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Basics of Pharmacogenomics for the Nuclear Pharmacist

Continuing Education for Nuclear Pharmacists
And
Nuclear Medicine Professionals

By

Todd A. Thompson, PhD
Assistant Professor of Pharmacogenomics
University of New Mexico College of Pharmacy
Research Incubator Building
And
Debra A. MacKenzie, PhD
Assistant Research Professor of Pharmacogenomics
University of New Mexico College of Pharmacy
Research Incubator Building

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Basics of Pharmacogenomics for the Nuclear Pharmacist

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Todd A. Thompson, Ph.D. and Debra A. MacKenzie, Ph.D.

BASICS OF PHARMACOGENOMICS FOR THE NUCLEAR PHARMACIST

STATEMENT OF LEARNING OBJECTIVES:

Upon completion of this course, participants will be able to:

1. Define pharmacogenomics.
2. Explain the difference between pharmacogenetics and pharmacogenomics.
3. Provide an explanation of how variability in response to drug action can occur within a population.
4. Describe how genetic differences can account for differences in drug response among individuals, just as genetics accounts for differences in appearance among individuals.
5. List the four base pairs found in DNA.
6. List five key features of a gene found in DNA.
7. Explain how genes may differ among individuals and how the DNA code can provide information that allows customizing drug administration on an individual basis.
8. Distinguish pharmacokinetic and pharmacodynamic parameters that are important to the pharmacogenomics of drug action and discuss how genetic variants can affect these parameters and alter drug treatment strategies.
9. List four distinct types of genetic variants that are known to occur in people.
10. Define the term single nucleotide polymorphism and discuss its relevance to genetic variation.
11. Describe how cancer pharmacogenomics is distinct from pharmacogenomic considerations of polymorphic variants.
12. Explain the difference between a mutation and a polymorphic variant.
13. Describe genetic syndromes and the associated genes that may be associated with sensitivity to radiation.
14. Explain the methods used to amplify DNA for genetic analysis and describe the key techniques currently used to identify genetic variants within DNA sequences.
15. Explain the importance of privacy for genetic information and discuss the components of GINA and laws that help reduce discrimination based on genetic information.

COURSE OUTLINE

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BASICS OF PHARMACOGENOMICS FOR THE NUCLEAR PHARMACIST

By

Todd A. Thompson, PhD and Debra A. MacKenzie, PhD
Assistant Professor(s) of Pharmacogenomics
University of New Mexico College of Pharmacy
Research Incubator Building

ABSTRACT

Pharmacogenomics is the study of drug action based on an individual person's genetics. Since the inception of pharmacogenetics in the 1940s and 50s, repeated examples of adverse drug reactions correlating with heredity have been reported. More recently, as a consequence of the revolution in the development of molecular biological techniques during the 1970s, 1980s, and 1990s, that enable direct associations between an individual's genetic makeup and the metabolism or activity of a drug, the field of pharmacogenetics has progressed to the study of pharmacogenomics, which integrates elements of pharmacology, genetics, and molecular biological technology. The Human Genome Project completed in 2003 provided the first complete sequence of human DNA, and is the most significant event in genomic innovations contributing to the field of pharmacogenomics. The ability to correlate an individual's specific DNA profile to responsiveness to a specific drug enables an "individualized" approach to pharmacy which will continue to have an impact on all areas of pharmacy practice including nuclear pharmacy. Thus, an understanding of pharmacogenomics among nuclear pharmacists will undoubtedly provide improvements in the field of nuclear pharmacy. Of special interest to nuclear pharmacists are familial genetic syndromes associated with sensitivity to radiation exposure. The purpose of this review is to provide nuclear pharmacists with an understanding of the field of pharmacogenomics as it stands today and an awareness of how it might affect pharmacy practice in the future.

Pharmacogenomics Background and History

The key mission of the field of pharmacogenomics is to enable the optimization of drug therapy based on a person's genetic make up in the hope of providing personalized medicine. Optimization of a drug therapeutic regimen involves insuring that a drug is administered in a manner that provides a favorable therapeutic response, which may require administration of higher or lower doses of drug than a

standard dose. In addition, pharmacogenomics endeavors to provide information to prevent toxicity resulting from drug administration. Pharmacogenomics emerged from the field of pharmacogenetics; i.e., the study of adverse drug reactions that are linked to heredity. Interest in optimizing therapeutic efficacy through genetic considerations originated from early clinical observations of adverse drug reactions with an underpinning biochemical relationship that was believed to be of genetic origin. These early studies of associations between adverse drug reactions and genetics spawned the field of pharmacogenetics. Although clinical reports of pharmacological observations associated with hereditary links were reported in the early 1900s, the initial recognition of genetic contributions to differences in drug action was more accurately noted in the 1950s (reviewed in Kalow, 2005) with the formal designation of pharmacogenetics occurring in the late 1950s (Vogel, 1959).

One of the first accounts associating genetic contributions to drug action was reported by Dr. Werner Kalow showing familial differences in pseudocholinesterase activity and deducing that differences in enzyme activity were genetically based (Kalow, 1956). At this point in our understanding of genetic considerations in drug therapy, developing personalized medicine was secondary to simply understanding variations in drug action. Many of the subsequent reports of variation in drug response were found to be related to differences in drug metabolism. This was in large part due to the ability to characterize drug metabolism using biochemical assays. This ability to measure drug metabolism biochemically initiated the field of pharmacokinetics related to pharmacogenomics and is currently a thriving area in pharmacogenomics. Throughout the 1950s, 1960s, and 1970s, reports on clinical observations of adverse drug reactions that were believed to have a genetic basis were described. In the mid- to late 1970s, the development of DNA sequencing provided the technology necessary to identify a more direct association of differences in DNA sequences that could be associated with genetic differences in specific genes. High-throughput DNA sequencing technology has progressed to the point that whole human genomes can now be sequenced. The completion of the Human Genome Project in 2003 has paved the pathway for individualized drug therapy. In the near future, the low cost of human genomic scans (e.g., genotyping) will give us a much more comprehensive understanding of the role of human genetics in differential drug responses, making the promise of pharmacogenomics and individualized medicine a reality.

As with all aspects of pharmaceutical science, variability in response to pharmaceuticals in nuclear pharmacy is poised to consider individual genetic variation for optimizing therapy. From the foundation of pharmacogenetics and pharmacogenomics, the field of nuclear pharmacogenomics is

emerging. Knowledge of basic pharmacogenomic principles will aid nuclear pharmacists in the development of the tools necessary to contribute to the growing understanding of the role of pharmacogenomics in nuclear pharmacy.

Variability of Drug Action in Populations

The driving force behind the field of pharmacogenomics in the development of individualized drug therapy stems from the observation that there is often significant heterogeneity in drug action among a population administered an equivalent dose of drug. This concept can be described as illustrated in *Figure 1*. The response to drug action in a population can broadly be divided into three categories which follow a typical distribution of quantitative traits; these include average responders, sensitive responders, and resistant responders. Most individuals are expected to demonstrate a favorable average response. However, a significant percentage of drug recipients may be resistant to the dose of drug they receive and thus do not benefit from the expected therapeutic outcome of a standard drug dose. For example, this may occur among individuals that rapidly metabolize and excrete the drug, thereby not allowing the levels of drug to reach a dose sufficient for therapeutic effect. Identification of individuals with genetic variants known to result in resistance would enable health care providers to increase the administered dose in order to reach therapeutic efficacy.

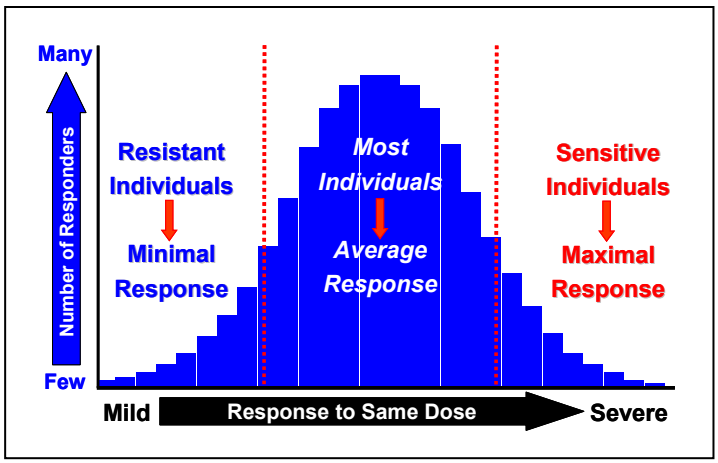


Figure 1. The variability in dose-response across a population, reflecting the distribution of quantitative traits. The number of individuals responding to the same dose of drug compared to the magnitude of response.

Other individuals might be highly sensitive to the actions of a drug resulting in toxicity at the dose known to be efficacious for the majority of recipients. This sensitivity may be due either to the lack of drug metabolism or due to genetic variability within the drug target itself resulting in increased responsiveness to the actions of the drug. Individuals identified with genetic variants that increase sensitivity could be given a dose more appropriate for their individual metabolic profile resulting in a decreased potential for toxicity.

The effects of the body on the drug action, such as the metabolism of the drug, represent a pharmacokinetic attribute of drug action. Differences in the interaction of the drug with the target protein due to alterations in target protein structure or to altered levels of target protein expression resulting in resistance or sensitivity to drug effects represent pharmacodynamic attributes of drug action. Examples of genetic variants that alter either the pharmacokinetics or pharmacodynamics of drug action have been reported. The key goal of pharmacogenomics is to identify and screen for genetic factors that will provide the information needed to optimize drug dosing and efficacy on an individual basis. A basic understanding of molecular genetics is necessary to achieve this goal.

DNA

Deoxyribonucleic acid, or DNA, is the cellular macromolecular storage form of genetic material among higher organisms. The function of DNA is to provide the information necessary for an organism to develop properly and for cells to function effectively when the organism is fully formed. At the molecular level, DNA is composed of a ribose sugar, a phosphate backbone, and four nucleic acids; the purines adenine (A) and guanine (G) and the pyrimidines cytosine (C) and thymine (T). The consecutive linking of these 4 nucleic acids in a strand of DNA is non-random. To facilitate the stabilization and increase the integrity of DNA, the nucleic acids in DNA pair with one another, where A pairs with T and G pairs with C. This pairing results in the formation of two anti-parallel and complementary strands. The linear order of the nucleic acids in a strand of DNA is the basis for the genetic code. DNA cellular organization and pairing of DNA bases in complementary strands is illustrated in *Figure 2*.

Cells and DNA

Apart from germ cells (i.e., ova and spermatozoa), all cells of the body possess the same DNA. Most cellular DNA is present in the nucleus; however, a small amount of DNA is contained within mitochondria. Because mitochondria are acquired from the female egg during fertilization, all mitochondrial DNA in an individual is of maternal origin (because of this, mitochondrial DNA is often used to trace maternal lineage). Nuclear DNA is organized into chromosomes. In humans, a maternal and paternal complement of homologous chromosomes is present in each cell. There are 46 chromosomes total in each non-germ cell (i.e., somatic cell). Twenty-three chromosomes are of maternal origin and twenty-three chromosomes are of paternal origin. Each set of twenty-three chromosomes represents a haploid compliment of DNA, with the combination from the mother and father representing a diploid compliment of DNA. The two sex chromosomes, the X and Y

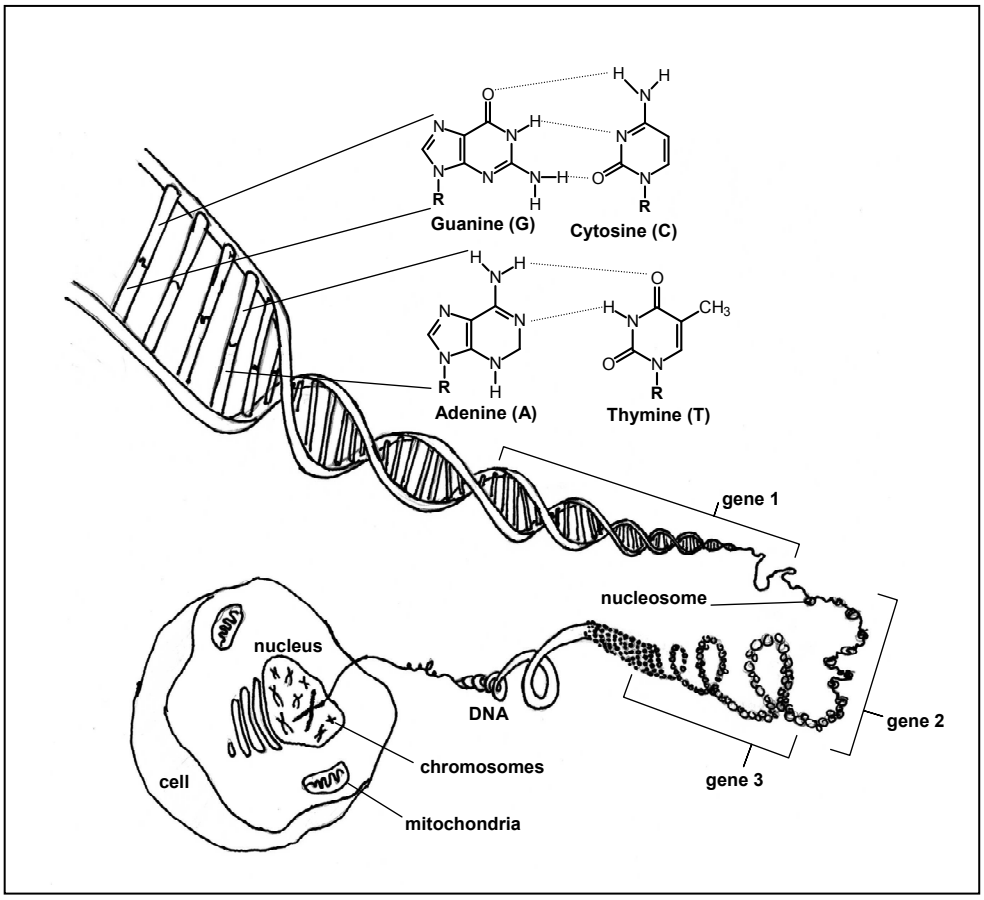


Figure 2. The organization of chromosomes, genes, and DNA in a cell. Somatic cells contain 46 chromosomes present in the nucleus. DNA stretched out from a chromosome illustrates the organization of DNA compaction, such as DNA in nucleosomes, which contributes to the ability of DNA to fit in the nucleus. Along the stretch of chromosomal DNA, specific genes are present (e.g., gene 1, gene 2, gene 3). DNA is present in complementary strands that perfectly hybridize with one another, where guanine bases (G) pair with cytosine bases (C) and adenine bases (A) pair with thymine bases (T). R represents the sugar-phosphate backbone of DNA.

chromosomes, determine gender. Two X chromosomes are present in females, whereas males possess a single X chromosome and a single Y chromosome (thus, all Y chromosome DNA is of paternal origin and can be used to trace paternal lineage). In addition to the sex chromosomes, there are 44 autosomal chromosomes in each somatic cell - half from the mother and half from the father. Chromosomes range in length from approximately 247 million base pairs in chromosome 1 to 50 million base pairs in chromosome 22, the smallest autosomal chromosome. In the nucleus, chromosomal DNA is condensed several thousand fold through a complex series of compactions facilitated by nuclear proteins, which enables these lengthy molecules to fit in the nucleus of the cell. The compacted nuclear DNA and proteins is referred to as chromatin. One of the first levels of compaction involves the wrapping of DNA around histone proteins, a structure referred to as a

nucleosome, which is also important in the functional operation of DNA. Understanding the organization of DNA and how regions of DNA lead to the production of proteins, which are among the most important targets for drug action, is necessary for a basic understanding of pharmacogenomics.

The Central Biological Dogma

Although virtually the same DNA is present in each cell of a person's body (with the exception of the gametes), DNA is a highly dynamic macromolecule. It is important to recognize that although all genes within a person's DNA are present in each cell, only a subset of genes are expressed within certain cells, resulting in the specific character associated with the unique types of cells in the body. Thus, one of the key roles of DNA is selective gene expression within distinct types of cells. Through the process of transcription, the synthesis of messenger RNA (mRNA), the first step toward protein production is achieved. Messenger RNA is processed in the cell nucleus and is translocated to the cytoplasm where it interacts with ribosomes on the rough endoplasmic reticulum. The code transferred from the nucleus by mRNA is translated by the ribosomes to introduce a sequential series of amino acids that make up the protein. Individual proteins are coded for by a triplet of three base pairs for different amino acids in the protein. This triplet code is very specific yet contains redundancy in that several series of three base pairs can code for the same amino acid. This process of DNA being transcribed to mRNA, which is in turn is translated to proteins, represents the Central Biological Dogma (*Figure 3*). Regions of particular importance along a stretch of chromosomal DNA that code RNA production are referred to as genes. Those genes that code for the amino acid sequence of proteins are referred to as structural genes. In this document, the term gene is used synonymous with structural gene.

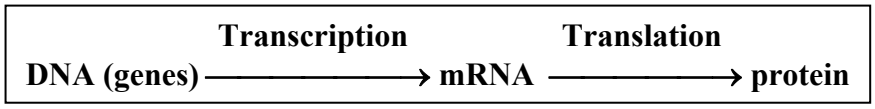


Figure 3. The Central Biological Dogma. Genes present in DNA are transcribed by a DNA-dependent RNA polymerase to messenger RNA (mRNA) in the nucleus, which translocates to the cytoplasm for protein production through translation.

Genes and Genomics

Structural genes are regions of DNA coding for the amino acid sequence of proteins. An understanding of genes and gene organization is critical to appreciate the value of pharmacogenomics in pharmacy practice. As illustrated by the Central Biological Dogma, structural genes are the fundamental code in DNA that is transcribed to mRNA and translated to the specific proteins expressed within a cell. In other words, genes are the key components of real estate in DNA that code for all the proteins that are the primary targets of drug action. A revelation resulting from the completion of the Human Genome

Project was the finding that there are fewer than 25,000 genes in human DNA coding for the exquisite complexity of the human organism. Thus, cellular identity is dictated by the subgroup of genes expressed in a specific cell, which is necessarily less than 25,000. All genes present in the DNA dictate an organism's genotype. However, it is the expression of specific proteins from the complete collection of genes that are responsible for the physical characteristics of a cell, constituting the cellular phenotype.

As described, genes are defined by those regions of DNA that code for the production of mRNA and ultimately proteins. However, gene structure itself varies a great deal and can stretch over thousands to many thousands of base pairs. Several hallmarks of gene structure are useful to know for understanding the impact of different types of genetic variants. The region in front of where transcription is initiated on the cell is the promoter region. The nucleotide where transcription starts in DNA is the transcriptional initiation site for the production of mRNA. Transcription continues over the DNA stretch of a gene until a DNA sequence, referred to as a polyadenylation signal, is recognized that terminates transcription. The nascent mRNA produced is spliced removing regions that are not incorporated in the final form of mRNA used to code for protein production. In comparing the mRNA to DNA, the regions in DNA that correspond to the spliced out regions of mRNA are called introns and the portions remaining in the mRNA correspond to regions of DNA are called exons. Thus, regions of DNA coding for proteins correspond to exons. Some genes exist that do not contain introns whereas other genes have been identified with over a 100 introns. Variants of genes can occur from differences in DNA present at any location along the gene.

Genes are arranged linearly along one of the 22 autosomal chromosomes, the X chromosome, or the Y chromosome (a few genes are also present in circular mitochondria DNA). In rare instances there is overlap between adjacent genes. However, most adjacent genes are separated from one another by thousands to tens or hundreds of thousands of base pairs. The orientation of genes on a chromosome is random. One can not predict the site where transcription is initiated on a gene from knowledge of the transcriptional initiation sites of adjacent genes. The regions of chromosomes associated with gene coding regions make up approximately 30% of the 3 billion base pairs of a haploid genome. Therefore, the majority of DNA present in a cell does not code for proteins. To date, the function of most of the non-coding regions of DNA is unknown. Thus, although non-coding regions of DNA (i.e., regions outside of identifiable genes) possess the majority of variants in DNA sequence between individuals, the impact of these variations on drug action is difficult to assess. The majority of genetic variants

having an impact on drug action are believed to be present in the regions of DNA that are known to code for proteins (i.e., genes). Because these genetic variants may lead to changes in the amino acid sequence of a protein and therefore, likely have an impact on the function of the protein, they are referred to as functional variants.

Polymorphic Variants of Genes and DNA

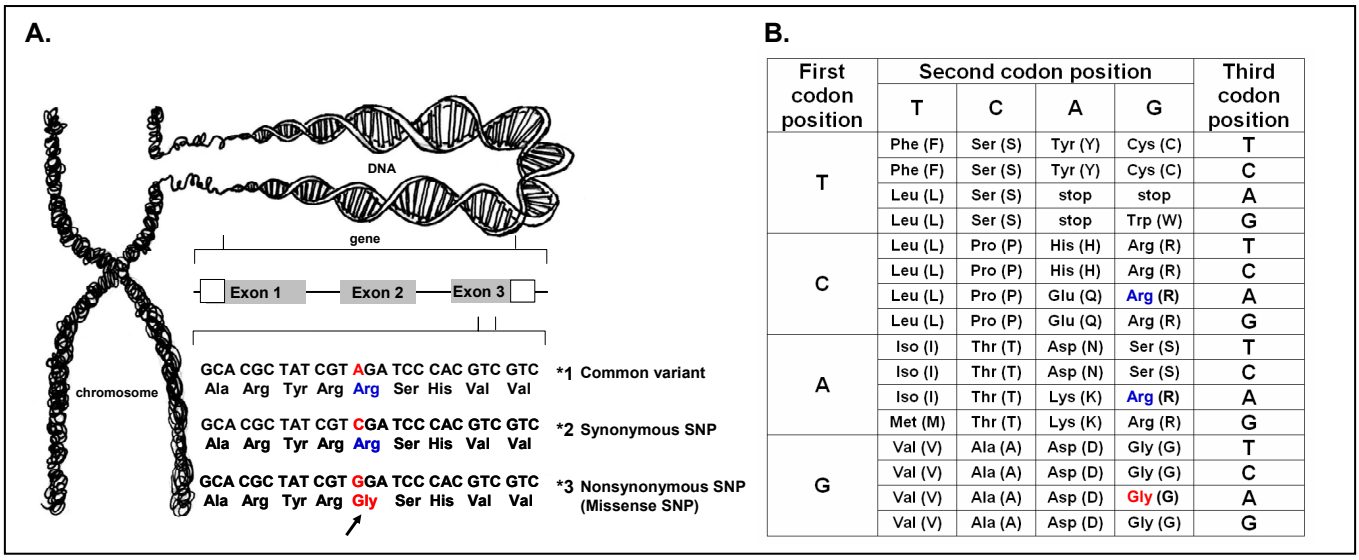
Any difference in the DNA sequence between two individuals that are present in a person's DNA at birth represents genetic polymorphic variants. The classical understanding of genetic variants was explored by the Austrian monk Gregor Mendel. Though the work of Mendel laid the foundation for developing the procedures to define genetic variants, an understanding of differences in genes at the DNA level has required the development of sophisticated molecular biological techniques, one of the most important of which is DNA sequencing, which identifies the specific sequence of base pairs within the DNA. In traditional genetics, variants are referred to as alleles, which historically were defined by phenotype (i.e., observable characteristics due to the expression of a gene). Today, polymorphic variants of genes can be defined by differences in DNA sequence, and thus are also defined by genotype.

Types of genetic variants that have a significant impact on pharmacogenomics and have been identified at the DNA level include: insertions or deletions of base pairs into the DNA (referred to as "indels"), duplications or amplifications of regions of DNA, and single nucleotide polymorphisms (alterations of single base pairs within the DNA). It is important to emphasize that these variants are present at birth (inborn) and are thus not considered mutations. Mutations are changes that occur within the DNA of specific cells over the lifetime of an organism (i.e., after embryonic development). These mutations would not be present in every cell of the body unless they all mutated in concert-an unlikely event. If a mutation occurs within a germ cell and is passed on to future generations, that DNA alteration becomes a polymorphic variant within that individual's genetic makeup which would then be passed on to their progeny. In the current literature, however, genetic variants are often referred to as mutations.

An example of mutations in cells that may develop in disease states include the genetic changes that occur in the development of cancer. In cancer, genetic modifications (i.e., mutations) occur in a select subpopulation of cells (i.e., the nascent cancer cell) that may facilitate the progression of the disease. In many instances, these genetic changes can be targeted therapeutically. Key examples include the

treatment of breast cancers over-expressing an amplified *Her-2/neu* gene (i.e., treated with trastuzumab; Pegram et al., 2000) and chronic myelogenous leukemia associated with translocations resulting in a novel fusion gene Bcr-Abl (i.e., treated with imatinib; Druker, 2002). However, most mutations found in the diseased cells, such as cancer cells, are not inherited as they would likely be detrimental to the development of the organism. Because drug therapy in cancer treatment may target proteins associated with gene mutations, genetic considerations in cancer pharmacogenomics is to a large degree distinct from pharmacogenomic considerations associated with inborn genetic variants.

The most frequent type of genetic variations occurring between individuals are single nucleotide polymorphisms. Single base pair differences in DNA between individuals are the basis of single nucleotide polymorphisms referred to as SNPs (pronounced "snips"). Simply stated, these are differences in a G, A, T, or C base pairs within an individual's DNA sequence. SNPs present in the coding region of DNA are divided into two subtypes; those that result in a change in the amino acids within the protein that is coded for (nonsynonymous or missense SNPs) and those that do not change the amino acid sequence of the protein (synonymous SNPs). A comparison of synonymous and nonsynonymous SNPs is illustrated in *Figure 4*. It is estimated that, on average, a variation in DNA between two individuals is present at approximately one in every thousand base pairs.



B.

First codon position	Second codon position				Third codon position
	T	C	A	G	
T	Phe (F)	Ser (S)	Tyr (Y)	Cys (C)	T
	Phe (F)	Ser (S)	Tyr (Y)	Cys (C)	C
	Leu (L)	Ser (S)	stop	stop	A
	Leu (L)	Ser (S)	stop	Trp (W)	G
C	Leu (L)	Pro (P)	His (H)	Arg (R)	T
	Leu (L)	Pro (P)	His (H)	Arg (R)	C
	Leu (L)	Pro (P)	Glu (Q)	Arg (R)	A
	Leu (L)	Pro (P)	Glu (Q)	Arg (R)	G
A	Iso (I)	Thr (T)	Asp (N)	Ser (S)	T
	Iso (I)	Thr (T)	Asp (N)	Ser (S)	C
	Iso (I)	Thr (T)	Lys (K)	Arg (R)	A
	Met (M)	Thr (T)	Lys (K)	Arg (R)	G
G	Val (V)	Ala (A)	Asp (D)	Gly (G)	T
	Val (V)	Ala (A)	Asp (D)	Gly (G)	C
	Val (V)	Ala (A)	Asp (D)	Gly (G)	A
	Val (V)	Ala (A)	Asp (D)	Gly (G)	G

Figure 4. SNPs present in a gene coding region. A. Graphical representation of a gene along a stretch of DNA from a chromosome with coding regions (contained in exons) separated by introns. A segment of exon 3 in the gene is shown coding for a protein containing the amino acid sequence AlaArgTyrArgArgSerHisValVal in the common variant *1 form. The *2 form contains a polymorphic variant that does not change the amino acid sequence (i.e., a synonymous SNP). The *3 has a variant that results in an amino acid change from Arg to Gly (., a nonsynonymous SNP). B. Codon chart set up for determining amino acids from a DNA sequence.

Nomenclature Associated with Polymorphic Variants

One of the most critical considerations in understanding pharmacogenomics is recognizing that the differences in response to drug action among individuals can be accounted for by genetic differences. As pointed out earlier, on average, it is estimated that there is a single base pair difference for every thousand base pairs in comparing the DNA between two individuals. Because there are six billion base pairs of DNA in the nucleus of somatic cells that largely comprise the cellular makeup of a person, there is on average a difference of six million base pairs between two individuals. At least some of these differences will result in alterations in proteins leading to variations in drug responses.

Unfortunately, a review of the literature on polymorphic variants can prove to be confusing when comparing sources of information related to variants of even the same gene. This is primarily because the nomenclature associated with the designation of polymorphic variants is not presently standardized. For example, a convenient means to refer to a single nucleotide polymorphism may include the particular base pair in question with the commonly recognized base pair preceding the base pair's location designation followed by the base pair variant that was identified. An example is the G2677T variant present in P-glycoprotein. P-glycoprotein (also referred to as the multidrug resistance protein 1, MDR1, or ABCB1) is a drug transporter protein, the over-expression of which may be associated with resistance to drugs such as paclitaxel (Kao et al., 2000). The variant designation of G2677T means that a G at position 2677 is present as a T in the variant. Confusion can be generated in this example in several ways. First, the numerical location of the base pair difference within a gene must specify how the base pair in question (i.e., position 2,677) is being located. This could be the location from a transcriptional initiation site (i.e., the beginning of the mRNA start site, which can vary slightly) or some other notable hallmark present in the gene, such as the translational initiation site. In reference to base pairs, the G most often designates a guanine base pair and the T a thymine. However, this nomenclature could also refer to amino acids. For example, the G could refer to a glycine and the T to a threonine amino acid, where the 2677 may refer to the 2,677th amino acid in the sequence of a polypeptide. Thus, in discerning the type of variant being referenced, it is often necessary to carefully evaluate the information source in order to determine precisely how the variant under investigation is being designated.

Early efforts to help clarify the designation of polymorphic variants led to the development of star terminology. An example of star terminology use is illustrated by the recent recognition by the FDA

that polymorphic variants of the cytochrome P450 2C9 gene (CYP2C9) can be used to optimize warfarin dosing. The form of CYP2C9 most commonly observed receives a *1 designation (CYP2C9*1). From *Table 1*, note that an individual homozygous (i.e., containing two of the same variant) for CYP2C9*1 has a mean clearance rate for warfarin of 0.065 mL/min/kg.

Table 1

RELATIONSHIP BETWEEN S-WARFARIN CLEARANCE AND CYP2C9 GENOTYPE IN CAUCASIAN PATIENTS		
CYP2C9 Genotype	**N**	**S-Warfarin Clearance/Lean Body Weight (mL/min/kg)Mean (SD)** [a]
*1/*1	118	0.065 (0.025) [b]
*1/*2 or *1/*3	59	0.041 (0.021) [b]
*2/*2, *2/*3 or *3/*3	11	0.020 (0.011) [b]
Total	**188**	

[a]SD=standard deviation.
[b]p<0.001. Pairwise comparisons indicated significant differences among all 3 genotypes.
Table 1 derived from Herman et al., 2005.

Other variants of CYP2C9 exist and known variants include CYP2C9*2 and CYP2C9*3, which are less active in metabolizing warfarin and thus result in lower clearance rates. Thus, individuals with the *2 or *3 variants of CYP2C9 may require a lower maintenance dose of warfarin than individuals homozygous for the CYP2C9*1 variant. Note that star terminology in no way provides knowledge relevant to the polymorphic variant under consideration. All that is established by star terminology is that variants exist. Thus, when star terminology is used, a key should be provided that describes the meaning of each variant being referenced. Unfortunately, keys are not always provided to define the star terminology in use. This leads to complications in deciphering the pharmacogenomics literature related to CYPs as there are 57 different cytochrome P450 genes (CYPs) in humans and variants for almost all CYPs have been identified and new variants are continually being discovered. This dilemma applies to the use of star terminology in general. Thus, without a standardized method for assigning star designations to each variant, the numerous known gene forms as well as newly discovered variants has become confusing. Although it is important to understand the use of star terminology in pharmacogenomics literature, it is likely that in the future other methods for designating gene variants will supplant its use.

A widely accepted method of designating single nucleotide polymorphisms is the reference SNP number or rs# method, which is facilitated by the National Center for Biotechnology Information (NCBI). In this case, when DNA variants are identified by a researcher, they are registered with the

NCBI. This results in the designation of an rs# for the polymorphic variant. Thus, each known polymorphic variant is provided a very specific designation that is characterized by the DNA sequence in the associated variant region. The use of rs# does not directly provide the precise genetic information associated with the polymorphic variant. However, it is straightforward to enter the rs# into the NCBI database, which in turn, provides extensive detailed information on the precise variant under consideration. Although the rs# system is rapidly being recognized as useful in organizing the many variants that are being discovered, it currently is backlogged for those variants that were identified before this system was implemented, resulting in well-established variants that are not currently identified by the rs# system. Interestingly, our understanding of DNA recombination in human populations has led to new methods to evaluate genetic variants. Thus, the routine use of rs#s in pharmacogenomic studies may also become supplanted in the ensuing years. Examples of gene polymorphic variants that are associated with modifications of drug response and nomenclature for their gene variant designations are shown in *Table 2*.

Table 2

EXAMPLES OF GENE POLYMORPHIC VARIANTS THAT MODIFY DRUG RESPONSES

Gene name	Gene symbol*	Drug examples affected by variants	Examples of gene variant designations
Thiopurine methyl transferase	TMPT	6-mercaptopurine	TMPT*1, *2, *3A, *3C
Vitamin K reductase C1	VKORC1	warfarin	VKORC1 SNPs at 381, 3673,5808, 6484, 7566, 9041
Cytochrome P450 2D6	CYP2D6	ondansetron	CYP2D6*1, *2, *3, *4, *5, *6, *7, *8, *10, *17,
Angiotensin-converting enzyme	ACE	ACE inhibitors	Insertion deletion (I/D)
ATP binding cassette protein B1 (P-glycoprotein)	ABCB1	paclitaxel	C1236T, G2677T, C3435T
Beta1 adrenergic receptor	BAR1	metoprolol	Ser49Gly, Arg389Gly
Cytochrome P450 2C9	CYP2C9	warfarin	CYP2C9*1, *2, *3
Dopamine 2 receptor	DRD2	clozapine	rs1125394
Glucose-6-phosphate dehydrogenase	G6PD	chloramphenicol	Over 300 variants
Leukotriene C4 synthase	LTC4S	aspirin	A-444C in promoter
UDP glucuronosyl transferase 1A1	UDPGT1A1	irinotecan	UDPGT1A1*28

* Gene symbol as used by the National Center for Biotechnology Information (NCBI).

Recent breakthroughs in our understanding of individual variants have progressed to the point that the analysis of single genetic variants, such as a single SNP, is not the most efficient means of distinguishing genetic differences between individuals. It has become clear that a group of SNPs that are in the same region of a chromosome may be inherited together (as a “block”) with enough

regularity to allow the analysis of only a designated set of SNPs in a region to define the sequence of the region that is inherited. Regions of DNA on a chromosome that are inherited without recombination and that can be defined by a group of distinct SNPs are known as haplotypes. Currently, nomenclature to distinguish haplotypes has not been established. However, in the future it is likely that analysis of haplotypes among individuals will be the primary means of indentifying genetic differences among individuals for pharmacogenomic purposes. In summary of nomenclature considerations for polymorphic variants, it is clear that care must be exercised when evaluating a polymorphic variant from several disparate sources. However, it should be recognized that the theoretical number of polymorphic variants is beyond comprehension (i.e., $4^{3billion}$). Thus, knowledge, patience, and perseverance are needed when examining the pharmacogenomic literature in an effort to understand optimized drug therapy based on genetic considerations.

Beyond genetic considerations of a single gene (i.e., monogenic), the variability in most drug responses is polygenic. That is, most variability in drug response arises not from variants of a single gene, but from the contributions of variants of many genes. For example, in optimizing warfarin therapy, we have discussed how different *CYP2C9* variants are considered in optimizing warfarin dosing. Recently, it was recognized that the target gene for warfarin activity, the vitamin K oxidoreductase C1 gene (i.e., *VKORC1*) has variants that should also be considered (Reider et al., 2005). Thus, as will be discussed in detail later, both *VKORC1* and *CYP2C9* variants should be considered for optimizing warfarin dosing.

Pharmacokinetic Considerations Important to Pharmacogenomics

The action of drugs as important considerations for pharmacogenomics can generally be divided into two broad classifications: Pharmacokinetics, or the action of the body on the drug (especially in relationship to the metabolic activity in the body that may alter drug action), and pharmacodynamics, or the action of the drug on the body (e.g., the action of a drug on a specific drug receptor). Pharmacodynamic considerations important to pharmacogenomics will be discussed in the following section. Pharmacokinetic considerations in pharmacogenomics were critical in the early observations of adverse drug reactions that led to an understanding of genetic contributions to drug activity. A key example is the finding that variants of CYP2D6 are important for the metabolism of antihypertensives such as debrisoquine. Numerous variants of CYP2D6 have been identified such that the metabolic profile for different CYP2D6 variants have been classified into groups of poor, intermediate, extensive, and ultra rapid metabolizers (Cascorbi, 2003). Pharmacokinetic considerations in drug action primarily

relate to metabolic actions that facilitate drug inactivation and excretion. However, drug metabolism may also have an important impact on drug activation (e.g., the metabolism of codeine to morphine).

Enzymes associated with the metabolism of drugs are commonly divided into categories of phase I and phase II enzymes. Phase I enzymes are usually associated with a modification of the drug structure that facilitates drug excretion. A key example of phase I metabolic activity is represented by the cytochrome P450s, which are monooxygenases that produce an oxidation of the drug structure. Other examples of phase I enzymes include reductases and hydrolases. Phase II enzymes are usually associated with the conjugation of a moiety to the drug, which usually further facilitates drug excretion. Examples of phase II enzymes include glutathione transferases, glucuronosyl transferases, sulfotransferases, acetylases, and methylases. For both phase I and phase II enzymes, each class of enzymes may has multiple members. As illustrated with CYP2D6, there are many variants of each drug metabolism enzyme such that the number of variants important to pharmacokinetics of pharmacogenomics is vast (e.g., *Table 2*).

Pharmacodynamic Considerations Important to Pharmacogenomics

Pharmacological considerations for most drugs address the action of a drug on the drug target. Many drug targets are proteins (i.e., gene products) with specialized cellular activity. The genes associated with the drug target may exist in variant forms, which may result in the production of different amino acid sequences in the gene product. Differences in amino acid sequence of these gene products may have pronounced functional consequences that impact the efficacy of drug action. Pharmacodynamic considerations in pharmacogenomics focus on the action of drugs on drug targets, which is usually relevant to specific disease states.

The scope of pharmacodynamics relevant to pharmacogenomics encompasses all drug targets for each drug. Therefore, a comprehensive coverage of this topic in the context of this monograph is impractical. Interestingly, even though there are many more drug targets than enzymes associated with drug metabolism, the field of pharmacogenetics related to pharmacokinetics (i.e., drug metabolism) is at present much farther along than the status of drug target pharmacogenomics. This is due in large part to the head start afforded by studies of drug metabolism using biochemical methods to analyze drug metabolism associated with adverse drug reactions. In contrast, the analyses of polymorphic variants of drug targets for the most part have progressed with the advent of DNA sequencing technology, which is a relatively modern breakthrough. A key example illustrating the immensity of investigating the

pharmacogenomics of drug targets includes G-protein coupled receptors (GPCRs), which as a gene family in humans contain over 700 members. As presented in *Table 2*, an example of polymorphic variants present in GPCRs is in the beta1 adrenergic receptor (*BAR1*) gene. For example, functional studies have shown that the Ser49Gly variant of *BAR1* is more sensitive to the inhibitory effect of metoprolol (Levin et al., 2002). As studies identifying variants in drug targets progress, there is a likelihood that pharmacodynamics related to pharmacogenomics will be a dominant contributor to personalized drug therapy.

Genetic Variants in DNA Replication and Repair Genes

Adverse drug reactions associated with the use of radiopharmaceuticals are rare. However, radiopharmaceuticals themselves represent a unique spectrum of pharmacogenomic considerations for the patient. This is in large part due to the necessary association of radiation exposure with radiopharmaceutical use. For most individuals, the impact of exposure levels of radiation can be estimated to help determine the potential for adverse reactions. However, in a number of rare genetic syndromes that affect DNA repair processes, further considerations of adverse events resulting from radiation exposure may be necessary. Examples of genetic variants in DNA repair pathways associated with cancer syndromes that may impact radiation exposure considerations are listed in *Table 3.*

Table 3

EXAMPLES OF CANCER SYNDROMES AND ASSOCIATED GENES		
Genetic Syndrome	**Gene Associated with Syndrome**	**Gene Product Activity**
Ataxia-Telangiectasia	ATM	Protein kinase – cell cycle control
Familial breast and ovarian cancer	BRCA1	Accessory factor for recombination
Bloom syndrome	BLM	Bloom syndrome helicase
Fanconi anemia	FANC(X)[1]	Involved in repair of DNA crosslinks
Hereditary nonpolyposis colon cancer type 2	MLH1	MutL homolog, forming heterodimers
Hereditary nonpolyposis colon cancer type 1	MSH2	Mismatch and loop recognition
Nijmegen breakage syndrome	NBN (NBS1)	DS break repair/ cell cycle control (?)
Rothmund-Thomson syndrome	RECQL4	DNA helicase
Multiple endocrine neoplasia type II A & B	RET (MEN2A/B)	Receptor tyrosine kinase
Li-Fraumeni syndrome	TP53 (p53)	Cell cycle regulation
Werner syndrome	WRN	Helicase/exonuclease
Xeroderma pigmentosum	XP(X)[2]	Binds damaged DNA

[1] The Fanconi anemia complementation group includes FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ, FANCL, FANCM, and FANCN.
[2] Forms of the *XP* gene include: *XPA, XPB, XPC, and XPD.*

Genetic syndromes with established relevance to radiation exposure include the genetic disease ataxia telangiectasia (AT). AT is a genetic disorder characterized by a high risk of cancer, immunodeficiency,

and progressive neurodegeneration (Lavin et al., 2007). AT patients are hypersensitive to radiotherapy, which has been recognized since the 1960s (Gotoff et al., 1967). AT is due to a variant of the ATM gene (ataxia-telangiectasia, mutated). The normal ATM gene product recognizes DNA damage and signals the damage to DNA repair machinery and cell cycle checkpoints with the consequence of minimizing DNA damage (Lavin et al., 2007). Radiation exposure to patients with AT has extremely high risks.

In addition to AT, other genetic syndromes have been documented that have increased radiation sensitivity, such as xeroderma pigmentosum (Arlett et al., 2006). However, the risk of radiation exposure for many genetic syndromes associated with DNA replication and repair genes is still unclear. For example, variants of the BRCA 1 gene product, which has a role in maintaining genomic stability, are associated with the development of familial breast and ovarian cancer. However, unlike patients with AT or xeroderma pigmentosum, patients with a non-functional *BRCA1* gene are not overtly recognizable. Radiation exposure concerns among patients with BRCA1 variants are under scrutiny (Pierce, 2002; Turnbull et al., 2006). Advancements in understanding the risks of radiation exposure among the variety of genetic syndromes associated with DNA replication and repair genes will be of great significance to nuclear pharmacy and are of critical importance to nuclear pharmacogenomics. Recent reviews on DNA repair pathways and hereditary cancer susceptibility syndromes that greatly expand on this topic are available (Spry et al., 2007). In addition, extensive databases of human DNA repair genes (Wood et al., 2005) are available to help keep abreast of those genetic syndromes that may be relevant to nuclear pharmacy.

Currently, changes in nuclear pharmacy practice can only be instituted for individuals with prior knowledge of existing genetic syndromes. The importance of developing screens for sensitivity to radiation exposure among patients with genetic syndromes has been recognized (Gatti, 2001). Interestingly, the importance of individualized tolerance limits for radiation therapy among patients has also been recognized (Peters, 1996), which directly reflects the interests of personalized medicine aligned with pharmacogenomics. In the future of nuclear pharmacy practice, it can be anticipated that genetic screens will identify individuals for whom extra precautions should be taken prior to exposure to radiopharmaceuticals. Screening for such genetic considerations is not necessarily straightforward. For any genetic syndrome, it is rare that a single polymorphic variant in a gene associated with the disease is responsible for the syndrome. Thus, to be most accurate, a screen must analyze all possible variants. A highly useful database for investigating most known variants associated genetic syndromes

is the Online Mendelian Inheritance in Man database (Hamosh et al., 2005), which is available through the NCBI website.

Methods Used for Pharmacogenomic Analyses

Although a detailed description of the many different methods used for pharmacogenomic studies is beyond the scope of this article, an overview of methods most critical to pharmacogenomic studies should prove helpful in evaluating the information provided in genetic analyses. An overview of three fundamental areas of molecular biological techniques is covered to facilitate an understanding of methods used for genetic analyses. These include DNA amplification methods, DNA sequencing methods, and methods based on DNA hybridization (i.e., microarray analysis).

DNA amplification methods – the Polymerase Chain Reaction

There are approximately six billion base pairs of DNA present in each cell of the body. Despite the seemingly large quantity of DNA per cell, in fact, each cell contains only two copies of each gene, one copy that is of maternal origin and one that is of paternal origin. Thus, if we are interested in analyzing a single gene for a genetic analysis, as is often the case, and if we consider that a gene itself represents a unique entity in a cell, then the analysis of this unique entity in the presence of all other cellular components is exceptionally difficult. Consider that exact copies of each gene are present in all somatic cells. The analysis of the DNA sequence of a single gene in a single cell is beyond the ability of current analytical methods. To analyze a gene effectively would require the presence of millions of copies of a specific gene. Thus, one method to examine a gene might be to isolate the DNA from millions of cells. However, even if sufficient DNA was removed from a large number of cells, the effort to identify the precise gene of interest in the presence of huge amounts of extraneous DNA that is co-isolated would be daunting. Thus, methods to specifically amplify a single region of DNA, such as a specific gene, have been developed that enable the precise analysis of a select region of DNA. One such method is the polymerase chain reaction (PCR).

PCR results in the generation of millions to billions of copies of DNA through amplification of a specific region of DNA that can span over ten thousand base pairs. This method is similar to the process by which DNA replication occurs *in vivo*, when new copies of DNA are synthesized from a "parent" strand by the enzyme DNA polymerase. Critical ingredients of a PCR reaction include the DNA of interest to be amplified (the "template"), DNA primers (short segments of DNA that are complementary to regions that flank the region of DNA to be amplified that are required for DNA

polymerase activity), a DNA polymerase that is stable at extreme temperatures (e.g., *Taq* polymerase, which is stable up to 100°C), and the deoxyribonucleotides that are incorporated in the growing DNA strand produced in the PCR reaction, as illustrated in *Figure 5*. Currently, specialized instruments called thermal cyclers are used to perform PCR reactions. All components are combined in a tube and placed in the thermal cycler. The temperature cycles from a "melting" temperature which separates (i.e., denatures) double-stranded DNA to an "annealing" temperature which allows for the primers to bind the template DNA, followed by an "elongation" temperature which allows for new DNA synthesis to occur using the single strands of DNA that "melted" apart as a template. This series of steps is repeated multiple times, which under optimal conditions, results in a doubling of the DNA of interest with each cycle (i.e., exponential DNA production). Thus, for a single gene, twenty cycles of the reaction theoretically can produce 2^{20} copies (i.e., over a million copies) of the DNA region of interest. The amplified DNA region can then be used or further genetic analysis, such as DNA sequencing or microarray analysis.

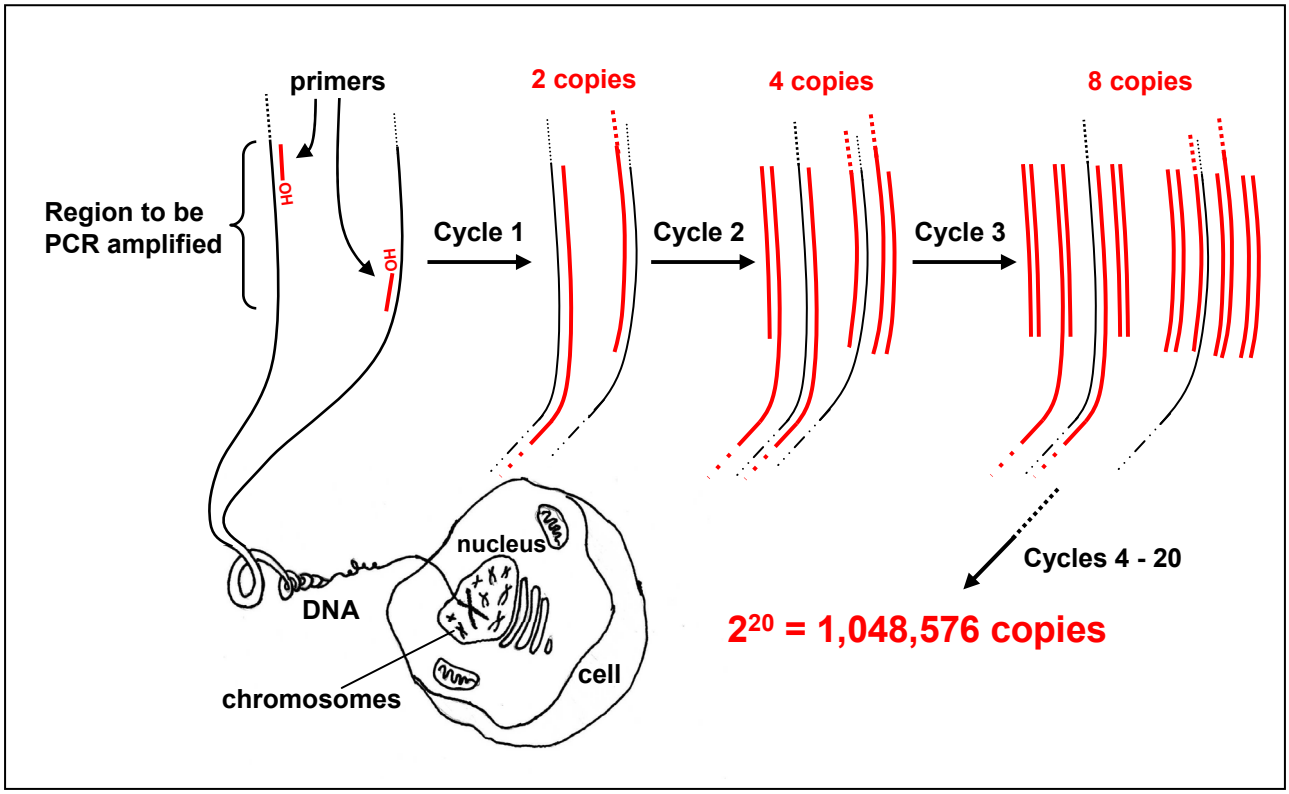


Figure 5. Illustration of the polymerase chain reaction used to amplify a segment of chromosomal DNA. First, DNA is denatured at high temperature (e.g. 95°C), then the temperature is decreased and primers homologous to DNA flanking the region to be amplified are annealed to the denatured DNA. In the elongation step, a polymerase (e.g., *Taq* polymerase) utilizes the 3′ OH of the primer to incorporate dNTPs creating a new DNA strand that is complementary to the region of interest. The newly formed double stranded DNA undergoes another round of denaturation, annealing and elongation. These steps are repeated in successive cycles. With each n cycle, 2^n segments of DNA are theoretically produced. The red strands represent the primers and PCR amplified DNA.

DNA sequencing methods

Pharmacogenetic studies have gained a new level of complexity with the onset of techniques such as DNA sequencing, which have contributed significantly to bringing about the era of genomics and hence the establishment of pharmacogenomics from pharmacogenetics. Because DNA sequence information is most useful when spanning relatively long regions, initial DNA sequencing methods were developed to provide as much DNA sequence information as possible while also providing accuracy in sequence determination. One of the first methods to achieve this goal is referred to as chain-termination (Sanger et al., 1977) or Sanger sequencing, named after Fredrick Sanger, who developed the method in the late 1970s for which he received the Nobel Prize in 1980. Although the details of Sanger sequencing are beyond the scope of this monograph, suffice it to say that improvements in this technology over the last two decades are largely responsible for the completion of the Human Genome Project. In fact, most DNA sequencing currently performed by commercial facilities utilizes variations of Sanger sequencing.

Fundamentally, genetic polymorphic variants are defined by the comparison of two seemingly homologous regions of DNA from different sources with the recognition that upon comparing DNA sequence data, the two sequences are in fact distinct. This exercise requires that the precise sequence of DNA of each region is determined. The series of steps for most DNA sequencing methods involve: 1. Isolation of cells providing a source of DNA, such as from a buccal swab, saliva, or white blood cells; 2. Purification of DNA from the DNA source; 3. Amplification of the region of DNA to be sequenced, which can effectively be performed by PCR; 4. Performance of the chain-termination chemistry using the amplified DNA region as a template, and finally; 5. Analysis of the DNA sequence using a DNA sequence analyzer. The output of DNA sequence information from a single chain-termination reaction usually ranges from 500 to 900 base pairs of consecutive G's, A's, T's, or C's. Programs are available that can take the sequence information from two individuals and determine precisely which base pairs, or even stretches of DNA sequence, differ between individuals. This information is used to precisely identify polymorphic variants that exist between individuals or even large groups of individuals.

Currently, high-throughput methods for DNA sequencing using techniques distinct from Sanger sequencing are under development. For example, 454 sequencing, developed by 454 Life Sciences (acquired by Roche Diagnostics) is a massively-parallel-sequencing system that allows far more rapid DNA sequencing over large stretches of DNA with higher efficiency and accuracy than the Sanger sequencing methods (Margulies et al., 2005; Rogers and Venter, 2005). In addition, numerous other

DNA sequencing systems are under development that will ultimately lead to the availability of high-throughput DNA sequencing in the clinical setting as a routine procedure. It is hopeful that these methods will result in the advancements necessary to move DNA sequencing technology forward for use in personalized medicine. However, before high-throughput DNA sequencing becomes a reality, the use of microarray-based technologies are sufficiently robust to provide a reasonably comprehensive DNA analysis for clinical purposes.

Microarray or chip analysis

Precise DNA sequence information is the most accurate method for assessing polymorphic variants, thus ideally, complete DNA sequence information would be utilized to analyze DNA for pharmacogenomic studies. However, as mentioned previously, the time and cost of most DNA sequencing methods is currently prohibitive for routine clinical analysis. In a clinical setting, routine DNA analysis can be performed using microarray or chip-based technologies. DNA microarray technology relies on the hybridization of complementary pieces of DNA present in relatively small stretches of DNA (i.e., approximately 25 base pairs = oligonucleotide). Over this stretch of DNA sequence, hybridization can be optimized so that only regions of DNA that match perfectly with one another will hybridize. It is this precise hybridization of DNA present as part of the microarray system with DNA from a patient sample that forms of the basis of microarray analysis. Many different types of microarray systems are available for nucleotide analysis. For the purposes of this monograph, the discussion is restricted to microarray technology relevant to DNA sample analysis of polymorphic variants.

Chip-based technology for DNA analysis refers to the surface upon which the procedure occurs, which can be similar to the silicon chips used in the computer industry. Microarray refers to the miniature array of regions on a chip where the hybridization occurs. Each tiny region on a chip that is a part of the array is referred to as a feature. The key component of each feature is usually single-stranded pieces of DNA with a known sequence, called an oligonucleotide, of approximately 25 base pairs, which is bonded to the chip surface at a specific and identifiable location. Thus, the precise sequence of the DNA oligonucleotide for a feature is known relative to its location on the chip. Numerous features, each containing a different DNA oligonucleotide sequence are bound to the chip next to one another, ultimately forming an array. Tens to hundreds of thousands of such features, each containing a known DNA sequence with a corresponding location on such a chip, the total size of which may be no larger than the surface of a nickel. The chip microarray is usually housed in a cassette that protects the

surface of the chip and provides a convenient receptacle to perform the sample hybridization steps. In addition to chip-based microarrays, bead-based microarray systems have also been developed that allow high-density sequencing, genotyping and gene expression analyses.

Unfortunately, sample DNA from a patient can not simply be placed on the microarray for analysis. The patient sample DNA must be prepared in a way that the precise hybridization on the features of the microarray can be detected. This usually involves PCR amplification of a DNA sample along with labeling of the amplified DNA segments in a way that allows the determination of a successful hybridization of the sample DNA to the microarray feature. Fluorescent chemicals are used to label the sample DNA in a manner that does not affect its ability to hybridize. Thus, precise hybridization of the sample DNA to the known DNA sequence present on the microarray feature is the key to a successful microarray analysis. After the sample has gone through a series of hybridization steps on the microarray surface, a specialized microarray analysis machine scans the microarray to determine which features have become fluorescent. Fluorescence of a specific feature indicates that DNA is present in the sample that is precisely complementary to the oligonucleotide present in the known microarray feature.

A strength and a weakness of microarray analysis is that the precise DNA sequence present at each feature is fixed. The strength is that this allows for the very rapid analysis of the DNA sequence information of interest. For example, different features on a microarray may correspond to different polymorphic variants of a gene. Thus, the analysis of a specific polymorphic variant can be very rapid and because tens to hundreds of thousands of features can be present on a single microarray, thus, a relatively thorough analysis of variants in a patient sample can be performed on a single microarray. A weakness of microarray analysis is that in order for a variant to be analyzed, the exact variant must be represented as a feature on the microarray. Because it is unlikely that all possible polymorphic variants will be identified, a comprehensive variant analysis is not realistically feasible using microarray technology. In other words, microarrays can only provide an analysis of known variants that are represented as features on the chip. Any unknown variants will not be identified. In contrast, DNA sequencing provides complete information regarding an individual's polymorphisms. Also, because most commercially useful microarrays are mass produced and proprietary, modifications of microarrays with newly discovered variants are not readily available. Despite these shortcomings, microarrays, microarray scanners, and the reagents necessary to utilize microarrays in the clinical setting are now available in a variety of platforms and have proven useful for relatively sophisticated

analysis of polymorphic variants. Thus, the use of microarrays for polymorphic variant analysis is having an enormous impact in advancing pharmacogenomic studies toward providing more personalized drug administration.

Identifying genes associated with variations in drug response

It is important to ask, "How can we identify gene variants that are responsible for differences in response to drug action?" The ability to address this question has become feasible due to the technological advances in genotyping, such as microarray analysis, that allow the performance of sophisticated studies of the genome. These efforts have evolved over the last twenty-five years from the relatively crude linkage analysis initially used to identify genes associated with genetic diseases. Optimally, linkage-based methods required that the condition under investigation showed a Mendelian inheritance pattern and that large amounts of pedigree information were available from families extending over many generations. These studies were relatively crude by modern standards because the ability to determine the specific region of DNA associated with the genetic disease under investigation had low resolution in considering the 6 billion basepairs of DNA and the limited number of genetic markers (e.g., tandem repeats) available for analysis. Despite what seemed to be an insurmountable task, these strategies facilitated notable discoveries of genes associated with genetic diseases, such as the cystic fibrosis gene (Kerem et al., 1989). At least two factors were prohibitive in utilizing such techniques for identifying genes associated with variations in drug response. First, the lack of pedigree data from large families showing differences in drug responses and, second, these studies were largely limited to phenotypes that resulted from alterations in a single gene. Both pharmacokinetic and pharmacodynamic considerations may contribute to differences in drug response among individuals resulting from the action of multiple gene products. Thus, due to the complexity of biological responses governing drug action, only a limited number of successes in identifying single genes associated with variable drug response have been achieved using a "candidate gene approach".

Technological advances have led to the development of high-resolution genotyping, which overcomes many of the hurdles previously associated with crude linkage analysis, allowing the identification of gene(s) associated with differences in drug action among individuals from varied populations. As pointed out previously, an important example of multiple gene products contributing to drug action is demonstrated by the identification of genetic variants responsible for the action of warfarin. The utility of genome-wide association studies (GWAS) is illustrated in research performed by Mark Rieder and

his colleagues (Cooper et al., 2008) showing that variants in the CYP2C9 and VKORC1 genes are vital in modulating warfarin's anticoagulant activity. A retrospective genome-wide association study was performed analyzing over 550,000 different SNPs using a microarray-based technology. The populations analyzed included an index warfarin population with DNA from 181 individuals and two independent replication patient populations with DNA from 374 individuals. The most significant independent effects from the index population were associated with CYP2C9*2 (rs# 4917639), CYP2C9*3 (rs# 1057910), and polymorphisms present in VKORC1 (e.g., rs# 9923231 and rs# 10871454), confirming that variants of these genes are important contributors to observed differences in warfarin activity among patients (Cooper et al., 2008). In the near future, studies similar to those performed to identify genes important in warfarin dosing will be expanded to identify genes important in dosing of many different drugs, especially those associated with serious toxicities or having a narrow therapeutic range.

Ethical, Legal, and Social Considerations of Pharmacogenomics

Genetic information is among the most important and most private information that a person possesses. Ultimately, genetic information will be defined to the point that it will allow a rough estimation of the physical and personality features of an individual. Importantly, this information will allow health care professionals to determine which disease predisposition an individual harbors. For health care purposes, genetic tests will be extremely useful in providing information that will allow a person to take preventive measures against disease development. However, exceptional care must be taken to insure that this information cannot be used to discriminate against certain individuals. Of particular concern is the potential for health insurance companies to deny coverage due to knowledge of a genetic predisposition to disease based on genetic information. This concern extends to the selective choice of employers for employees based on genetic information. A comprehensive exposition on concerns relevant to the social, legal, and ethical concerns of pharmacogenomics has been chronicled (Rothstein, 2003). More recently, legislation was passed in the United States prohibiting genetic discrimination by employers and insurers that will have a profound impact on the public's concerns in allowing the field of pharmacogenomics to move forward.

On May 21st, 2008, the Genetic Information Non-discrimination Act (GINA) was signed into law (Hudson et al., 2008). For this law, "Genetic Information" includes information about a person's genetic tests (i.e., genotype analysis, variant analysis, or chromosomal analysis), genetic tests of a person's family members, and participation of a person or family member in research that includes

genetic testing, counseling, or education. GINA prohibits group and individual health insurers from using a person's genetic information in determining eligibility or premiums. In addition, GINA prohibits an insurer from requesting or requiring that a person undergo a genetic test. With regard to employers, GINA prohibits using a person's genetic information to make employment decisions such as hiring, firing, or job assignments and GINA prohibits an employer from requesting, requiring, or purchasing genetic information about persons or their family members. GINA is enforced by the Department of Health and Human Services, the Department of Labor, the Department of Treasury, along with the Equal Opportunity Employment Commission. GINA is a necessary law for the promise of pharmacogenomics to be realized, helping to insure that all individuals can feel secure that their personal genetic information will not be used to discriminate against them.

SUMMARY

Providing the most effective use of pharmaceutical agents is a goal for all pharmacists. Achieving this goal requires many considerations. Increasingly, the genetic make up of each person is being factored into providing the most effective use of pharmaceutical agents. Pharmacogenomic considerations in nuclear pharmacy will have a specialized niche in helping to achieve the grand goal of personalized health care.

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ACRONYMS AND ABBREVIATIONS

A	adenine
bp	base pair
C	cytosine
CYP	cytochrome P450
DNA	deoxyribonucleic acid
G	guanine
GINA	Genetic Information Non-discrimination Act
GPCR	G-protein coupled receptor
GWAS	Genome-wide association studies
Indels	insertions/deletions
mRNA	messenger RNA
NCBI	National Center for Biotechnology Information
PMV	polymorphic variant
RNA	ribonucleic acid
SNP	single nucleotide polymorphism
T	thymine
VKORC1	vitamin K oxido-reductase complex 1

ASSESSMENT QUESTIONS

1. Pharmacogenomics is:

    a. The study of drug action based on an individual person's genetics
    b. The area of medicine with the goal to optimize drug therapy based on a person's genetic make up
    c. The hope of providing personalized drug therapy
    d. The field of genetics that investigates genetic differences among individuals which may be associated with differences in drug action in populations, including adverse drug effects
    e. All of the above.

2. The critical distinguishing feature between pharmacogenetics and pharmacogenomics is that pharmacogenomics integrates:

    a. An understanding of how DNA interacts with nuclear proteins
    b. Epigenetics
    c. Breakthroughs in molecular biological technologies
    d. Polymerase chain reaction
    e. A basic understanding of Mendelian genetics

3. A goal of pharmacogenomics is to:

    a. Sequence the DNA of every man, women, and child
    b. Prevent the spread of disease through knowledge of pathogen genetics
    c. Optimize drug therapy through knowledge of individual patient genetics
    d. Improve health insurance policies by instituting genetic screens
    e. Increase efficacy of drug therapy through epigenetics

4. Which statement is TRUE regarding the expression of quantitative traits among a population that may affect drug response?

    a. Genetic considerations are secondary to environmental factors in dictating drug response within populations
    b. Distribution of drug response in a population always follows a perfect Gaussian distribution
    c. Genetic contributions to drug response are monogenic and therefore highly predictable
    d. Genetics does not contribute to population differences in drug response
    e. None of the above

5. Which of the following is an example of differences in genotype?

    a. Brown compared to blue eye color
    b. Different alleles of the gene responsible for eye color
    c. Blond compared to brown hair color
    d. Straight blond hair compared to curly brown hair
    e. Ruffled compared to smooth peas

6. The nucleic acid components of DNA include a ribose sugar and phosphate backbone as well as which four bases?

    a. adenine, cytosine, guanine, and thymine
    b. alanine, cysteine, glycine, and threonine
    c. adenine, cysteine, guanine, and uracil
    d. arginine, cytosine, guanosyl, uracil
    e. adenylic acid, cystenoic acid, glycinic acid, and tymiric acid

7. Which statement most accurately describes the human chromosomes present in the somatic cells of the body?

    a. All DNA in a cell is present in the cell nucleus
    b. Gametes contain only autosomal chromosomes
    c. Chromosomes are different in each cell of the body, which is why each cell is unique
    d. Somatic cells contain 44 autosomal chromosomes and 2 sex chromosomes, which are contributed by both the mother and the father
    e. All of the above

8. The Central Biological Dogma can be explained as:

    a. DNA is transcribed to proteins
    b. DNA contains genes which are transcribed to messenger RNA which is in turn translated to proteins
    c. Proteins are degraded to nucleic acids which are the fundamental building blocks of DNA
    d. DNA is made up of sugars, phosphates, and bases
    e. Proteins come from jeans

9. There are approximately how many genes in the human genome?

    a. 250
    b. 2,500
    c. 25,000
    d. 250,000
    e. 2,500,000

10. The following components are found in genes, EXCEPT:

    a. Coding regions for proteins
    b. Amino acid codons
    c. Exons
    d. Introns
    e. Transcriptional initiation sites

11. Which of the following best describes a mutation?

    a. Mutations are changes in DNA basepair sequence that occur before embryonic development.
    b. Mutations are changes in DNA basepair sequence that occur during embryonic development.
    c. Mutations are changes in DNA basepair sequence that occur after embryonic development.
    d. Mutations are changes in DNA basepair sequence that are equivalent to polymorphic variants.
    e. Mutations do not affect the DNA basepair sequence.

12. In the vernacular of pharmacogenomics, SNP is most appropriately an abbreviation for:

    a. A single gene mutation
    b. Sanguine normal paternity
    c. Simple natured people
    d. Synonymous neutral particle
    e. Single nucleotide polymorphism

13. Which definition most accurately describes a synonymous SNP?

    a. A basepair change in a codon wobble site that does not result in an amino acid change
    b. A basepair change present in an intron region that does not result in an amino acid change
    c. A basepair change in a coding region that would result in an amino acid change
    d. A basepair change outside of the coding region that may affect gene expression
    e. A basepair change in the polyadenylation sequence of a gene that would disrupt transcription

14. There are on average how many variants in DNA between two individuals?

    a. 1 in a 10,000
    b. 1 percent
    c. 1 in a 1,000
    d. 10 percent
    e. 1 in a 100,000

15. All of the following are examples of genetic differences in DNA between individuals that qualify as examples of polymorphic variants, EXCEPT?

    a. Introns
    b. Single nucleotide polymorphisms
    c. DNA duplications
    d. DNA deletions
    e. Nonsynonymous SNPs

16. Which of the following statements is accurate concerning star terminology used to designate genetic variants?

    a. The star one variant (*1) is always used to reference the wild-type gene variant
    b. Star variants may designate any type of variant, even duplications of a gene
    c. A star one variant is the most frequently found variant, a star two variant is the second most common variant, and so on
    d. Star terminology is obsolete and no longer used to designate genetic variants
    e. Star terminology is most commonly used to designate SNPs of metabolic enzyme genes

17. Regions of chromosomal DNA in linkage disequilibrium that can be defined by a group of SNPs known as:

    a. Equilotypes
    b. Complotypes
    c. Simplotypes
    d. Haplotypes
    e. Silent types

18. Which of the following are examples of genes that represent the involvement of pharmacokinetics related to pharmacogenomics?

    a. Polymorphic variants of a gene producing a drug target
    b. Genetic syndromes associated with premature aging
    c. Genetic variants of genes associated with insulin signaling
    d. Polymorphic variants of genes associated with the effect of the body on drug action (e.g., genes for drug metabolism enzymes)
    e. Genetic variants of genes involved in neurotransmitter signaling

19. Which of the following genes is an example of a gene that would represent the involvement of pharmacodynamics related to pharmacogenomics?

    a. Beta1 adrenergic receptor
    b. Cytochrome P450 2C9
    c. Glutathione transferase pi
    d. Glucuronosyl transferase
    e. Cytochrome P450 3A5

20. Which of the following set of gene polymorphic variants may be of particular importance to nuclear pharmacogenomics?

    a. Genes associated with DNA recombination and repair
    b. Genes involved in drug metabolism
    c. Genes important in pulmonary function
    d. Genes regulating sodium and potassium homeostasis
    e. Genes functioning in neuronal regulation

21. A genetic syndrome associated with increased sensitivity to ionizing radiation is:

    a. Amyotrophic lateral sclerosis
    b. Multiple sclerosis
    c. Ataxia telangiectasia
    d. Gilbert's syndrome
    e. Sickle cell anemia

22. Cancer pharmacogenomics, in addition to optimizing drug therapy due to genetic variants that are important to drug response, may also consider:

    a. The genes expressed in the cancer's tissue of origin
    b. SNPs present in oncogenes
    c. The activation of tumor suppressor genes
    d. Mutations present in the cancer cells that are involved in the progression of the cancer (e.g., amplification of Her2/neu)
    e. The balance of oncogenes and tumor suppressor genes in the cancer

23. Which of the following is a revolutionary molecular biology method used to amplify segments of DNA?

    a. SNiP CHIPs
    b. DNA microarrays
    c. Sanger sequencing
    d. Chain-termination sequencing
    e. Polymerase chain reaction

24. Microarray technology is dependent of which biochemical feature of nucleic acids?

    a. Advancements in the computer industry and silicon technology
    b. The ability of DNA to code for genes
    c. Nucleic acid hybridization between complementary strands
    d. Measurements of messenger RNA expression
    e. Fluorescence of DNA basepairs

25. The Genetic Information Nondiscrimination Act is aimed at reducing discrimination from:

    a. Local, state, and federal governmental agencies.
    b. Pharmaceutical companies
    c. Insurance companies and employers
    d. Physicians, hospitals, and other health care facilities
    e. Memberships to clubs