Genetic Mechanisms for Variability in Drug Response and Toxicity

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Abstract

It is now well established that many proteins involved in the metabolism or pharmacodynamic action of drugs and foreign compounds exhibit structural polymorphism and variation in their level of expression. This variation leads to dramatic phenotypic differences in response to medicines or susceptibility to carcinogenesis. Some of the changes in the phenotypic expression of proteins are secondary to variation in the nucleic acid sequence of their respective genes. The science of pharmacogenetics links differences in gene structure (polymorphism) with pharmacologic differences in drug action and disposition of foreign compounds. Through discussion of four examples, we will emphasize the variety of genetic mechanisms that can potentially influence the phenotypic response to xenobiotic challenge and pharmacotherapy. The first example illustrates how structural variation in the coding region of drug metabolizing enzymes influences risk of drug toxicity. A second example demonstrates how genetic variation can influence gene transcriptional regulation and how the resulting dysregulation may be linked to increased susceptibility to exposure-linked cancer. The third example illustrates how genetic polymorphism can selectively influence the pharmacodynamic response to medication, and the final example of warfarin response illustrates how genetic variation in more than one gene can account for broad extremes in phenotypic response.

Introduction

Pharmacologic management of disease is a proven approach to improved human health. However, because of substantial individual differences in response to therapeutic agents, treatment of certain patient groups is compromised. The relationship between a drug dose or exposure to a toxic substance and the ultimate physiologic response is governed by a variety of mechanisms. These mechanisms generally fall into two traditional aspects of drug response, pharmacokinetics (PK) and pharmacodynamics (PD), and rely on fundamental structure-function relationships (Figure 1) (1). The PK of foreign compounds is controlled by enzymes and transport proteins that facilitate and influence the pharmacokinetic components of absorption, distribution, metabolism, and clearance. These variables, in concert with the exposure dose and frequency, establish the systemic concentration of the drug or other foreign compound and also drive the PD response. The PD or toxicologic response is likewise subject to a number of mechanisms involving a variety of cellular proteins (Figure 1). A genetic basis for intersubject variation in drug response or susceptibility to exposure-linked toxicity and carcinogenesis has been recognized for over 50 years. With the advent of improved techniques for the study of gene structure and expression, a number of mechanisms that alter the structure and function of expressed proteins have been discovered. We will briefly review fundamental concepts of genetic variation and then provide four examples of genetic mechanisms for variability in drug response with an emphasis on how an understanding of mechanism can lead to corrective action or discovery of novel therapeutic approaches.

Fundamental Concepts: Gene Structure in Genetic Variation

Review of gene structure

A gene, in the most fundamental sense, is a linear sequence of nucleotides. You will recall that nucleotides are joined to one another in sequence via a phosphodiester bond between the 5' and 3' carbons of the deoxyribose moiety of the nucleotide. This arrangement provides a basis for structural orientation. The deoxyribonucleic acid is a double-stranded molecule with antiparallel polarity. The coding strand (that strand which is transcribed into RNA) is referred to as the sense-strand and is conventionally depicted in the 5' to 3' orientation. The antisense strand is thus complementary (A-T, G-C) in sequence to the sense strand and is depicted by convention in the 3' to 5' orientation. The linear sequence of a gene includes a minimum of four structural domains. The most 5' domain is the regulatory region, which includes the structural attributes (nucleotide sequence) required for transcriptional control of

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gene expression including cell-type or tissue specific regulation and the regulatory response to intra- or intercellular signals. This domain terminates at the nucleotide where the RNA polymerase initiates transcription. This nucleotide is the +1 position of the gene. Nucleotides 5' to the start site of transcription are numbered with sequential negative numbers and conversely nucleotides 3' to the start-site of transcription are numbered with positive numbers. Downstream or 3' to the +1 nucleotide, the general structure of a gene includes the nucleotide sequence domains (exons) which ultimately direct the amino-acid structure of the protein gene product and may be interrupted by nucleotide sequence domains (introns), which, in some genes, play a variety of roles including fine-tuned transcriptional regulation.

Introduction to genetic variation

With the general structure of genes in mind we can now turn to mechanisms of variation in gene structure referred to as genetic polymorphism. Genetic polymorphism occurs in the form of gross structural changes including complete gene deletion, gene duplication and genetic translocation where portions of similar genes are combined creating a new gene hybrid. By far, the most common form of genetic polymorphism is single nucleotide polymorphisms (SNPs) where the nucleotide sequence at one specific position is changed, inserted, or deleted. Each of these changes in the gene structure introduces a variant form of the gene into the population gene pool and is designated an allele of the original gene. (Some standards suggest that a gene variant can be classified as an allele when the frequency of the variation is >1%). Thus, an allele is an inherited gene, present in each nucleated cell of the body. Because of the diploid structure of the human genome, each cell carries two copies of each gene. Two copies of the same allele yields a homozygous genotype, and any combination of two different alleles yields a heterozygous genotype.

A convention for allele nomenclature has been developed. For the majority of genes where genetic polymorphism has been characterized the most common sequence is denoted by the gene abbreviation followed by an asterisk and the number 1. This allele then serves as the reference sequence to which all other alleles of that gene are compared. For example, one gene discussed further in this review is the gene encoding the enzyme thiopurine methyltransferase (TPMT). The most common allele is designated TPMT*1. There are at least nine alleles of this gene, each having a specific designation (e.g., TPMT*2, TPMT*3, etc.). Further alleles may share common deviations from the wild-type or reference gene sequence. In this case, the primary structural change associated with altered function or expression of the gene product is designated by the number, and the additional structural change is designated by a capital letter. For example, the TPMT*3A, *3B, and *3C alleles share a single nucleotide polymorphism (SNP). In addition, the TPMT*3B and *3C alleles each share this SNP in addition to unique SNPs found in the TPMT*3B and *3C alleles.

The various types of genetic polymorphism can be generally classified by their resulting influence on protein expression or phenotype. Genetic polymorphism resulting in gene deletion invariably leads to loss of function and no production of the gene product. In contrast gene duplication and multiduplication most commonly leads to increased expression of the gene product and a hyperactivity phenotype. An exception to this is duplication of an allele which includes additional structural variation leading to loss of function. Genetic translocation typically yields a non-functional gene. SNPs can result in a variety of changes in the expressed protein function depending upon where the polymorphism occurs in the overall gene structure. SNPs in the 5' regulatory domain may influence gene regulation (2). SNPs in the coding exons only influence function if there is a resulting amino-acid change that alters the protein function. SNPs within the intron regions are typically silent unless the SNP alters a nucleotide critical for splicing of the RNA during maturation, which typically leads to loss or decrease in protein function.

The following four sections provide examples of the variety of mechanisms by which genetic variation leads to intersubject variability in drug response. These mechanisms include polymorphism of the gene encoding the drug metabolizing enzyme thiopurine methyltransferase which alters the protein function, polymorphism of the apo-lipoprotein E gene and its influence on the treatment of Alzheimers disease, and polymorphism of the cytochrome P4502E1 gene which alters transcriptional regulation. In the final example, genetic mechanisms for viability in response to the anticoagulant warfarin are discussed. This example provides an opportunity to consider how two independent genetic mechanisms may interact to yield hybrid genotypes with indistinguishable phenotypes.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Representative therapeutic substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome P4502D6</td>
<td>Desipramine</td>
</tr>
<tr>
<td>N-acetyltanserase</td>
<td>Procainamide</td>
</tr>
<tr>
<td>Cytochrome P4501A2</td>
<td>Theophylline</td>
</tr>
<tr>
<td>Cytochrome P4502C19</td>
<td>Omeprazole</td>
</tr>
<tr>
<td>Cytochrome P4502C9</td>
<td>Phenytoin</td>
</tr>
<tr>
<td>UDP-glucuronosyl transferase</td>
<td>Irinotecan</td>
</tr>
<tr>
<td>Dihydropyrimidine dehydrogenase</td>
<td>5-Flouroucil</td>
</tr>
</tbody>
</table>

Figure 1. Overview of the pharmacologic process. (Revised from Linder and Valdes [reference 1]).

Table I. Drug Metabolizing Enzymes with Genetic Polymorphism
Genetic Mechanism for Changes in Drug Pharmacokinetics

In terms of PK variability, genetic variation resulting in loss or increase of enzyme activity can have profound effects on the relationship between drug-dosage and observed plasma concentrations. Such dramatic differences account for severe toxicity and therapeutic failure. Several examples exist where structural variation in genes encoding drug metabolizing enzymes leads to an enzyme deficiency or over expression of active enzyme. Genetic polymorphism leading to enzyme deficiency results in a poor metabolizer phenotype (PM) with decreased drug clearance which may ultimately lead to toxic drug concentrations in individuals treated with standard drug dosages. Alternatively, enzyme deficiency can lead to subtherapeutic effects of pro-drugs due to loss of bioactivation. Genetic polymorphism may also include duplication or multiduplication of the active gene. This increase in gene copy number leads to increased expression of active enzyme and an ultrarapid metabolizer phenotype (UM). Ultra-rapid metabolizers may fail therapy because of unexpectedly low drug concentrations when administered standard drug dosages. Table I lists a number of drug metabolizing enzymes with known genetic polymorphism and representative pharmaceutical substrates.

The thiopurine S-methyltransferase (TPMT) is an excellent example of a genetic mechanism for altered drug pharmacokinetics leading to risk of toxicity in patients treated with standard drug dosages. TPMT is a cytosolic enzyme that catalyzes the S-methylation of aromatic and heterocyclic sulfhydryl therapeutic agents including 6-mercaptopurine, azathioprine (converted to 6-mercaptopurine in vivo), and 6-thioguanine. These drugs have cytotoxic and immunosuppressive properties and are used in the treatment of acute lymphoblastic leukemia (ALL), rheumatoid arthritis, organ transplantation, and autoimmune/inflammatory diseases. The thiopurine drugs are inactive pro-drugs requiring metabolism to thiopurine nucleotides via the purine salvage pathway to exert cytotoxicity. Alternatively, these drugs are inactivated by xanthine oxidase to thiouric acid or by thiopurine methyltransferase (TPMT)-catalyzed S-methylation to S-methylated bases. In hematopoietic tissues, xanthine oxidase activity is virtually absent, and thus TPMT catalyzed S-methylation of these agents is the principle step in the pharmacokinetics of these drugs controlling cytotoxicity in hematopoietic tissues. Patients who have TPMT deficiency, and thus less efficient methylation, have greater conversion to active thioguanine nucleotides which accumulate, leading to potentially fatal hematopoietic toxicity when treated with standard doses of thiopurines (3,4). Patients who have intermediate activity accumulate ~ 50% more thioguanine nucleotides and are at increased risk of hematopoietic toxicity (3).

Thiopurine methyltransferase phenotype is defined by erythrocyte 6-mercaptopurine methylation (5). There is a tri-modal distribution of TPMT-activity leading to a 40-fold range of activity among healthy populations (6). Approximately 90% of subjects have high activity, ~10% intermediate activity, and ~0.3% demonstrate deficiency in TPMT activity (7). These phenotypic groups are accounted for in part by genotypic differences of the TPMT gene. Extremely elevated TPMT activity has also been reported; however, a genetic basis for this phenotype has not been elucidated.

Genetic polymorphism of TPMT

Nine non-functional TPMT alleles are associated with inheritance of low enzymatic activity TPMT *2-*8 (8,9) with the TPMT*1 representing the most common active allele. The nucleotide changes, resulting change in the structural protein, and the allele frequencies are given in Table II. Subjects with high activity are believed to be homozygous for the common active TPMT allele, intermediate subjects heterozygous with one TPMT allele variant, and low-activity subjects homozygous with two variant TPMT alleles. The most common TPMT allele variants in order of decreasing frequency are the TPMT*3A, TPMT*3C, followed by TPMT*2 (4).

Because of the potential threat of fatal hematopoietic thiopurine toxicity resulting from reduced TPMT activity, it is recommended that a testing strategy with the highest level of sensitivity be adopted. Although marked differences in the prevalence of the individual PM alleles exists between populations of differing ethnic origin, the identity of the alleles is constant, allowing for a uniform pharmacogenetic screening approach for all populations studied to date. The TPMT*3A allele includes two nucleotide sequence variants G460-A and A719-G. Each of these nucleotide variants has been shown independently to result in a dramatic decrease in TPMT expression and catalytic activity as these variants are found in isolation within the context of the rare TPMT*3B and TPMT*3C alleles, respectively. By performing analysis for both variants (e.g., the TPMT*3A allele) in the process, the TPMT*3B and TPMT*3C alleles will be revealed. For example, an individual heterozygous for the TPMT*3A and TPMT*3B alleles will demonstrate heterozygosity for the A719-G variant and homozygosity for the

### Table II. Thiopurine Methyltransferase Alleles

<table>
<thead>
<tr>
<th>Allele</th>
<th>Functional nucleotide change</th>
<th>Amino-acid change</th>
<th>Enzyme activity</th>
<th>Associated phenotype</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPMT*1</td>
<td>None</td>
<td>None</td>
<td>Normal</td>
<td>EM</td>
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<tr>
<td>TPMT*2</td>
<td>G235C</td>
<td>Ala71Pro</td>
<td>Decreased</td>
<td>PM</td>
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<tr>
<td>TPMT*3A</td>
<td>G460A, A719G</td>
<td>Ala154Thr, Tyr246Cys</td>
<td>None</td>
<td>PM</td>
<td>0.035–0.065</td>
</tr>
<tr>
<td>TPMT*3B</td>
<td>G460A</td>
<td>Ala154Thr</td>
<td>None</td>
<td>PM</td>
<td>0.0035</td>
</tr>
<tr>
<td>TPMT*3C</td>
<td>A719G</td>
<td>Tyr246Cys</td>
<td>None</td>
<td>PM</td>
<td>0.007</td>
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<tr>
<td>TPMT*4</td>
<td>G to A in intron 9/exon 10</td>
<td>Splicing defect</td>
<td>None</td>
<td>PM</td>
<td>0.000</td>
</tr>
</tbody>
</table>
C460-A variant. In contrast, an individual heterozygous for the TPMT*3B and TPMT*3C alleles will demonstrate heterozygosity for both variants. This individual cannot be differentiated from individuals heterozygous for TPMT*1*3A. However, subjects heterozygous for both variants can be presumed to be heterozygous TPMT*1*3A based on the extremely low frequency of TPMT*3B*3C heterozygotes estimated at < 1 in 100,000.

Interpretation of TPMT genotyping

The clinical use of the thiopurine anti-metabolites is an important component of successful therapy of a number of diseases. The utilization of these compounds can be directed based on the knowledge of the individual’s TPMT activity determined either directly or inferred from the pharmacogenetic analysis. TPMT deficiency occurs as a result of homozygosity for two variant TMPT alleles and is associated with grossly elevated thioguanine nucleotide concentrations and severe hematopoietic toxicity in individuals treated with standard dosages. In contrast, very high TPMT activity is associated with a higher risk of therapeutic failure and disease relapse. Based on pharmacogenetic analysis, > 95% of TPMT-deficient individuals can be correctly identified (10). Identification of patients with TPMT deficiency can be applied to the successful treatment of these individuals with thiopurine medications by reducing the dose to 6–10% of the conventional dosage (11–13). Individuals with high TPMT activity appear to benefit from doses near the upper limit of the dosage generally recommended (14).

It should be noted that genetic polymorphism accounts for only two-thirds of the total variance in TPMT activity, suggesting the possibility of additional genetic factors beyond that currently described which might regulate TPMT activity (15).

Genetic Mechanism of Altered Gene Regulation

Changes in gene structure may occur throughout the entire gene sequence including the coding regions, introns and the 5’-regulatory domains. Changes in the gene structure of the 5’-regulatory domain may result in an alteration in the transcriptional activity of the gene. The following example illustrates how this genetic mechanism may influence risk of exposure-linked cancer.

In one of the clearest examples of a cause and effect relationship involving chemical carcinogenesis, occupational exposure to vinyl chloride (VC) gas was found to cause cell-type and tissue-restricted cancers of the liver (angiosarcoma, ASL) and brain (16–18). There is substantial evidence that exposure to VC leads to ASL in a sub-population of subjects (19), which may represent genetic predisposition (20).

As depicted in Figure 2, the molecular mechanism of vinyl chloride induced carcinogenesis is believed to involve epoxidation by cytochrome P4502E1 (21) to form chloroethylene oxide (CEO), primarily in the liver (22). Conjugation of CEO and other metabolites of VC to glutathione through the action of glutathione S-transferases (GST) is thought to provide detoxification of these metabolites. Unconjugated CEO induces specific genetic lesions through DNA-adduct formation. The principle DNA adducts observed following exposure to VC (its active metabolites CEO and CAA) are (N-2,7-oxoethyl) guanine and the cyclic etheno-bases (Figure 2) (23–25).

Cytochrome P4502E1 is a member of a multigene superfamily of hemithiolate proteins which catalyze the oxidative metabolism of a variety of endogenous and exogenous compounds. This isoenzyme is involved in the activation of a number of pro-carcinogenic substances including butadiene, benzene, and urethane (23). The gene encoding this enzyme exhibits structural polymorphism. Three alleles of CYP2E1 have been identified (CYP2E1*5B, CYP2E1*6, and CYP2E1*7B) and associated with increased susceptibility to cancer or to altered functionality of the transcriptional promoter. The nucleotide sequence variants of the CYP2E1*5B and CYP2E1*7B alleles are in the 5’ transcriptional regulatory region of this gene, (26–28) whereas, the CYP2E1*6 is defined by a sequence variant located in intron 6 of the CYP2E1 gene (1,25). There is evidence both in vitro and in vivo that the CYP2E1*5B and *7B alleles lead to increased phenotypic expression of the active enzyme. Polymorphism at nucleotide position G(-35) T (CYP2E1*7B) and associated with increased susceptibility to cancer or to altered functionality of the transcriptional promoter. Polymorphism at nucleotide position G(-35) T (CYP2E1*7B) is associated with increased activity of the CYP2E1 promoter (25). However, no association...
has been made between the CYP2E1*7B allele and a phenotypic consequence such as increased CYP2E1 expression or altered susceptibility to cancer or other disease.

We found that the CYP2E1*7B allele frequency was increased fourfold among subjects with ASL over nondiseased subjects with comparable exposure. These data strongly support a role for the CYP2E1*7B allele in increasing susceptibility to this cancer (29).

Having discovered the association between the CYP2E1*7B allele and VC-induced angiosarcoma, we were compelled to understand what influence this genetic polymorphism had on the phenotypic expression of CYP2E1 and to understand the mechanisms involved. Generally speaking, it is now well understood that the nucleic acid sequences located upstream (5') of the start site of transcription for many genes are important to the tissue-specific, developmental, and regulated expression of their protein products. These regulatory sequences, referred to as responsive DNA elements, provide a structural site for specific interaction with nuclear proteins governing diverse regulatory responses in gene expression. The structural specificity of these interactions is determined by the nucleotide sequence of the responsive DNA element. Therefore, comparison of the nucleotide sequences of interest with conserved nucleotide sequence motifs for known transcriptional regulatory proteins is the first step to identifying the regulatory aspects of the nucleotide sequence of interest.

Figure 3 illustrates structural similarity between the polymorphic region of the CYP2E1 promoter in comparison to well characterized gene structure domains which are known to form specific high affinity binding sites for transcriptional regulatory proteins including γ-interferon (γ-IFN). These nucleotide sequence elements have been thus designated as γ-interferon activation sequences (GAS). We have demonstrated γ-IFN dependent specific binding to this structural domain of CYP2E1 (30) and are in the process of elucidating the function of this structural domain and the influence of the G35 T nucleotide sequence polymorphism. This example illustrates one potential mechanism where structural variation in the regulatory domain of genes can alter the phenotypic response to challenge by exogenous compounds.

### Genetic Variation Influencing the Mechanism of Drug Action

The following example illustrates how genetic variation in a protein whose function is central to the mechanism of drug action, leads to an attenuated pharmacodynamic response to the therapeutic agent.

Alzheimer's disease (AD) is characterized by a severe loss of cholinergic activity in the central nervous system. Currently, cholinesterase inhibition, directed towards increasing synaptic acetyl choline, is the most commonly applied approach to pharmacologic management of symptoms of AD (31). The most commonly used drug to treat patients with AD is Tacrine, a centrally active cholinesterase inhibitor (32). Although this drug has proved beneficial in some patients, up to 75% of treated patients have failed to demonstrate improvements in memory or cognitive abilities (32). This observation has led to the pursuit of a biochemical basis for therapeutic failure. In the search for methods to identify patients who are likely to respond to Tacrine, apolipoprotein E (APOE)-genotype was found to be related to response to Tacrine. Patients who were carriers of the APO E4 allele demonstrated lessor response to Tacrine therapy than subjects whose APOE genotype included the e2 or e3 alleles (33). The mechanism linking Tacrine response to APOE genotype is believed to involve the central role of APOE in membrane remodeling associated with synaptic plasticity (34). Apolipoprotein E and its cognate receptor (LDL) are important for the delivery of phospholipids, phosphatidyl choline, and phosphatidyl ethanolamine and important in regulating the synthesis of acetylcholine (35). The gene encoding apolipoprotein E exhibits structural polymorphism, resulting in three common alleles e2, e3, and e4 encoding three major apoE isoforms. The apoE e4 allele leads to decreased brain apoE concentration; thus, a hypothesis was developed that drugs (including Tacrine) designed to take advantage of the residual cholinergic activity present in the AD brain should be less effective in subjects deficient in apoE function (e.g., apoE e4 allele carriers). In fact, a superior response to Tacrine has been demonstrated in patients who do not express the apoE e4 allele (36). A similar relationship between inefficacy of the acetylcholinesterase inhibitor Memantone was also related to the apoE e4 allele. In contrast, Xanomeline a cholinergic agonist which would not rely on the ability to synthesize acetyl choline for function, has been shown to yield beneficial effects in patients of the e3/e4 genotype. Patients homozygous e4/e4 did not respond as well as non-e4 individuals. Two other non-cholinomimetic drugs have in fact demonstrated improved response of e4 carriers versus non-e4 subjects (37,38). Thus, drug response is dependent on mechanism of action. Drugs that augment the cholinergic system fail in apoE e4 carriers because of the intrinsic dysfunction of this pathway resulting from apoE functional deficiency. In contrast, AD drugs that do not rely on this fundamental mechanism tend to act either independently of apoE genotype or even superior in apoE e4 carriers.

### Multigene Mechanism Leading to Pharmacogenomic Interactions

This final example illustrates a situation where multiple genetic mechanisms influence the dose-response relationship for a single therapeutic drug. The central concept of this example is to illustrate how multiple mechanisms potentially interact and thus influence the ultimate phenotype of the individual depending on whether they are present in isolation or in combination.

Warfarin is widely prescribed for the prevention and control of thromboembolism (39). This drug is a racemic mixture of R- and S-enantiomers and displays both stereoselective metabolism and pharmacologic potency with the S-enantiomer approximately three times as potent as the R enantiomer (40).
Steady-state concentrations of warfarin are maintained through the balance of dose administered, cytochrome P4502C9 metabolism of the S-enantiomer, and renal elimination of inactive hydroxy-metabolites (41).

The mechanism of warfarin action is believed to be inhibition of vitamin K1,2,3-epoxide reductase (VKOR) (42). The reduced vitamin K is a required cofactor for the glutamate carboxylase that converts precursor forms of blood clotting factors to active coagulation zymogens. Concomitant with this reaction, reduced vitamin K is converted to vitamin K 2,3-epoxide. The epoxide is then chemically reduced by enzymes in the liver to the active cofactor for the carboxylase enzyme (43). This cyclic conversion establishes an oxidation/reduction cycle known as the vitamin K cycle. Two unrelated enzymes have been identified as parts of the cycle. The flavoprotein DT-diaphorase reduces the quinone form of vitamin K but cannot reduce the epoxide. The other enzyme is the warfarin-sensitive vitamin K epoxide reductase, which reduces the epoxide as well as the quinone and is thus essential for completion of the cycle. Inhibition of this reductase by warfarin limits the amount of reduced vitamin K cofactor available for the synthesis of essential proteins involved in the clotting cascade (44).

Effectiveness of anticoagulant therapy is routinely monitored as the international normalized ratio (INR), which is a ratio of the time required for the patient's blood to coagulate relative to a standardized coagulation time. Epidemiologic evidence has established that the warfarin dose required to achieve a given target INR may vary by as much as 120-fold between individuals (45,46). These distributions typically demonstrate that ~ 7% of subjects require dosages of < 15 mg warfarin/week and that ~ 4% of patients require dosages of > 60 mg/week. This degree of variation in response presents a dangerous and costly dilemma for both patients requiring maintenance anticoagulation therapy and their physicians (47).

Proposed mechanism of warfarin hypersensitivity

A high affinity hepatic S-warfarin 7-hydroxylase ascribed to cytochrome P4502C9 is responsible for both regioselective and stereoselective metabolism of S-warfarin (40). The cytochrome P4502C9 enzyme is encoded by the CYP2C9 gene. This gene exhibits structural polymorphism leading to two gene variants (alleles) which encode proteins with differing catalytic activities with respect to the 7-hydroxylation of S-warfarin. The allele expressing the wild-type protein is designated CYP2C9*1. This gene sequence is the reference sequence to which all variants are compared. The CYP2C9*2 allele is defined by a C416T nucleotide substitution resulting in substitution of cysteine for arginine at amino acid position 144. The CYP2C9*3 allele is defined by an A1061C nucleotide substitution resulting in substitution of leucine for isoleucine at amino acid position 359.

The influence of these CYP2C9 alleles on warfarin dose requirements is currently a subject of great interest. Multiple studies have clearly demonstrated increased frequency of CYP2C9 allele variants in patients stabilized on low-dose warfarin therapy (50-53) and have established relationships between genetic deficiency in CYP2C9 with increased likelihood of extremely elevated INR's and major bleeding complications compared to the general clinic population. Although a recent study did support the association between low warfarin dose requirement and CYP2C9 polymorphisms (53), this study did not observe increased likelihood of severe over-anticoagulation. Thus, these data clearly implicate genetic polymorphism of CYP2C9 in low warfarin dose requirement and suggest that the influence of this genetic variation on outcome is somewhat controversial and may be related to intensity of surveillance. Also, among those subjects requiring the lowest warfarin dosages, 20% of these were homozygous for the common active CYP2C9*1 allele, raising the possibility that additional unidentified CYP2C9 allele variants are present within the population (49).

The limited information available on human subjects and the in vitro activity assessment of the CYP2C9*2 and CYP2C9*3 variant proteins suggests that these alleles will demonstrate non-equivalent effects on warfarin metabolism and dose requirement. Therefore, more in depth studies which include identification of all relevant CYP2C9 alleles and measurement of plasma S:R-warfarin concentrations and ratios are needed to strengthen the relationship between CYP2C9 genetics and warfarin maintenance dose requirements.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Functional nucleotide change</th>
<th>Amino-acid change</th>
<th>Enzyme activity</th>
<th>Associated phenotype</th>
<th>Allele frequency</th>
</tr>
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<tbody>
<tr>
<td>CYP2C9*1</td>
<td>None</td>
<td>None</td>
<td>Normal</td>
<td>EM</td>
<td>0.819 (0.793-0.844)</td>
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<td>CYP2C9*2</td>
<td>C-T exon 3</td>
<td>Arg164Cys</td>
<td>12% of wild-type</td>
<td>PM</td>
<td>0.107 (0.086-0.127)</td>
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<tr>
<td>CYP2C9*3</td>
<td>A-C exon</td>
<td>Ile135Leu</td>
<td>5% of wild-type</td>
<td>PM</td>
<td>0.074 (0.056-0.091)</td>
</tr>
</tbody>
</table>

Potential mechanisms of warfarin resistance

Two potential forms of hereditary warfarin resistance have been identified in humans. One form appears to be a pharmacokinetic mechanism where the clearance of (S)-warfarin was increased on the order of fourfold compared to control subjects. The mechanism of this increased warfarin clearance has not been defined. Gene duplication or muti-duplication of cytochrome P450 enzymes has been described and accounts for an ultra-rapid metabolism phenotype (54). Duplication of CYP2C9 has not been reported to date, but is a potential explanation for increased warfarin clearance. A second form of
hereditary warfarin resistance has been described in both rats (55) and humans (56,57) and appears to be dependent upon the pharmacodynamic mechanism of the drug action. This form of hereditary warfarin resistance is characterized by relatively high dosages of warfarin required to achieve poisoning or therapeutic effect, normal warfarin pharmacokinetics, extremely high warfarin concentrations in blood, normal concentrations and half-lives of blood clotting proteins, and hyper-responsiveness of warfarin-resistant individuals to anticoagulant reversal by vitamin K administration.

The pharmacodynamic activity of warfarin is inhibition of the vitamin K1 2,3-epoxide reductase, which limits the amount of reduced vitamin K available for the synthesis of blood clotting zymogens. Vitamin K1 2, 3 epoxide reductase activity (VKOR) is an activity attributed to a lipo-protein complex of the hepatic endoplasmic reticulum (58). Two protein components of the active complex have been identified as microsomal epoxide hydrolase (mEH) (59) and soluble glutathione S-transferase (GST) (26). Specificity of vitamin K1 2, 3 epoxide binding is attributed to the mEH of the complex whereas the site of warfarin action on this enzyme complex is through direct inhibition of the GST moiety of the complex. The cDNA and protein structure of the mEH component of the VKOR complex is unaltered in warfarin-resistant rats, suggesting that differences in the GST component may account for differential response to warfarin. Cain et al. (60) demonstrated that ~40% of rat hepatic microsomal GST activity is inhibited in the presence of warfarin. In contrast, GST activity of warfarin-resistant rats is not inhibited in the presence of warfarin. Thus, there is a direct relationship between warfarin action on GST activity and VKOR activity of warfarin-resistant rats, suggesting that the molecular mechanism of warfarin-resistant VKOR activity is structural variation of the GST component of the VKOR protein complex. The mechanism of hereditary warfarin resistance in rats may serve as a model system for a similar mechanism existing in humans.

Application of CYP2C9 genotyping to warfarin therapy

The information available to date strongly supports the association between homozygosity with the CYP2C9*3 allele and a low warfarin dose requirement. In addition the frequency of the CYP2C9*2/CYP2C9*2 and CYP2C9*2/CYP2C9*3 genotypes and to a lesser extent the CYP2C9*1/CYP2C9*2 and CYP2C9*1/CYP2C9*3 genotypes tend to be increased in the population of subjects who require low warfarin doses. Thus, this information can alert the physician to the likelihood that the patient may be of the hypersensitive phenotype and warrant consideration of a conservative approach to induction of warfarin therapy. However, numerous subjects heterozygous for either the CYP2C9*2 or CYP2C9*3 alleles and at least one subject homozygous CYP2C9*2/CYP2C9*2 are documented as requiring standard to even unusually high warfarin dosages. Explanations for these apparent discrepancies between CYP2C9 genotype and warfarin dose requirement may lie in the discovery of additional CYP2C9 variants that confer a deficiency in metabolism and thus partially explain the low dose requirements of subjects currently classified as CYP2C9*1/CYP2C9*1. In addition, a better understanding of warfarin resistance and the potential genetic basis for this phenotype may provide some insight as to why some individuals with an apparent CYP2C9 deficiency continue to require standard to high warfarin dosages.

Conclusions

A brief review of basic principals in pharmacology reveals a large number of genes with potential for altering individual response to challenge by foreign compounds. Examples reviewed in this text illustrate a variety of molecular mechanisms contributing to interindividual variation in the response to therapeutics or risk of cancer when exposed to carcinogens. They also provide a framework for beginning to understand how characterization of these mechanisms can potentially lead to improved therapeutic management, risk assessment, and the discovery of rational therapeutic approaches.

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