

Gene-Based Drug Prescribing: Clinical Implications of the Cytochrome P450 Genes

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ABSTRACT

The Institute of Medicine recently mandated an increased effort to improve patient safety and reduce medical error. With the description of genetic polymorphisms in the drug metabolizing enzymes, the field of pharmacogenetics may improve medical care through a reduction in both therapeutic failure and adverse drug reaction. Investigators at the Marshfield Clinic in central Wisconsin are piloting the process of gene-based drug prescribing in a variety of contexts. This paper reviews the field of cytochrome P450 (CYP) genetics and explores factors that impact the utility of this information in clinical practice.

INTRODUCTION

Therapeutic failure (lack of efficacy due to suboptimal drug dosing) and adverse drug events (ADEs) both represent significant areas of patient safety concern.¹ In general terms, ADEs include both compliance issues and medication dispensing errors.² Adverse drug reactions (ADRs), on the other hand, are complications that occur despite appropriate dispensing of the correct medication at the "intended dose." Since drug outcome is determined in part by genetic variation, the intended dose may not be the correct dose for all individuals. Therefore, in many situations, it may be helpful to have prospective access to genetic information that might predict efficacy and/or toxicity.

GENETIC VARIATION AND THE CYTOCHROMES P450 (CYPS)

Many drugs are lipid soluble and tend to be retained

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in the body until they are rendered more polar. Drug metabolizing enzymes (DMEs) impact drug polarity, and most are expressed within a variety of tissues. Since DMEs alter drug solubility, variations in their structure can alter a drug's route of excretion (ie, renal versus hepatobiliary) and predispose patients to a therapeutic failure or an increased risk of developing an ADR.

Drug metabolism is typically classified as either Phase I (oxidation/reduction reactions) or Phase II (conjugation). Phase I reactions tend to alter the structure of the parent drug and any subsequent metabolites that are produced. Phase II reactions modify the drug covalently. Both Phase I and Phase II drug metabolizing enzymes (DMEs) can impact a drug's activity. In some cases, a prodrug may require bioactivation by a DME.

Phase I DMEs include tissue esterases, dehydrogenases, flavin monooxygenases, and the cytochromes P450 (CYPs).³ CYPs represent the most common family of Phase I DMEs, and they are expressed at high levels within the human liver. All enzymes contain a heme moiety that combines molecular oxygen with drug substrate. While over 50 known human CYP isoforms have been characterized, only 4 appear to be responsible for the majority (>75%) of all P450 activity observed in vivo.⁴ These are CYP3A4 (40%), CYP2D6 (20%), CYP2C9 (15%), and CYP2C19 (5%). The remaining P450-mediated metabolism is carried out primarily by CYP1A2, CYP2A6, and CYP2E1.⁴

Variation in the activity of CYP enzymes between individuals is a very common cause of inter-individual variation in drug response. All 7 CYP enzymes introduced above are known to be encoded by genes that vary structurally within most populations. A comprehensive discussion of population-based variability in CYP DNA structure is beyond the scope of this review, but an up-to-date list of all known human CYP alleles can be accessed on-line at www.imm.ki.se/CYPalleles/. (Accessed July 27, 2005.) The clinical relevance and substrate specificity of these enzymes is summarized in Table 1.

Table 1. CYP Substrate Examples, Shown with Summary of CYP Gene Variability

DME Gene	Representative Substrates	Gene Variability*
CYP1A2	Caffeine SSRIs (fluvoxamine, paroxetine and sertraline) Theophylline Imipramine	As many as 23 polymorphisms
CYP2A6	Nicotine Aflatoxin B1 Nitrosamines Volatile anesthetics (e.g. halothane) Antiepileptics (valproic acid) Disulfiram	More than 20 polymorphisms Possible association between the CYP2A6 gene and nicotine use
CYP2C9	Warfarin Losartan and irbesartan (ARBs) NSAIDs (ibuprofen, naproxen, indomethacin) COX 2 inhibitors (celecoxib) Glypizide and tolbutamide Phenytoin (to some degree)	13 polymorphisms described Gene products metabolize ~15% of all commonly prescribed drugs CYP2C9 gene-based dosing models currently being tested in the context of warfarin therapy
CYP2C19	Minor impact (<30%): Phenytoin, Propranolol Modest impact (~50%): Benzodiazepines (diazepam, flunitrazepam) TCAs (imipramine, lomipramine) SSRIs (citalopram and sertraline) Major impact (>80%): PPIs, and Mephenytoin	18 polymorphisms described Gene products metabolize ~5% of all commonly prescribed drugs Differences described in rates of GERD healing with treatment Differences described in rates of <i>H.Pylori</i> healing with treatment
CYP2D6	Beta blockers (propranolol, metoprolol, timolol) Class IC anti-arrhythmics (encainide, flecainide, propafenone) Psychotropics (amitriptyline, sertraline, haloperidol) Codeine	>75 polymorphisms have been identified to date, but it appears that 4 alleles account for most of the functional variation Gene products metabolize ~20% of all commonly prescribed drugs
CYP2E1	Endogenous substrates (e.g., acetone, acetaldehyde) Exogenous substrates (e.g., benzene and ethylene glycol) Also contributes significantly to the metabolism of a variety of drugs that are metabolized primarily by other DMEs	As many as 10 polymorphisms
CYP3A4 and CYP3A5	Acetaminophen Antibiotics (e.g., erythromycin) Benzodiazepines (e.g., midazolam) HMG CoA reductase inhibitors (e.g., atorvastatin)	>30 polymorphisms proposed within the CYP3A4 gene >10 proposed for CYP3A5 Involved in the metabolism of ~50% of all prescription drugs

* <http://www.imm.ki.se/CYPalleles/>. Accessed July 27, 2005. SSRI=selective serotonin reuptake inhibitor; TCA=tricyclic antidepressant; PPI=proton pump inhibitors; GERD=gastroesophageal reflux disease

THE CLINICALLY RELEVANT CYPs

CYP1A2 demethylates caffeine, and it oxidizes carcinogenic arylamines.⁵ Some prescription drugs oxidized by CYP1A2 include antidepressants (imipramine, fluvoxamine, paroxetine, and sertraline), antipsychotics (clozapine), and theophylline.^{6,7} In vivo, CYP1A2 enzyme activity appears to be highly variable. This variability is due to a combination of environmental (tobacco smoke)

and genetic influences. CYP1A2 gene variability is distributed according to a codominant or recessive pattern of inheritance. While many variations in CYP1A2 gene structure have been reported in the scientific literature, most remain poorly characterized with respect to clinical drug outcomes.

CYP2A6 metabolizes nicotine, nitrosamines, and some prescription drugs (including disulfiram and

valproic acid). It also metabolizes volatile anesthetics (halothane and methoxyflurane).⁸ More than 20 polymorphisms have been reported in the CYP2A6 gene. Because of the importance of CYP2A6 in nicotine metabolism, variation in the CYP2A6 gene may influence smoking behavior.⁹

CYP2C9 metabolizes approximately 15% of all prescribed drugs. Clinically relevant substrates include angiotensin receptor blockers (losartan, irbesartan), oral sulfonyleurea agents (glypizide, tolbutamide) and, to some degree, phenytoin. CYP2C9 is also the primary route of metabolism for a number of agents known to alter hemostasis. These include warfarin (stereoselective oxidation of S-warfarin), as well as the older (ibuprofen, naproxen, indomethacin) and newer (celecoxib) non-steroidal anti-inflammatory drugs.^{8,10,11} Approximately 30% of the general population appears to express at least 1 variant CYP2C9 allele,¹² and investigators at the Marshfield Clinic have recently developed a multivariate CYP2C9 gene-based dosing model that explains much of the variance in maintenance warfarin dose.¹³ Prospective gene-based dosing trials are currently being organized, in an effort to determine if CYP2C9 genotyping will reduce the rate of bleeding complications associated with the use of this drug.¹⁴

CYP2C19, another member of the CYP2C enzyme family, handles a smaller proportion of all the available prescription drugs (~5%). Many are commonly used to treat chronic medical problems. CYP2C19 substrates include antiepileptics (phenytoin, mephenytoin), benzodiazepines (diazepam, flunitrazepam), tricyclic antidepressants (imipramine, clomipramine), selective serotonin receptor inhibitors (citalopram, sertraline), and proton pump inhibitors (PPIs; omeprazole, lansoprazole, pantoprazole, and rabeprazole).^{8,15} Although CYP2C19 allele frequency varies with ethnicity, a poor metabolizer (PM) phenotype has been detected in most populations.¹⁵ In PMs, the plasma concentration of proton pump inhibitors appears to be elevated several-fold with respect to the rest of the population.¹⁶ This increase in circulating concentrations of PPI may be associated with greater acid suppression and improved therapeutic efficacy.

CYP2D6 metabolizes approximately 20% of all commonly prescribed drugs.⁸ Examples include beta-blockers (metoprolol, timolol) and a variety of psychotropic agents (amitriptyline, haloperidol). CYP2D6 is also a pivotal metabolic step during the *in vivo* bioactivation of codeine, an otherwise inactive oral opioid analgesic.⁸ CYP2D6 gene expression is commonly lost through several types of gene disruption. Although the CYP2D6 gene is clearly the most polymorphic of all

known human cytochrome P450 (more than 75 polymorphisms have been identified), it appears that 4 alleles account for >95% of the functional variation observed in the general population.

CYP2E1 contributes to the metabolism of a variety of drugs that are metabolized primarily by other DMEs.⁸ Like CYP2A6, this enzyme catalyzes the oxidation and inactivation of a number of volatile anesthetics (halothane and enflurane). CYP2E1 also represents an alternative demethylation pathway for a variety of substrates metabolized by CYP3A4. Although studies have suggested that some CYP2E1 gene polymorphisms may reduce catalytic activity *in vitro*, their clinical relevance remains undefined.

CYP3A4 is highly expressed within the liver and the epithelial lining of the gut. CYP3A4 (and the related gene product, CYP3A5) may be involved in the metabolism of approximately half of all prescription drugs in use today. Typical CYP3A substrates include analgesics (eg, acetaminophen), antibiotics (eg, erythromycin) and benzodiazepines (eg, midazolam), as well as many of the HMG CoA reductase inhibitors (eg, atorvastatin).⁸ Until recently, the CYP3A enzyme family has been considered highly conserved, ie, CYP3A genes have been thought to have minimal variability in most populations. In the past few years, however, several polymorphisms have been observed in the CYP3A4 gene.^{17,18} Their functional relevance remains unproven. Interestingly, a related gene, CYP3A5, appears to be in linkage disequilibrium with the most well characterized CYP3A4 polymorphism.¹⁹ The CYP3A5 gene appears to be expressed by a variety of extra-hepatic tissues, and a considerable amount of energy is currently being applied to the clinical characterization of CYP3A5 gene variation.²⁰

PHARMACOGENETICS IN CLINICAL PRACTICE

The field of pharmacogenetics is advancing rapidly. Nonetheless, most of the clinical work to date has been conducted retrospectively. The extent to which this field will translate prospectively into practice therefore remains largely undetermined. It is conceivable that, if ADRs are defined as a disease state (ie, undesirable outcome resulting from an interaction between gene and environment), then the CYP enzyme gene polymorphisms discussed in this review are likely to represent some of the most common inheritable risk factors for the development of "disease."

Consider the clinical relationship between polymorphism in the CYP2C9 gene and the hemorrhagic

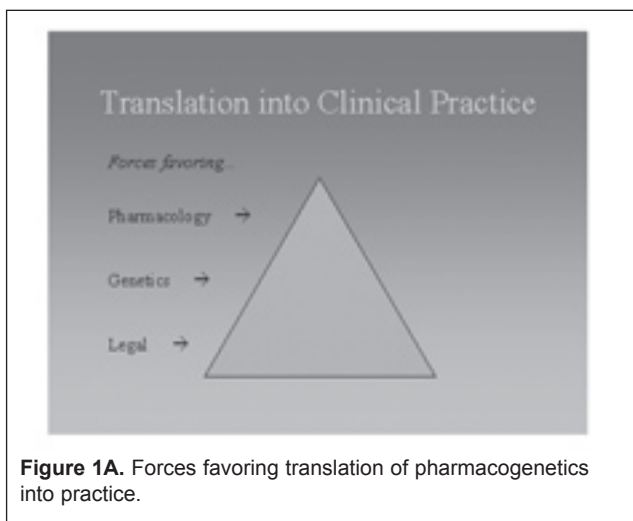


Figure 1A. Forces favoring translation of pharmacogenetics into practice.

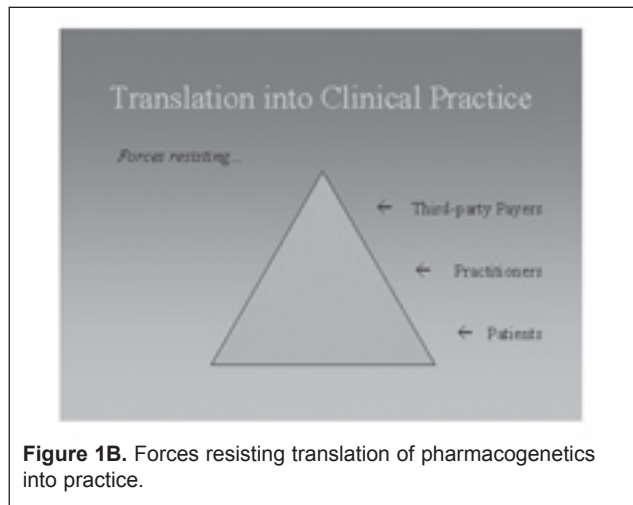


Figure 1B. Forces resisting translation of pharmacogenetics into practice.

complications associated with warfarin therapy.²¹ As mentioned earlier, S-warfarin (the active enantiomer) is primarily metabolized by CYP2C9. The frequency of variant CYP2C9 alleles appears to be as high as 30% in the general population,¹² and it has been suggested that heterozygosity (ie, the presence of only a single variant copy of the CYP2C9 gene) is capable of inducing a clinically recognizable alteration in phenotype (eg, increased rate of warfarin-related bleeding complication).²¹

FORCES FAVORING PHARMACOGENETICS IN PRACTICE

Pharmacologists and genetic epidemiologists continue to explain increasing amounts of the variance associated with clinical outcomes in population-based studies of drug toxicity. Hence, a growing interest in the area of drug safety is tending to move the clinical community in the direction of prospective gene-based drug prescribing (Figure 1A). This is particularly true if the drug

has a relatively narrow therapeutic index (median toxic dose/median effective dose) and the nature of the adverse reaction is serious.

Translational (ie, Bench-to-Bedside) pharmacogenetics is already becoming a clinical reality in the context of cancer therapy. Consider the relationship between 6-mercaptopurine (6-MP) and variation in the gene for thiopurine methyltransferase (TPMT).²² Although a detailed discussion of the TPMT gene (a Phase II DME) is beyond the scope of this review, it is worth noting that inherited deficiency in TPMT activity has been shown to predispose patients to an increased risk for the development of potentially life-threatening toxicity when 6-MP is used to treat acute leukemia.²³ Last year, the Food and Drug Administration (FDA) reviewed evidence that administration of the usual doses of 6-MP to patients with variant TPMT alleles may result in an increased risk of bone marrow suppression. FDA subsequently mandated that a discussion of TPMT gene variation—and the availability of TPMT gene testing—be included in the product label for 6-MP.²⁴

Other drugs with narrow therapeutic indices and severe ADRs are likely to soon undergo similar review. As introduced earlier, warfarin is known to be metabolized by CYP2C9, and as many as 30% of all patients express at least 1 variant CYP2C9 allele. In 2002, investigators at the University of Washington found that patients expressing an abnormal CYP2C9 genotype were at increased risk for the development of warfarin-related adverse bleeding events.²¹ Since then, a variety of large clinical centers have been working to clarify the extent to which genotype influences warfarin dosing requirement.

Several groups have begun developing rationale gene-based dosing models that quantify the impact of CYP2C9 genotype in the context of clinical covariates. At the Marshfield Clinic, investigators have recently demonstrated that a model including age, gender, body size, CYP2C9 genotype, comorbidity, and concomitant medication can explain 34% of the variance in stable warfarin dosing.¹³ With the characterization of additional genes impacting warfarin's mechanism of action,²⁵ it is anticipated that gene-based models will explain increasing amounts of warfarin-related outcome variability.

FORCES RESISTING PHARMACOGENETICS IN PRACTICE

As the clinical community moves toward prospective gene-based drug prescribing, a number of issues will need to be addressed. These include patient education, security

of the genetic information, physician time allocation, and the overall socioeconomic impact of gene-based prescribing (Figure 1B). Even if patient receptiveness is robust (as preliminary studies suggest),¹⁴ practitioners will still need to find the time to educate patients about the science (as well as the risks and benefits) prior to embarking on a course of gene-based dosing. Since most practitioners are experiencing increased economic pressure (ie, being encouraged to see more patients, in some cases with less time), it is likely that patient education will be a substantial barrier to implementation.

Furthermore, large populations may need to be genotyped in order to identify a small number of patients who will benefit. Consider the relationship between CYP2C19 genotype and outcomes related to the use of proton pump inhibitors (PPIs). Clinical studies have revealed that CYP2C19 gene polymorphisms are associated with variable cure rates in patients with gastroesophageal reflux disease, and variable eradication rates in patients being treated for *H. pylori* infection.²⁶ Clinical pharmacologists have therefore begun modeling the economic utility of gene-based treatment for these conditions.¹⁵ It is conceivable that extensive metabolizers (EMs) need higher doses of PPIs. It has also been suggested that gene-based treatment of patients with duodenal ulcers containing *H. pylori* would be more cost effective if EMs were given a combination drug regimen containing an H₂-histamine receptor blocker rather than a proton pump inhibitor. The latter model predicts a break-even cost between \$89.20 and \$118.96 per patient genotyped.²⁷

Although the above margin of savings appears to be relatively modest, it was generated using an example of gene-based prescribing that was (1) based on efficacy, and (2) based on a class of medications known to have a wide therapeutic index. The magnitude of gene-based cost savings is likely to be amplified when one considers the utility of genotyping for (1) the prevention of toxicity, and (2) in the context of a class of drugs known to have a narrow therapeutic index. This is particularly likely if the manifestation of toxicity for that class of drugs is clinically severe (eg, requiring hospitalization). Consider the relationship between CYP2C9 genotype and warfarin-related bleeding events. It is anticipated that the potential cost savings realized by avoidance of hemorrhagic ADRs will be substantial.²⁸ Based on data (dosing and adverse event rates) obtained in their seminal paper on the impact of CYP2C9 genotype,²¹ Higashi and colleagues have predicted a relatively low number needed to treat (NNT = 13 patients), for the cost of adverse event(s) to be offset by the cost of genotyping.²⁹

CONCLUSION

As prospective gene-based drug prescribing trials emerge, and the economic benefits of gene-based dosing are more rigorously characterized, it is likely that forces slowing the translation of pharmacogenetics into practice will yield. This field may soon become an essential part of every practitioner's fund of knowledge.

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