Pharmacogenomics

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Abstract

This chapter discusses the essential terms and concepts of drug–gene interactions or how genetics affect pharmacology. This subset of pharmacology is also known as pharmacogenomics.

Since the inception of pharmacology, it has been acknowledged that different patients respond differently to the same medication. While many non-genetic factors (i.e., age, weight, organ function, concomitant therapy, etc.) can influence how a patient reacts to medication, the existence of large population variability is consistent with genetic factors as the major determinant of drug response. In fact, it is estimated that genetics are responsible for 20% to 95% of the variability observed in a drug's pharmacokinetics and pharmacodynamics.[1] Consideration of this inter-patient variability in drug response is critical in the clinical setting, as pharmaceuticals are one of the most common causes of adverse events, resulting in morbidity, mortality, and increased cost of treatment. In 1994, it was estimated that even when drugs were appropriately administered, over 2.2 million hospitalized patients suffered severe adverse drug reactions (ADRs), and over 100,000 had fatal ADRs, making ADRs between the fourth and sixth leading cause of death in the United States.[2]

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Recognition that specific genetic variants were responsible for differences in drug effects began as early as the 1950s, giving rise to the field of pharmacogenetics.[3,4] However, today the field has evolved into a genome-wide approach that explores inherited variations in genes that dictate drug response and investigates the ways these variations can be used to predict patient's drug response. This genome-wide approach is what is now referred to as pharmacogenomics (although the two terms are currently used synonymously). The development of this field has challenged the previous notion of one drug and dose fits all, with the promise of a more individualized approach to drug therapy. The essential clinical benefit of pharmacogenomics is the ability to predict a patient's drug response prior to administration, thereby preventing possible ADRs, improving drug therapy and improving overall therapeutic outcome.

How Genetics Affect Pharmacology

To understand the utility of pharmacogenomics, it is important to understand how genetics can affect the therapeutic action of a drug. A drug's pharmacokinetic action is dependent on the absorption, distribution, metabolism, and elimination (ADME) of the compound. Proteins such as membrane transporters and metabolizing enzymes control the ADME of every drug, determining the concentrations of drug at the site of action at different points in time. Once at the target, the interaction of the drug with the receptor results in the pharmacodynamic action of the compound. This combination of pharmacokinetics and pharmacodynamics controls the therapeutic outcome for all drugs, and the proteins that ultimately determine these factors are regulated by a patient's genome.

Every human is born with a unique DNA sequence inherited from our parents, which encodes that person's genome. This sequence is comprised of the four nucleotides adenosine (A), thymine (T), guanine (G), and cytosine (C), which carry the messages to construct specific proteins in the following process. Select portions of a DNA sequence, that encode information for specific genes, are transcribed into pre-messenger RNA (pre-mRNA). The pre-mRNA is further processed by splicing-out non-coding regions of RNA (introns), leaving only the coding regions of RNA (exons), which are spliced together to form mature mRNA. The structures of proteins are encoded in mRNA by three nucleotide units (codons), which correlate to specific amino acids. Specialized protein aggregations, called ribosomes, translate these codons to form chains of amino acids that ultimately form proteins. This highly organized and controlled process results in the production of every protein found in an individual's body.

With the completion of the Human Genome Project, it was discovered that despite the human genome containing roughly three million base pairs, only 1.1% of the DNA was comprised of exons, while introns accounted for 24% and inter-genic

DNA spanned the remaining 75% of the genome.[5] Furthermore, despite the size of the genome, interpersonal variation in DNA sequences are relatively rare, with only one nucleotide differing for every 1000 to 2000 bases.[6] When these variations occur in more than 1% of the population, they are classified as "polymorphisms," while less common variations are classified as "mutations" or "rare inborn errors." The most common variations result from single nucleotide polymorphisms (SNPs, pronounced "snips"), which are single-base variants in a DNA sequence. Over 1.4 million SNPs have been identified in the human genome. The vast majority of these SNPs are found in the non-coding and intronic regions of DNA and they usually have no functional consequences; they are therefore known as "silent" SNPs. However, non-coding segments of DNA can contain promoter regions that control the expression of various genes. SNPs found within these promoter regions can result in increased or decreased protein expression. Similarly, SNPs found within intronic DNA can produce changes in the splicing of pre-mRNA, resulting in truncated, nonfunctioning proteins. Relatively fewer SNPs (approximately 60,000) are found within the coding regions of DNA. SNPs within an exon result in an altered codon, which encodes for a specific amino acid. In some cases these altered codons will still encode for the proper amino acid, known a "synonymous substitution," resulting in a normal protein. Conversely, "non-synonymous" SNPs results in codons that change an amino acid; with the outcome of this substitution being a change in protein structure or function.

Despite SNPs being a relatively small change, they have been associated with clinically relevant changes in the pharmacological action of various drugs. Consequences of SNPs have been linked to 1) decreased clearance of drugs, leading to "functional overdose"; 2) rapid clearance of drugs, resulting in loss of efficacy; 3) failure to convert prodrugs to active compounds; 4) altered pharmacodynamics; and 5) idiosyncratic toxicities. In this chapter, we focus on therapeutic consequences of various SNPs. This chapter is not meant to be a comprehensive review, but rather to provide relevant examples to illustrate the utility of pharmacogenomics as a molecular diagnostic method to improve drug therapy.

Metabolism

One of the first examples of the utility of pharmacogenetics involves a protein well known to anesthesiologists, butyrylcholinesterase (BCHE).[7] This enzyme is responsible for the hydrolysis of ester-containing compounds such as the neuromuscular blocking drugs (succinylcholine and mivacurium) and local anesthetics (procaine, chloroprocaine, and cocaine). Succinylcholine is rapidly metabolized and inactivated by BCHE, which accounts for its short half-life of approximately 1 to 3 minutes. However, it has been well documented that in some individuals a typical dose of succinylcholine results in prolonged muscle paralysis. Various polymorphisms in the coding region of the BCHE gene have since been identified, and account for this

prolonged paralysis. The most frequent SNPs of BCHE are the atypical and Kalow variants, which describe the nucleotide substitution of A with G at position 209, and of G with A at position 1615, respectively. These substitutions result in amino acid changes from aspartic acid to glycine at codon 70, and from alanine to threonine at codon 539, respectively. Both variants are associated with BCHE enzymes that have decreased ability to hydrolyze succinylcholine, producing prolonged muscle relaxation.[8]

Cytochrome P450 (CYP) enzymes are a superfamily of microsomal drug-metabolizing enzymes that catalyze the vast majority of Phase I drug metabolism. As the primary metabolizers of clinically relevant drugs, polymorphisms found within the genes of CYPs can have profound effects on drug therapy and potential ADRs. A specific enzyme in this family, CYP2D6, is one of the most extensively studied and classic examples of pharmacogenetics in drug metabolism.[9] CYP2D6 is of particular interest because it metabolizes numerous drugs as diverse as codeine, dextromethorphan, propranolol, nortriptyline, and tramadol. Furthermore, to date, 85 different variants have been described for this one gene (www.cypalleles.ki.se). These polymorphisms greatly influence the enzyme's metabolic efficiency, resulting in patients being classified into four major phenotypes: 1) poor metabolizers, subjects with little to no enzyme activity; 2) intermediate metabolizers, subjects with decreased enzyme activity; 3) extensive metabolizers, subjects with a normal range of 2D6 activity; and 4) ultra-rapid metabolizers, subjects with multiple copies of the *CYP2D6* gene, resulting in greater than normal enzyme activity. Identification of a patient's 2D6 genotype would greatly enhance the ability to predict the metabolism of various drugs used in the pre-, intra-, and postoperative setting.

With the high incident rate of cardiovascular disease in America, patients undergoing ß-blocker therapy are commonly encountered in the clinical setting. Patients with the 2D6 poor metabolizer genotype demonstrate decreased clearance of ß blockers, such as metoprolol and propranolol, leading to an increase in their pharmacodynamic effects, and potential ADRs.[10] In the case of metoprolol, patients with the CYP2D6 ultrarapid metabolizer genotype have approximately 100% greater clearance of metoprolol compared to rapid metabolizers (see Chap. 127, "**Too Slow To Flow**").

CYP2D6 poor metabolizers demonstrate poorer clearance of the R-enantiomer of carvedilol (an α-receptor antagonist and ß-receptor antagonist), leading to a much greater alpha-blockade than would otherwise be predicted.[11] As this is becoming a popular adjunct in the treatment of heart failure, the implications of 2D6 isozyme variation in patients treated with carvedilol undergoing anesthesia could be dramatic. Ondansetron is also at least partly metabolized by 2D6 and 2D6 ultrarapid metabolizers are thought to metabolize the medication too quickly to gain a therapeutic benefit from the drug. In these cases, the appropriate response is not to administer more ondansetron, but to change the therapeutic approach altogether (see also Chap. 162, "**Bounce Back**").[12]

The ultra-rapid metabolizer genotype and phenotype can have a profound effect on the efficacy of normally benign prodrugs. Codeine and tramadol are both prodrugs that are converted via CYP2D6 to the active analgesic opioid receptor agonists morphine and *O*-desmethyltramadol, respectively. While standard dosages of these compound usually result in mild analgesia and central nervous system depression, 2D6 ultrarapid metabolizers have been shown to have serious ADRs, such as respiratory depression, with even small dosages (see also Chap. 63, "**Too Much of a Good Thing**").[13,14] Conversely, patients who are 2D6 poor metabolizers exhibit limited conversion of codeine and tramadol to their active metabolites, and they will therefore be more likely to provide beneficial analgesia. Prior knowledge of a patient's 2D6 genotype would allow alteration of dosage or substitution of medication to prevent ADRs and optimize therapy.

The commonly prescribed vitamin K antagonist warfarin is a CYP2C9 substrate. At least two CYP2C9 variants exist, with alleles designated as *CYP2C9*2* and *CYP2C9*3* respectively. The presence of either of those alleles in an individual can greatly decrease warfarin clearance, and patients with these polymorphisms are much more likely to experience excessive anticoagulation and bleeding.[15] Warfarin's genomics are complex however, as the activity of the drug is also dependent on the effectiveness of vitamin K epoxide reductase (VKORC1).[16] Studies have demonstrated that approximately 25% of the coumadin variability can be attributed to the presence of a single nucleotide polymorphism (SNP) present in this gene. Together, variations of CYP2C9 and VKORC1 have significant implications for perioperative care and complications. 2C9 is also an important metabolizer of the popular nonsteroidal anti-inflammatory drug (NSAID) celecoxib (Celebrex®), a cyclooxygenase-2 inhibitor. Known genetic polymorphisms in the *CYP2C9* gene lead to an inability of this enzyme to effectively metabolize this drug. The result is a dramatic increase in drug plasma levels, and has lead to a manufacturer drug information warning to use caution when administering celecoxib to "poor metabolizers of CYP2C9 substrates."[17]

A final example in which the pharmacokinetics of a drug commonly used to anesthetize patients is influenced by an individual's genome is that of propofol. For some individuals, a very small propofol dose can produce profound sedation. These patients likely have a "functional polymorphism" in the gene for the propofol metabolizing enzyme, CYP2B6.[18] This polymorphism, which carries a frequency of homozygous carriers in the Caucasian population of approximately 2%, leads to reduced CYP2B6 activity, and thus enhanced propofol action.

Transporters

Transport proteins play an important role in the pharmacokinetics of drugs because they regulate the absorption, distribution, and excretion of drugs and their metabolites. Because these transporters are one of the major factors controlling the

duration and concentrations of drugs at their sites of action, polymorphisms that alter the functioning of these proteins can have profound effects on a compound's therapeutic outcome. The ATP-binding cassette (ABC) family of membrane transporters is one of the most important families of transporters involved in drug distribution. Of the 49 known human ABC transporters, P-glycoprotein (encoded by the *ABCB1* gene, also called *MDR1*) is the most recognized, due to its wide tissue distribution throughout the body, and its role in transporting a wide array of compounds, including digoxin, quinine, vinblastine, dexamethasone, cyclosporine, and loperamide. One of the most important functions of P-glycoprotein is to prevent the accumulation of xenobiotics in the brain through efflux of compound across the blood–brain barrier. Of importance to anesthesiologists, P-glycoprotein is a major determinant of opioid bioavailability in the brain, thereby affecting systemic analgesia. A SNP in the *ABCB1* gene (substitution of C with T at position 3435) has been associated with decreased expression of P-glycoprotein. Patients with this polymorphism have demonstrated increase concentrations of P-glycoprotein substrates, such as digoxin and morphine, in their cerebrospinal fluid.[9,14] Although clinical significance of this polymorphism remains uncertain, it demonstrates the ability of transporter polymorphisms to effect the ADME of relevant compounds.

Receptors

While polymorphisms of drug metabolizing enzymes and transporters affect the concentration of drug at the target, genetic variation within the targets (i.e., receptors) can have profound effects on drug efficacy. Severe pain can be therapeutically addressed with opioid analgesics that act through the opioid receptors. The majority of clinically relevant opioid analgesics work through the μ_1-opioid receptor (encoded by the *OPRM1* gene). The *OPRM1* gene has been shown to have several polymorphisms that result in meaningful functional consequences.[19,20] One of the most common *OPRM1* SNPs (10.5%-18.8% allelic frequency) is the nucleotide substitution of A with G at position 118, resulting in an amino acid change from asparagine to aspartate at codon 40.[21] Patients who are carriers (heterozygous) for this polymorphism were found to require higher dosages of analgesics (i.e., alfentanil and morphine) to obtain pain relief than non-carriers of this SNP. Interestingly, carriers of this SNP were also found to tolerate the higher plasma levels of opioids, without increases in opioid toxicities or side effects, such as nausea and vomiting. Therefore, while this SNP requires an increase in opioid demand to achieve therapeutic analgesia, it may also broaden the therapeutic index of opioid analgesics.[22]

The $ß_2$-receptor, a site of action for some ß-agonist drugs, is coded by the gene *ADBR2*, a gene associated with significant variability. Polymorphisms in the *ADBR2* gene produce a $ß_2$-receptor that can be more or less sensitive to therapy leading to

differences in bronchodilation.[23] Oddly, however, the same polymorphism associated with bronchoconstriction is associated with increased survival in trauma patients.[24] Both factors may be of use to future anesthesia providers attempting to tailor care to individual patients.

Although we have discussed a few significant pharmacogenomically influenced anesthesia agents, it is important to remember that this field is relatively new, and additional data should be expected. Additional anesthetic/pharmacogenetic data are emerging regarding metabolism of midazolam (extensive metabolism via variant CYP3A5), local anesthetics (sodium channel variations and metabolism via CYP3A4) and volatile agents (variation in GABA receptors and melanocortin receptor 1).[25,26] The latter variation in volatile agent effect correlated with the same melanocortin-1 variant that produces red hair has received extensive press coverage.[27] It should be expected that public interest in the potential of pharmacogenomics will only increase; thus our attention to this field is vital.

Take-Home Points

- It has long been recognized that variations of specific genes can affect the pharmacology of a drug, giving rise to the field of pharmacogenetics. However, because the therapeutic effects of drugs are determined by the interplay of various genes (i.e., those coding for transporters, receptors, and metabolizing enzymes), the ability to characterize the polygenic determinants is advantageous in predicting a drug's therapeutic outcome.

- With the advances in human genomics and completion of the Human Genome Project, the ability to use a genome-wide approach to individualize a patient's drug therapy is rapidly becoming more realistic.

- Currently, individualization of therapy based on genomics is only reasonable for very few drugs, due to the costs of genetic mapping and our limited knowledge of functionally important polymorphisms. Currently, genotyping is done most commonly for the CYP2D6 and CYP2C19 isozymes.

- However, genotyping techniques are rapidly improving to the point where it is possible to screen for a panel of thousands of SNPs in genes that affect ADME and pharmacodynamics.

- As our knowledge of functionally relevant polymorphisms continues to advance through research, the practicality of pharmacogenomics in widespread clinical practice will only continue to increase.

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