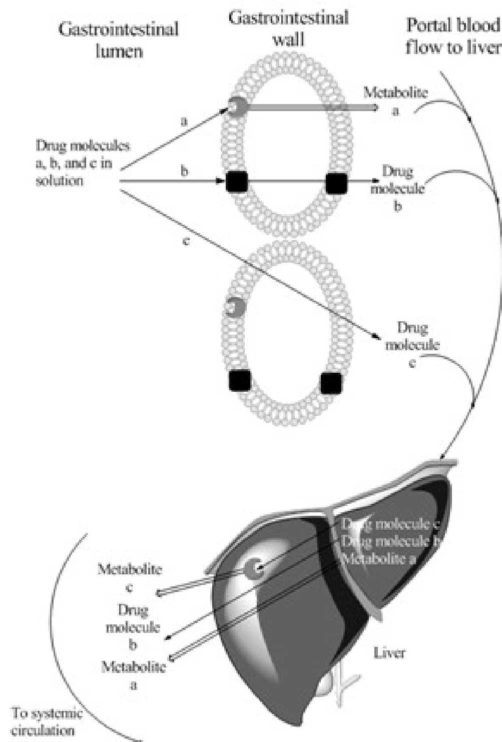


Figure 2-7 Drug being absorbed from the gastrointestinal tract. Upon oral dosing of a given drug, drug molecules a, b, and c, in solution in the gastrointestinal tract, are presented for absorption. Drug molecule (a) is metabolized by an enzyme in the gastrointestinal wall and does not reach systemic circulation and is therefore not bioavailable. The resulting metabolite travels to the liver, via portal blood flow, and then to systemic circulation. Drug molecule (b) is transported, via a protein, from the gastrointestinal lumen to portal blood flow, where it travels to the liver. The molecule moves through the liver and reaches systemic circulation, thus being bioavailable. Drug molecule (c) passively diffuses through the gastrointestinal wall and travels to the liver via portal blood flow. The drug molecule does not reach systemic circulation and is not bioavailable as it is metabolized in the liver (i.e., first-pass metabolism).

Equations 5, 6, and 6a represent relationships for a one-compartment pharmacokinetic model where a drug distributes efficiently throughout the body and administration and elimination are into and from a single compartment. This model describes drugs that exhibit a single declining slope on a semi-log plot, following the maximum concentration, when concentrations are observed over time following drug administration.

Following drug administration, many drugs however exhibit more than one declining log-concentration slope over time, suggesting that the drug distributes at different rates into different tissues and that the rate of elimination is slower than the rate of distribution. In this case, the drug concentration versus time data are best described by multi-compartment models. Here, these models describe drug typically administered into the initial volume (V_1), which represents a component of the total volume of distribution (V_{ss}). Ideally, the initial volume of distribution is calculated by dividing the intravenous push (bolus) dose by the initial drug concentration observed immediately after administration of the intravenous push dose. By definition, volumes of a multi-compartment model are additive, thus V_1 (the volume of the first compartment) is smaller than the V_{ss}. Typically, for a multi-compartment model, V_1 is relatively small because immediately after a push dose drug has not yet moved into slowly perfused tissues (i.e., the drug has not equilibrated with other tissue volumes). Also in these models, drug is typically shown to be eliminated from V_1 because the major, “high blood flow” eliminating organs (i.e., kidneys and liver) are considered to be in V_1. Relationships of

pharmacokinetic parameters and calculation of the loading dose for a multi-compartment drug as shown in Equations 7 and 7a are related to a multi-compartment model and are similar to the equations for a one-compartment model (Equations 6 and 6a).

$$D_L = C \cdot V_1 \text{ or } D_L = C \cdot V_{ss} \qquad \text{(eq. 7)}$$

$$C = \frac{D_L}{V_1} \text{ or } C = \frac{D_L}{Vss} \qquad \text{(eq. 7a)}$$

For calculation of the loading dose for a drug that has its concentration versus time profile best described by a multi-compartment model, V_1 is used when distribution from the first compartment to the other compartments is relatively slow, whereas V_{ss} is used when distribution from the first compartment to other compartments is relatively rapid.

The distribution of a given drug may depend on the function of a drug transporter such that its overexpression or underexpression alters the volume of distribution, which then may alter the half-life. Additionally, as we have learned more regarding the location and function of certain transporters, it has become clear that in some cases there is a relationship between the volume of distribution and clearance, a measure of drug removal from the body, that may or may not influence the half-life.[16]

As noted earlier, P-gp, an efflux transporter, is expressed in many tissues in the human body, including the liver, kidney, lung tissue, and, to a lesser extent, muscle, mammary glands, and other tissues. As P-gp works to keep drugs out of tissues, underexpression of P-gp would allow for greater distribution of a P-gp substrate drug; the drug would not be removed from the tissue as it would if P-gp were normally expressed. This would increase the volume of distribution of the drug. If the Vd or V_1 (and hence V_{ss}) of the drug were to be the only altered parameter, it would be expected, that the $t_{1/2}$would be increased also (Equation 5). It also is noted that the calculated loading dose would be higher (Equations 6 and 7). The above scenario implies

that the tissue "protected" by P-gp would not serve to metabolize and/or eliminate the given drug, because the volume of distribution was the sole parameter that was altered, with clearance remaining unchanged.[12,16]

Genetic–Kinetic Connection: Drug Distribution, Vd, CL, $t_{1/2}$, and Drug Concentration

An individual with a reduced-function allele for OATP1B1 (resulting in underexpression in liver tissue) is receiving atorvastatin for the treatment of hypercholesterolemia. Atorvastatin is a substrate for OATP1B1 and is metabolized in the liver. The genetic constitution of this individual results in a decreased volume of distribution and a decreased clearance of atorvastatin:

$$\leftrightarrow t½ = \frac{0.693 \cdot Vd \downarrow}{CL \downarrow}$$

Here, conceptually, it would be expected that the drug concentration would increase because the initial dose is administered into what is effectively a smaller volume of distribution in this individual:

$$\uparrow C = \frac{D_L}{Vd \downarrow}$$

Additionally, as a maintenance dose (D_M) is continued in this individual, the average steady-state concentration will be increased further as the clearance is decreased (Equation 8): $D_M = CL \cdot C_{ave}^{ss}$

$$\uparrow C_{ave}^{ss} = \frac{F \cdot Dose}{\downarrow CL \cdot \tau} \quad \text{(eq. 8)}$$

Although the t½ may not be altered, increases in the drug concentration put the patient at potential risk of toxicity.

Table 2-2 Examples of Overexpression of Drug Transporters in Tissues in Humans and Effects on the Volume of Distribution and the Drug Concentration

Transporter (Type)	Example Tissue	Gene	Effect on Volume of Distribution	Effect on the Plasma Drug Concentration
P-gp (efflux)	Liver	*ABCB1*	Decrease	Increase
OATP1B1 (influx)	Brain	*SLC01B1*	Increase	Decrease
OCT1 (influx)	Kidney	*SLC22A1*	Increase	Decrease

The influx transporter OATP1B1, an organic anion transporter, is expressed in human liver tissue. If a given drug is an uptake substrate for OATP1B1 and also is metabolized in the liver, alterations in the expression of OATP1B1 will have an effect on the volume of distribution and on the clearance of the drug.[17–19] Here, Vd and CL will change in the same direction. The magnitude of change in each parameter will determine whether the $t_{1/2}$ remains constant or is altered (Equation 5). In this situation, clearance is related to the Vd of the drug.

The relationship between Vd and CL can be thought of as a

relationship between the physical volume in which the drug resides and the functional mechanism of drug elimination that occurs in that volume.

The genetically controlled tissue expression of a given drug transporter is critical in understanding how a drug's pharmacokinetics are related to the drug concentration. **Table 2-2** shows the tissue distribution of example drug transporters in humans and how genetic variation influences drug distribution and drug concentration.

Metabolism

Many drugs are not excreted from the body unchanged; therefore, they require metabolic conversion to be inactivated and primed for removal via excretory pathways. Genetic variation in the expression and/or activity of drug metabolizing enzymes can have a profound effect on the concentration versus time profiles of these drugs and, more importantly, on the therapeutic outcomes of drug therapy. With two phases of drug metabolism, the potential exists for genetic variability to disrupt drug metabolism, especially for a drug which undergoes each phase of metabolism.

Phase I metabolism refers to chemical reactions involving oxidation, reduction, or hydrolysis. These reactions work to make the drug more polar by adding functional amino, sulfhydral, hydroxyl, and carboxyl groups that make the given drug more hydrophilic, thus promoting excretion of the drug from the body, such as being eliminated in urine.[20] **Phase II metabolism** typically refers to conjugation reactions, including glucuronidation, sulfation, acetylation, and methylation, among other reactions. Phase II metabolism, like phase I metabolism, works to make molecules more water soluble, promoting drug excretion. Both phase I and phase II metabolic reactions are under genetic control, and polymorphisms have been identified for specific enzymes that perform these metabolic functions.

A drug undergoing phase I metabolism may be converted to

inactive metabolite(s) that may be excreted or act as substrate(s) for phase II metabolic reactions. Alternatively, a drug may undergo phase I metabolism, resulting in the drug being "activated,"which is the premise for the development of prodrugs. Finally, in some cases, the "inactivation" of a drug by phase I and/or phase II metabolism may result in the formation of a toxic metabolite. In this case, the drug is inactivated and no longer produces the desired therapeutic response; however, the metabolite is toxic, eliciting an adverse reaction or unwanted effect.

With respect to phase I oxidative metabolism, the **cytochrome P450 enzyme (CYP)** superfamily has been the focus of significant research. Although there are numerous CYP enzyme families, three families in particular (CYP1, CYP2, and CYP3) encompass the major drug metabolizing enzymes, with CYP3A being the most prominent.[21] **Table 2-3** presents examples of the CYP enzymes with polymorphisms involved in drug metabolism and the tissues in which these enzymes are expressed. **Figure 2-8** shows the nomenclature for cytochrome P450 enzymes, and **Figure 2-9** presents the contribution of various CYPs in drug metabolism.

Table 2-3 Examples of Cytochrome P450 Drug Metabolizing Enzyme Tissue Expression, Allele Variation, Metabolic Consequence, and Influence on Drug Concentration

CYP Enzyme	Example Tissue Expression	Gene (SNP) rs#[a]	Primary Pharmacokinetic Alteration	Effect on Drug Concentration
CYP2C9	Small intestine/ liver	*CYP2C9**2 (C.430C>T) rs1799853	Poor metabolizer: increased *ffp*, decreased CL	Increased fraction of drug dose presented to the liver. Increased concentration.
CYP2C19	Liver	*CYP2C19*2* (681G>A) rs4244285	Poor metabolizer: decreased CL	Increased concentration.
CYP2D6	Liver	*CYP2D6**4 (1846G>A) rs3892097	Poor metabolizer: decreased CL	Increased concentration.

[a] Reference SNP (refSNP) number. These numbers are unique and consistent identifiers of the given SNP.

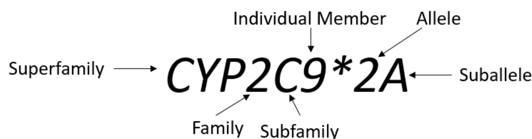


Figure 2-8 Nomenclature for the cytochrome P-450 (CYP) alleles. The established nomenclature system for alleles of the cytochrome P-450 (CYP) superfamily designates "CYP" followed by family number, subfamily letter, and individual subfamily number. Allelic differences are defined by number or a number and letter following an asterisk (*).

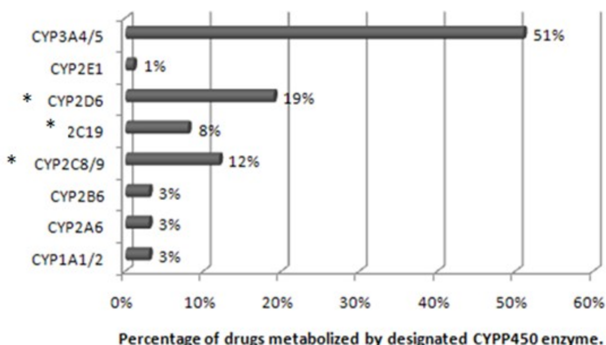


Figure 2-9 Percentage of drugs metabolized by designated cytochrome P450 (CYP) enzymes. Polymorphic (*) expression of certain CYP enzymes confounds the concentration vs. time profile of the drug and may alter the therapeutic response in individuals, thus requiring specific dosing considerations.

Because metabolism of a given drug influences the clearance of that drug and clearance is used to calculate the maintenance dose, identifying a single nucleotide polymorphism (SNP) related to a given CYP enzyme can aid in "personalizing" an individual's dose. Single nucleotide polymorphisms can result in a patient handling a drug in such a manner that they would be considered to be a particular "type" of metabolizer. Homozygous individuals with a polymorphism resulting in the expression of a "loss-of-function"/"no function" (inactive) or "reduced-function" CYP enzyme would be considered a poor metabolizer. These individuals would have a decreased clearance of substrate drugs and would require a lower maintenance dose to achieve the desired therapeutic response. Individuals who

are heterozygous, with one allele producing a loss-of-function or decreased-function enzyme and the other producing a normal-function enzyme, are termed intermediate metabolizers. These individuals may require a lower maintenance dose because they would have a decreased clearance. However, the reduction in clearance would not be as great as that seen in a poor metabolizer, and the required maintenance dose would not be as low. Extensive/normal metabolizers are individuals who have two normal function alleles, and would receive the "normal" maintenance dose. The fourth type of individual would be one in whom there is gene duplication with a consequential overexpression of the CYP enzyme, resulting in a high clearance of the drug, necessitating a higher maintenance dose. These individuals are called **ultrarapid metabolizers (UM)**.

Terminology used to describe the types of phenotypic metabolizers insinuates two concepts related to drug metabolism. The first is the extent of metabolism, and the second is the rate of metabolism. These terms are related to pharmacokinetics in that the phenotypic category of a given individual (i.e., poor metabolizer, intermediate metabolizer, extensive/normal metabolizer, or ultrarapid metabolizer) implies the characteristics of specific pharmacokinetic parameters and dosing requirements. As described above, clearance is the primary pharmacokinetic parameter that is affected by an individual's genetic constitution. This will result in a potential alteration in the half-life because it is dependent on the clearance (and the volume of distribution). Also, an altered clearance will impact the maintenance dose, and the half-life will influence the dosing interval. **Table 2-4** describes the impact of **phenotype** on pharmacokinetic parameters related to metabolism and dosing considerations.

It is important to understand that an individual's **genotype** may *not* match their phenotype, in that influences other than genetics can alter the expression of a metabolizing enzyme. For instance, an individual with the *1/*1 genotype for *CYP2C19* (*CYP2C19**1/*1) would be considered an extensive/normal metabolizer. However, if this individual is receiving a certain

proton pump inhibitor (PPI), such as omeprazole, for the treatment of esophageal reflux disease, the PPI may inhibit the function of CYP2C19, thus causing the individual to effectively be a poor metabolizer.[22] Here, due to the drug interaction, the individual has the phenotype of a poor metabolizer. It is always important to consider drug–gene interactions and drug–drug interactions simultaneously, here considered a drug-drug-gene interaction.

Table 2-4 Metabolic Phenotypes: Pharmacokinetic and Dosing Consequences[a]

Phenotype	Pharmacokinetic Parameter	Consequence	Dosing	Potential Consequence
Poor metabolizer (PM)	k_e; elimination rate constant/$t_{1/2}$; half-life (related to k_e)	↓↓/↑↑	Dosing frequency	↓↓
	CL; clearance	↓↓	Maintenance dose	↓↓
Intermediate metabolizer (IM)	k_e; elimination rate constant/$t_{1/2}$; half-life (related to k_e)	↓/↑	Dosing frequency	↓
	CL; clearance	↓	Maintenance dose	↓
Extensive/Normal metabolizer (EM/NM)	—	—	—	—
Rapid Metabolizer (RM)	k_e; elimination rate constant/$t_{1/2}$; half-life (related to k_e)	↑/↓	Dosing frequency	↑
	CL; clearance	↑	Maintenance dose	↑
Ultrarapid metabolizer (UM)	k_e; elimination rate constant/$t_{1/2}$; half-life (related to k_e)	↑↑/↓↓	Dosing frequency	↑↑
	CL; clearance	↑↑	Maintenance dose	↑↑

[a] Relative to the extensive/normal metabolizer being considered "normal," with the same dose being administered to each individual with a given phenotype: ↓ = decreased, ↑ = increased. The number of arrows indicates the relative magnitude of the consequence.

Genetic–Kinetic Connection: Drug Metabolism, CL, Dose, and Dosing Interval

An individual with inheritance of alleles resulting in *CYP2D6* gene duplication is receiving doxepin for the treatment of depression. Doxepin, a tricyclic antidepressant, is metabolized by CYP2D6. The genetic constitution of this individual results in the individual being an ultrarapid metabolizer, exhibiting a significantly higher clearance of doxepin. This individual has been taking the drug, but has not been responding. This could be due to the increased clearance of the drug, resulting in low concentrations and drug exposure (Equation 9):

$$\downarrow\downarrow C_{ave}^{ss} = \frac{F \cdot Dose}{\uparrow\uparrow CL \cdot \tau}$$

$$\downarrow\downarrow AUC = \frac{Dose}{\uparrow\uparrow CL} \qquad \text{(eq. 9)}$$

The increased clearance will require an increased maintenance dose to achieve the desired concentration that would maximize the probability of a therapeutic response:

$$\uparrow\uparrow D_M = \uparrow\uparrow CL \cdot C_{ave}^{ss}$$

Additionally, the significantly higher clearance seen in an individual who is an ultrarapid metabolizer will result in a shorter half-life:

$$\downarrow\downarrow t½ = \frac{0.693 \cdot Vd}{\uparrow\uparrow CL}$$

If the individual is to remain on the drug, the frequency of administration will need to be increased to maintain therapeutic concentrations. The dosing interval **tau (s)** can be estimated for a rapidly absorbed drug as (Equation 10):

$$\downarrow\downarrow \tau = \frac{\ln(\frac{C\max}{C\min})}{\uparrow\uparrow ke} \qquad \text{(eq. 10)}$$

or specifically for an intravenous medication (Equation 10a):

$$\downarrow\downarrow \tau = \frac{\ln(\frac{C\max}{C\min})}{\uparrow\uparrow ke} + ti \qquad \text{(eq. 10a)}$$

where t_i is the infusion time.

Excretion

As previously mentioned, drug influx and efflux transporters are found in many tissues and play a role in the distribution of drugs throughout the body. Not only do these transporters affect

distribution, but they can influence the drug's removal from the body through drug excretion.

The renal excretion of a drug, moving the compound from the blood to the urine, can be a consequence of genetically mediated drug transport.[4,23,24] Renal filtration occurs in the glomerulus, and active secretion occurs in the nephron tubules. Both of these sites are "excretory" because drug is moved from the blood to the urine. Relative to tubular secretion, numerous transporters have been identified in kidney tissue, including P-gp (MDR1), OCT1, OAT1, MRP2, cMOAT, and ENT1, among others. **Table 2-5** presents examples of drug transporters in the kidney and their influence on renal drug handling. Transporter distribution among different populations may explain differences in renal excretion of drugs, and SNPs may further delineate drug removal in given individuals.

Table 2-5 Examples of Renal Drug Transporters Responsible for Urinary Excretion

Example Drug	Example Transporter	Renal Drug Process
Cefamandole[a]	OAT1	Renal tubular excretion
Cimetidine[b]	OAT3	Renal tubular excretion
Acyclovir[b]	OCT1	Renal tubular excretion
Amoxicillin[a]	PEPT1	Renal tubular excretion
Zidovudine[b]	OAT4	Renal tubular reabsorption

[a] Inhibitor of transporter. [b] Substrate of transporter.

Genetic–Kinetic Connection: Renal Drug Excretion

An individual receiving metformin for treatment of type 2 diabetes may have the genetic constitution that results in the expression of a less active form of the drug transporter OCT1 found on the apical side of the proximal and distal tubules in the kidney. This expression results in decreased uptake of metformin from the plasma, resulting in decreased renal clearance, and hence overall clearance because the clearances are additive (Equation 11):

$$\downarrow CL = \downarrow CL_R \cdot C_{Other} \quad \text{(eq. 11)}$$

However, OCT1 is also found in liver tissue, and the decreased activity in this tissue results in decreased hepatic uptake of metformin, which may alter the drug effect (pharmacodynamics).

Similar to renal drug excretion, biliary excretion is another mechanism of drug elimination. Efflux transporters (MDR1, MDR3, and others) move drug from the hepatocyte into the

biliary canaliculi. The drug/metabolite then is moved to the small intestine, where it may be reabsorbed through enterohepatic cycling or excreted from the body in the feces. Therefore,

changes in the level of expression/activity of these transporters within the hepatocytes would be expected to impact biliary drug excretion.

Chapter Summary

The pharmacokinetics of a drug are determined by evaluating the concentration of drug in biologic fluids over time. Drug metabolizing enzymes and drug transporters may influence all aspects of the concentration–time profile, including transporters affecting the volume of distribution, which is used in calculating the loading dose and metabo-lizing enzymes influencing the clearance, which is used in calculating the maintenance dose. Both the volume of distribution and the clearance influence the elimination rate constant, and hence the half-life, which is used to calculate the dosing interval. It is clear that genetic variation in transporters and metabolizing enzymes are responsible for the varied dosing regimens of the same drug required by different individuals.

Review Questions

1. The study of a gene involved in response to a drug is referred to as:
   a. pharmacokinetics.
   b. pharmacodynamics.
   c. pharmacogenetics.
   d. pharmacogenomics.

2. The _______ is the main site of drug absorption due to its large surface area, membrane permeability, and capillary blood flow.
   a. liver
   b. large intestine
   c. small intestine
   d. stomach

3. If an individual is an extensive/normal metabolizer of a drug relative to an intermediate metabolizer or a poor metabolizer, what happens to the k_e and T_{max} of that drug?
   a. k_e is decreased and the drug is eliminated more slowly; therefore, the T_{max} will occur later.
   b. k_e is decreased and the drug is eliminated faster; therefore, the T_{max} will occur sooner.
   c. k_e is increased and the drug is eliminated more slowly; therefore, the T_{max} will occur later.
   d. k_e is increased and the drug is eliminated faster; therefore, the T_{max} will occur sooner.

4. The _______ of drug absorption is expressed by T_{max}, and the _______ of drug absorption is defined by C_{max} and AUC.
   a. rate; extent
   b. extent; rate
   c. concentration; time
   d. time; concentration

5. With respect to drug metabolism, which individual, relative to metabolizer status, may be at risk of experiencing toxicity from a standard dose of a particular drug (not referring to a prodrug)?
   a. Poor metabolizer
   b. Intermediate metabolizer
   c. Extensive/normal metabolizer
   d. Ultrarapid metabolizer

6. Compared to an extensive/normal metabolizer, an ultrarapid metabolizer will need _______ dosing frequency.
   a. a decreased
   b. an increased
   c. the same
   d. Not enough information has been provided to answer this question.

7. If an individual has a genetic constitution that results in the decreased production of gastrointestinal influx transporters, what will happen to the bioavailability and concentration of a drug that is a substrate for the transporters?
   a. Bioavailability will increase; the concentration will decrease.
   b. Bioavailability will increase; the concentration will increase.
   c. Bioavailability will decrease; the concentration will decrease.
   d. Bioavailability will decrease; the concentration will increase.

8. How might treatment outcome be affected if less of a dose of drug avoids gastrointestinal wall metabolism in a patient?
   a. the patient may be at risk of treatment failure due to low drug concentrations.
   b. he patient may be at risk of toxicity due to high drug concentrations.
   c. he patient may be at risk of treatment failure due to increased bioavailability.
   d. he patient may be at risk of toxicity due to increased bioavailability.

9. _______ or _______ activity of gastrointestinal efflux transporters results in decreased bioavailability and potentially lower systemic drug concentrations.
   a. Underexpression; decreased
   b. Underexpression; increased
   c. Overexpression; decreased
   d. Overexpression; increased

10. With regards to the following equation, if the fraction of a drug that avoids gastrointestinal wall efflux decreases, what would happen to the resultant concentration of the drug in the blood?

$$C_{ave}^{ss} = \frac{F \cdot Dose}{CL \cdot \tau}$$

a. Decreased
b. Increased
c. No change
d. Not enough information provided to answer the question.

11. An individual has the genetic constitution that shows "loss-of-function" of the drug metabolizing enzyme CYP2C19, and this individual is taking a drug that is metabolized by this isozyme. With regards to hepatic first-pass metabolism, _______ of the given drug avoids metabolism, resulting in ________ bioavailability of the drug.
a. more; increased
b. more; decreased
c. less; increased
d. less; decreased

12. On average, which cytochrome P450 enzyme has the highest percentage of presence in both the gut wall and the liver?
a. CYP2C9
b. CYP3A4/5
c. CYP2C19
d. CYP2D6

13. An individual overexpresses the efflux protein P-gp and also is an ultrarapid metabolizer, overexpressing CYP2C19. If a drug is a metabolic substrate for CYP2C19 and is subject to efflux by P-gp, what would be the effect on bioavailability? Consider that: $F = (ff \cdot fg) \cdot ffp$

    a. Bioavailability would decrease.
    b. Bioavailability would increase.
    c. Bioavailability would not change.
    d. Not enough information has been provided to answer the question.

14. Influx and efflux transporters in the gastrointestinal epithelium can influence _______ and _______.
    a. distribution of a drug; bioavailability
    b. distribution of a drug; drug absorption
    c. drug absorption; bioavailability
    d. volume of distribution; bioavailability

15. The volume of distribution influences the half-life and is used to calculate a drug's:
    a. maintenance dose.
    b. loading dose.
    c. dosing interval.
    d. a and c

16. _______ is a primary pharmacokinetic parameter that is affected by an individual's genetic constitution.
    a. k_a
    b. $t_{½}$
    c. k_e
    d. CL

17. If a patient has underexpression of the influx transporter OATP1B1 in the liver, and the volume of distribution and clearance are decreased by the same magnitude, what change would need to be made to the dosing interval of the drug?
    a. The dosing interval would need to be decreased.
    b. The dosing interval would need to be increased.
    c. The drug would need to be discontinued.
    d. The dosing interval would not need to be changed.

18. A homozygous individual with a polymorphism resulting in a loss-of-function CYP enzyme would be considered a (n) _______ and would have _______ clearance requiring a _______ maintenance dose.
    a. poor metabolizer; increased; higher
    b. extensive/normal metabolizer; increased; higher
    c. poor metabolizer; decreased; lower
    d. extensive/normal metabolizer; decreased; lower

19. An individual with depression has *CYP2D6* gene duplication and is considered to be an ultrarapid metabolizer. If this individual is taking the antidepressant doxepin, a CYP2D6 metabolic substrate, what would be the likely treatment outcome and what could be done to correct this?
    a. The individual would likely experience adverse drug reactions due to the relatively high clearance and would require an increased maintenance dose or the use of another drug.
    b. The individual would likely experience adverse drug reactions due to the relatively low clearance and would require a decreased maintenance dose.
    c. The individual would likely experience treatment failure due to the relatively high clearance and would require an increased maintenance dose or the use of another drug.
    d. The individual would likely experience treatment failure due to the decreased clearance and would require a decreased maintenance dose.

20. With regards to renal excretion, if an individual has overexpression of the ABCB1 gene coding for the P-gp (MDR1) transporter in the kidney, what effect would this have on clearance and the drug concentration?
    a. Increased clearance and increased drug concentration.
    b. Decreased clearance and decreased drug concentration.
    c. Increased clearance and decreased drug concentration.
    d. Decreased clearance and increased drug concentration.

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CHAPTER Three

Pharmacogenomics and Pharmacodynamics

Learning Objectives

Upon completion of this chapter, the student will be able to:

1. Recognize the influence of genetic polymorphisms on the efficacy and affinity of drugs.

2. Explain how a specific polymorphism would affect the design of a patient's drug dosing regimen.

3. Differentiate among receptors, enzymes, and transporters as drug targets, and explain how genetic polymorphisms of these drug targets can influence drug selection.

4. Propose alterations to a patient's dosing regimen based on pharmacogenomic influence on pharmacodynamic parameters.

The student should demonstrate an understanding of how drug targets are influenced by genetic variation. The student should understand that variation in these proteins results in variation in pharmacodynamics, potentially influencing how an individual responds to a given drug.

Key Terms	Definitions
affinity	The strength of the reversible binding between a drug and drug target (receptor).
agonist	An endogenous or exogenous ligand that activates a drug target to induce a response.
antagonist	An endogenous or exogenous ligand that inhibits another endogenous or exogenous ligand from binding to a drug target to induce a response.
dissociation constant (K_D)	Describes the ratio of free drug (D) and free receptor (R) concentration to drug–receptor [DR] concentration. Used to determine the affinity of an agonist.
drug resistance	The inability of a drug to produce a pharmacodynamic response at a standard dose.
drug target	Endogenous binding site for drugs. Drug targets can include receptors, enzymes, and membrane transporters.
EC_{50}	The half-maximal (50%) effective concentration of a drug producing a specific response.
efficacy	The effect (E) elicited by a drug (D) and the concentration of drug–receptor complex [DR].
ligand	Endogenous or exogenous agent that binds to a drug target.
pharmacodynamics (PD)	The relationship between drug exposure and pharmacologic response.
potency	The dependence of the pharmacologic effect(s) of the drug on the drug concentration.
serotonin reuptake transporter (SERT)	A transport protein that regulates the amounts of serotonin in the synaptic cleft.

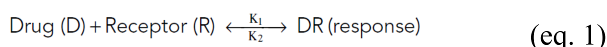
Key Equations	Description
$\text{Drug (D)} + \text{Receptor (R)} \underset{K_2}{\overset{K_1}{\longleftrightarrow}} \text{DR (response)}$	The relationship between free drug concentration (D), free receptor (R) concentration and drug–receptor complex (DR) and drug response.
$K_D = \frac{[D][R]}{[DR]}$	Describes the strength of the reversible interaction between a drug and receptor (affinity). K_D is proportional to the free drug concentration and the concentration of unoccupied receptors and is inversely proportional to the drug–receptor complex concentration.

Introduction

The mechanisms of drug action are the fundamental underpinning of pharmacodynamics. Drugs elicit their mechanism(s) of action through biochemical and physiological interactions with drug targets. Thus, the pharmacodynamic effects of a drug determine its overall therapeutic utility. **Pharmacodynamics (PD)** is the relationship between drug exposure and pharmacologic response, with elicited effects being related to drug binding to target proteins such as receptors, enzymes, and membrane transporters. Drugs bind to these targets

through a combination of chemical bonding interactions, such as covalent, hydrogen, hydrophobic, ionic, and van der Waals. Because these **drug targets** are all proteins, they are susceptible to the effects of single nucleotide polymorphisms (SNPs). Single nucleotide polymorphisms in the DNA encoding these proteins can result in reduced drug binding (e.g., decreased ability for chemical bonding interactions) and subsequently induce **drug resistance**. Drug resistance is the inability of a drug to produce a pharmacodynamic response at a standard dose. Therefore, the ability to detect SNPs in drug targets represents a method for improving the therapeutic response to drugs.

The **affinity** of a drug for a drug target, such as a receptor, is measured by the strength of the interaction between the drug and the target. The relationship between a drug (D) and receptor (R) determines the drug's overall affinity and efficacy. Affinity describes the strength of the reversible interaction between a drug and drug target. This interaction is described in the following equation:



(eq. 1)

In this equation (Equation 1), the effect (response) of a drug is directly dependent on the DR interaction. Therefore, the ratio of K_2 to K_1, or the dissociation of the drug from the receptor, determines the overall effect of the drug. This ratio (K_2/K_1) is known as the **dissociation constant (K_D)**:

$$K_D = \frac{[D][R]}{[DR]} \quad \text{(eq. 2)}$$

A high affinity of a drug for a receptor means a small K_D. The generation of a response from the DR complex is determined by the drug's **efficacy**. Efficacy describes the effect (E) elicited by a drug (D) and the concentration of the drug–receptor complex [DR]. Efficacy is a measure of the relative **potency**, or likelihood of a drug to induce a response. Thus, the potency of a drug is determined by the affinity and efficacy of a drug at the receptor. The potency of a drug is also influenced by receptor density and

responsiveness at the target tissue.

Genetic–Dynamic Connection: K_D
An individual may have the genetic constitution that results in the reduced expression of a receptor (R). In this case, the patient is considered to be drug resistant. As the concentration of drug–receptor complex [DR] decreases, the concentration of free drug [D] increases, and the overall KD increases (therefore the numerator is increasing while the denominator is decreasing in Equation 2). An increase in K_D means a lower affinity of a drug for a receptor, and the patient could appear to be resistant to the drug's effects.

In general, as the concentration of a drug increases, so does the pharmacologic response. Plotting the magnitude of response against the dose of the drug generates a dose–response curve, as depicted in **Figure 3-1**. Note that the *x*-axis is the log drug concentration [drug]. Semilogarithmic plots allow for graphing of doses that may span several orders of magnitude. Once

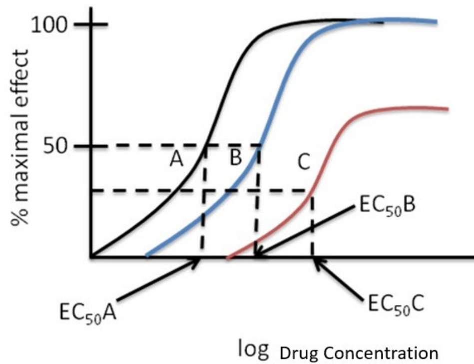


Figure 3-1 Classic dose-response curves. Based on the EC_{50}, the potency of the drugs are in the following order A > B > C. However, drugs A and B have the same efficacy because both reach the same E_{max}. Drug C is less efficacious than both drugs A and B.

the maximal drug–receptor complex [DR] concentration is reached, a 100% maximal effect (E_{max}) is achieved, and the dose–response curve plateaus. The drug concentration [D] that produces a 50% maximal response is designated as the effective concentration 50% (**EC_{50}**). The lower the EC_{50} for a drug, the more potent the drug.

Genetic–Dynamic Connection: Dose–Response Curves (Agonist Example)
An individual may have the genetic constitution that results in a heterozygotic genotype, resulting in decreased drug affinity for a given receptor. If curve A in Figure 3-1 is a consequence of the normal expression of the receptor, then curve B could represent an individual who displays a heterozygotic genotype resulting in decreased drug potency (↑EC_{50}). In this example, the drug's overall efficacy (E_{max}) did not change. However, a heterozygotic and/or homozygotic genotype of the same receptor could induce the response depicted in curve C; resulting in a decrease in both potency (↑EC_{50}) and efficacy (↓E_{max}).

Drugs that block the ability of the endogenous **ligand** to bind to the receptor are classified as **antagonists**. In the classic dose–response curves (Figure 3-1), a competitive antagonist shifts the curve to the right (shifting curve A toward curve B). With competitive antagonism, the effects of the antagonist can be reversed by adding sufficient concentrations of agonist. By comparison, noncompetitive antagonism cannot be reversed by adding high concentrations of agonist (shifting curve A toward curve C).

Pharmacogenomics and Receptors as Drug Targets

Endogenous receptor ligands bind to a receptor to stimulate a biochemical and physiological response. For example, during the fight-or-flight response invoked by the activation of the sympathetic nervous system, epinephrine is released in order to activate a cascade of physiologic effects. The epinephrine that is released binds to β_2-adrenergic receptors (β_2AR) in the bronchiolar smooth muscle to induce bronchodilation and increase oxygen exchange, which is required during the fight-or-flight response. In this example, epinephrine is serving as the endogenous ligand, activating β_2-adrenergic receptors as an **agonist**. The β_2-adrenergic receptor is a cell-surface receptor composed of 413 amino acid residues (see **Figure 3-2**). These amino acids are arranged in such a way that the receptor contains seven transmembrane-spanning domains with an extracellular N-terminus and an intracellular carboxy terminus. To date, 49 SNPs have been reported in the β_2-adrenergic receptor. Five of these

have been associated with nonsynonymous (missense)

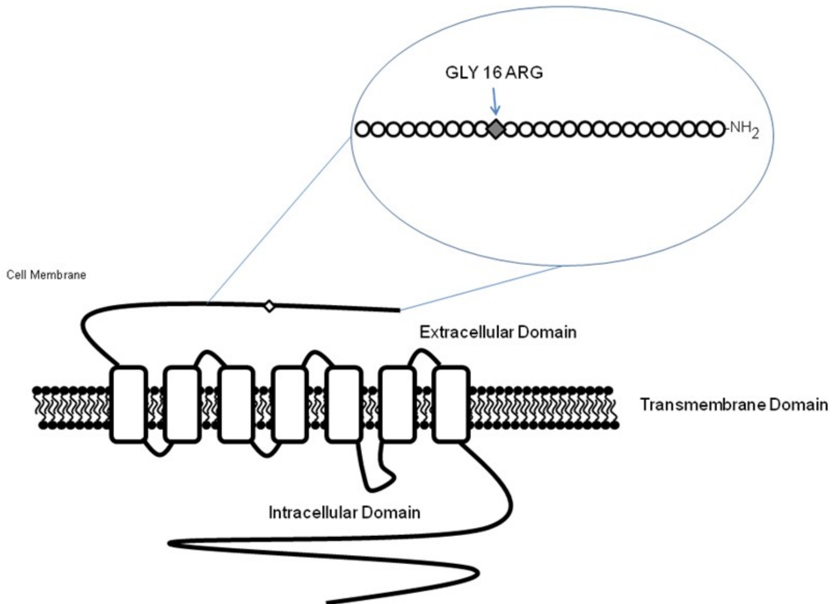


Figure 3-2 The β_2-adrenergic receptor is composed of 413 amino acids. The Gly16Arg amino acid change in the receptor (indicated by the diamond) predisposes patients to nocturnal asthma and influences asthma severity. Note that this amino acid switch occurs in the external-binding domain.

polymorphisms resulting in a change in the amino acid sequence: Ser220Cys, Thr164Ile, Val34Met, Gln27Glu, and Gly16Arg.[1] The Gly16Arg amino acid change in the receptor (protein) predisposes patients to nocturnal asthma and influences asthma severity.[2]

With respect to pharmacodynamics, nonsynonymous SNPs encoding for either Arg or Gly at position 16 have been linked to altered responses to short-acting β_2AR agonists, such as albuterol (Gly at position 16 imparts a better response than Arg at position 16).[1]

Pharmacogenomics and Enzymes as Drug Targets

Enzymes also serve as pharmacodynamic targets for drugs. Like receptor targets, enzymes are composed of amino acids that not only regulate the enzyme's endogenous activity but also the ability of the drug to bind to the enzyme to produce a pharmacodynamic response. Asthma is characterized by increased responsiveness of the tracheobronchial tree to a multiplicity of stimuli. The cysteinyl leukotrienes (LTC4, LTD4, and LTE4) serve as a stimulus to increase bronchiolar smooth muscle contraction and mucus secretion, triggering an asthmatic response. 5-lipoxygenase is an enzyme essential to the biosynthesis of cysteinyl leukotrienes, and it serves as the pharmacodynamic target for drugs such as zileuton (see **Figure 3-3**). By inhibiting 5-lipoxygenase, zileuton decreases the synthesis of cysteinyl leukotrienes, and therefore provides symptomatic relief for the asthma patient.[3] Polymorphisms in the 5-lipoxygenase gene promoter region are associated with differential responses to 5-lipoxygenase inhibitors.[4] Insertion- or deletion-type mutations have been identified in the promoter region of the gene in 22% of Caucasians.

Genetic–Dynamic Connection: 5-Lipoxygenase

Genetic information received through a saliva sample obtained from a patient reveals a deletion polymorphism within the promoter region of the 5-lipoxygenase gene. This deletion results in an altered amino acid sequence within the binding region of the enzyme and decreased zileuton binding to the enzyme. The following inhibitory E_{max} model best describes the altered binding of zileuton:

In this example, the deletion polymorphism within the promoter region results in an increased EC_{50}, and therefore an overall decrease in the patient's sensitivity to zileuton. In this scenario, the patient would require a dose of zileuton that is higher than the standard dose.

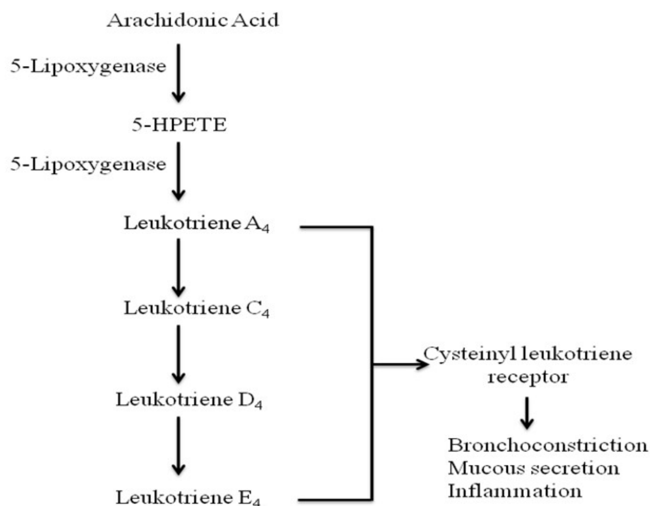


Figure 3-3 The role of 5-lipoxygenase in cysteinyl leukotriene synthesis. The 5-lipoxygenase enzyme catalyzes the initial steps in the synthesis of the cysteinyl leukotrienes LTA4, LTC4, LTD4, and LTE4. These leukotrienes mediate bronchoconstriction, mucous secretion, and the recruitment of inflammatory cell mediators through the activation of the cysteinyl leukotriene receptor. 5-HPETE = 5-Hydroperoxyeicosatetraenoic acid.

Pharmacogenomics and Membrane Transporters as Drug Targets

The termination of neurotransmitter effects in the central nervous system predominantly occurs as a result of neurotransmitter reuptake into the secreting neuron. For example, serotonin is released into the synaptic cleft to activate postsynaptic receptors, inducing a physiologic response (see **Figure 3-4**). Serotonin's effects are terminated in large part by reuptake mediated by the **serotonin reuptake transporter (SERT)**. Once taken back up by the neuron, the serotonin is recycled for later use. The selective serotonin reuptake inhibtors (SSRIs), such as fluoxetine, induce their pharmacodynamic effects through the inhibition of SERT. Inhibition of SERT increases serotonin levels in the synaptic cleft, thereby enhancing serotonin-mediated effects. A polymorphism in the promotor region of the SERT gene has been identified.[5] This

polymorphism is often referred to as SERTPR, in reference to the promotor region. Two forms of polymorphisms have been identified in the SERTPR:

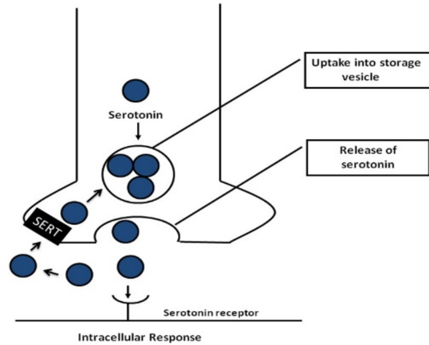


Figure 3-4 Serotonin released into the synapse is recycled back into the neuron for later release and use. The reuptake of serotonin into the sertonergic terminal is mediated by the serotonin reuptake transporter (SERT).

long (l) and short (s). Depressed patients who are homozygotic (l/l) or heterozygotic (l/s) for the variant have demonstrated a better response to SSRIs than those homozygotic (s/s) for the short variant.[6] Subsequently, other indications for SSRIs (e.g., anxiety) have also demonstrated similar variations in responsiveness.[7]

Pharmacogenomics and Pharmacodynamics Application

DC is a 29-year-old African American male who presents to his primary care physician's office for initial evaluation in the pharmacotherapy clinic. Today, DC reports wheezing, coughing, and shortness of breath at rest and during activity. DC states he has a history of coughing and wheezing beginning in his teenage years and has been treated for multiple episodes of bronchitis. DC reports that he has had numerous unscheduled doctor visits in the last year, about 15 emergency department visits in the past five years, and that he has been hospitalized seven times in his lifetime, with the last hospitalization being four months ago. DC also complains that

he has difficulty exercising and doing some daily activities. DC states that most recently he is feeling short of breath, has a runny nose/nasal congestion, wheezes a few times a week, has night-time symptoms of coughing, and is using his albuterol inhaler daily. His current medications include: albuterol inhaler, two puffs as needed (patient states he has been using it three to four times daily with no relief); Claritin 10 mg, once daily; and Flonase, two inhalations each nostril, as needed.

DC states that he lives alone. His dog sometimes sleeps in the same bed with him; he also has one cat. Carpeting is present throughout his house except for the kitchen and the bathrooms. With pharmacogenomic testing, DC is found to be homozygous for the SNP that results in the Gly16Arg amino acid sequence change in the β_2 receptor. As stated, his asthma pharmacotherapy consists of a short-acting β_2-agonist, albuterol, which is not providing relief, indicating uncontrolled asthma. Because of the patient's nonsynonymous (homozygotic) SNP resulting in the amino acid change at position 16, he is not responding to his albuterol. Based on the fact that he is frequently self-dosing to no avail, adding an alternative treatment may be of benefit. Two alternatives with different pharmacologic mechanisms to consider would be the addition of a low-dose inhaled corticosteroid or a mast cell stabilizer, such as cromolyn sodium. Additionally, DC should be counseled on the proper use of his medications as well as lifestyle modifications, including no longer allowing his dog to sleep in his bed. Additionally, DC should consider removing the carpeting from his house.
Note that in cases where the patient is homozygotic, switching to an alternative drug with a different mechanism of action may be warranted. In cases where the patient is heterozygotic, increasing the dose may provide a therapeutic response. If it does not, it would be prudent to switch the drug choice to a compound from an alternative pharmacologic class.

Chapter Summary

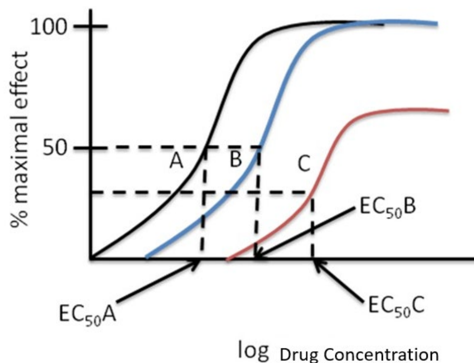
Pharmacodynamic variability is usually greater than pharmacokinetic variability. The variability results in different

responses among patients, related to efficacy and/or toxicity. Here, differences in drug receptors based on an individual's genetic constitution can have a significant influence on whether a patient will respond to a given drug therapy. This represents a genetic–dynamic interaction. In the case where genetic constitution results in a kinetic variance that alters the drug concentration, resulting in a varied clinical effect, we have a genetic–kinetic–dynamic interaction.

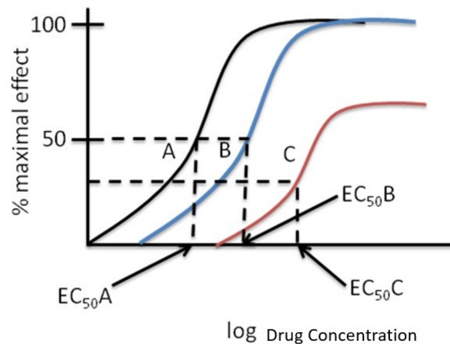
Review Questions

1. The study of the relationship between the plasma concentration of a drug and the observed pharmacologic effects is referred to as:
   a. pharmacokinetics.
   b. pharmacodynamics.
   c. pharmacogenetics.
   d. pharmacogenomics.

2. If curve A in the following figure represents the dose–response curve to an agonist, which curve would represent the addition of a noncompetitive antagonist?
   a. Curve A
   b. Curve B
   c. Curve C



3. If curve B in the following figure represents the dose–response curve of an agonist, which curve would represent the addition of a competitive antagonist in a patient who is homozygotic for a SNP resulting in a conformational change in the receptor so the antagonist cannot be overcome?
   a. Curve A
   b. Curve B
   c. Curve C



4. An increase in K_D:
   a. results in a lower affinity of a drug for a receptor.
   b. means that a patient could appear to be resistant to the effect of the drug.
   c. could result from a heterozygotic SNP coding for a receptor with decreased drug affinity.
   d. All of the above

5. A SNP that ultimately results in an increased EC_{50} produces:

   a. an overall increase in drug sensitivity.
   b. an overall decrease in drug sensitivity.
   c. no change in drug sensitivity.

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