CYP2C19 Testing: An Opportunity to Improve Patient Care

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Cytochrome P450 2C19 metabolizes 5% of the prescription drugs in use today. Some of the commonly used and largest selling therapeutics for heart disease, depression, and fungal treatments are all metabolized by CYP2C19.[1] Understanding a patient's CYP2C19 genotype may provide insight into how they will respond to drugs.[2]

Introduction

The discovery of genetic factors such as the cytochrome P450 (CYP) drug metabolizing genes and several years of subsequent clinical research have added to our understanding of the clinically relevant single nucleotide polymorphisms (SNPs) that may help predict drug response. Several genes are responsible for differences in drug metabolism and response; the cytochrome P450 (CYP) genes are among the most common. They encode the cytochrome P450 class of metabolic enzymes found primarily in the human liver. Many of these enzymes play an instrumental role in the metabolism and clearance of clinically prescribed drugs.

Cytochrome P450 2C19 (CYP2C19) Enzyme

Drugs may be metabolized by more than one pathway involving several enzymes of the cytochrome P450 class. Cytochrome P450 enzyme 2C19 (CYP2C19) metabolizes many clinically important drugs including proton pump inhibitors, antidepressants, the antiplatelet drug clopidogrel, and the antifungal voriconazole.[3]

Drugs metabolized by CYP2C19 are called 'substrates' (Table 1a). Drugs that decrease CYP2C19 activity are called inhibitors, and drugs that increase activity are called inducers (Table 1b). Inhibitors are likely to increase the plasma concentrations of certain medications, and in some cases, can cause adverse drug reactions (ADRs) to occur. Inducers are likely to decrease the plasma concentration of medications, and potentially render them ineffective. For example, CYP2C19 activity can be inhibited by such compounds as fluoxetine, fluvoxamine, lansoprazole, pantoprazole, and ticlopidine, and can be induced by compounds such as phenobarbital and rifampin.[4]

Table 1a. Common Substrates of CYP2C19[5]

Common Substrates of CYP2C19	
Proton Pump Inhibitors	• Lansoprazole/Dexlansoprazole • Omeprazole/Esomeprazole • Rabeprazole • Pantoprazole
Antiepileptics	• S-Mephenytoin • Diazepam • Phenobarbital • Phenytoin • Primidone
Antidepressants	• Amitriptyline • Citalopram • Clomipramine • Moclobemide • Imipramine • Desipramine • Sertraline
Antibiotics	• Chloramphenicol
Antifungals	• Voriconazole
Anticancer	• Nilutamide • Cyclophosphamide • Neniposide
Other	• Clopidogrel • Carisoprodol • Indomethacin • Mephobarbital • R-warfarin* • Hexobarbitol • Nelfinavir • Propranolol • Progesterone • Proguanil

*Warfarin consists of a mixture of equal parts S-warfarin and R-warfarin. S-warfarin has more potent anticoagulant activity, and is metabolized by CYP2C9.[5]

Table 1b: Common Inhibitor and Inducers of CYP2C19[5]

Therapy	Inhibitors	Inducers
Antibiotic	Chloramphenicol	Rifampicin
Antidepressants	Moclobemide	
Antifungal	Ketoconazole	
Antiepileptic	Topiramate Oxycarbazepine	Phenobarbital Carbamazepine
Antiplatelet	Ticlopidine	
Corticosteroid		Prednisone
Contraceptive		Norethindrone
Eugeroic	Modafinil	
Proton Pump Inhibitors	Lansoprazole Omeprazole Pantoprazole Rabeprazole	
Receptor Antagonist (H2)	Cimetidine	
SSRI	Fluvoxamine Fluoxetine	
NSAID	Indomethacin	
Uricosuric	Probenicid	

Pharmacogenetics 101[6]

- Single Nucleotide Polymorphism (SNP)—Genetic variation arising from substitution of one base pair in DNA for another base pair is referred to as a SNP. SNP is a genetic mutation in the DNA that can result in a disease phenotype.
- Haplotype—Combinations of several SNPs together on the same chromosome.
- Alleles—Alternative forms of a gene that arise by mutations in the DNA.
- Genotype—An individual's collection of genes. The term also can refer to the two alleles inherited for a particular gene. The genotype is expressed when the information encoded in the gene is used to make protein.
- Phenotype—The expression of the genotype contributes to the individual's observable traits.

Genetics of CYP2C19

Every individual has two CYP2C19 alleles (except for individuals with gene duplications), one inherited from each parent. The combination of these two alleles ("genotype") determines the overall level of CYP2C19 enzyme activity, or phenotype, particular to that combination.

All of the identified polymorphisms associated with CYP2C19 are autosomal recessive. For example, only individuals who are homozygous (such as *2/*2) or compound heterozygous (such as *2/*3) for these polymorphisms are poor metabolizers. Individuals who are heterozygous, with one normal gene and one variant gene (*1/*3), will have lower metabolic activity compared to *1/*1 individuals, and are considered intermediate metabolizers.[3]

The mutations in the CYP2C19 gene are heritable. Up to 34 different variations in the gene sequence have been described for CYP2C19.[7] The CYP2C19*1 allele is considered the wild-type, or "normal" allele, with "normal" enzyme activity.

Cytochrome P450 2C19 Nomenclature[8]

- CYP = cytochrome P450
- 2 = genetic family
- C = genetic sub-family
- 19 = specific gene
- *1 = allele
- *NOTE that this nomenclature is genetically based—it has NO functional implication.

Other CYP2C19 alleles have various mutations compared to CYP2C19*1, that results in different levels of enzyme activity compared to the wild-type. For some alleles, the sequence changes compared to the *1 allele may only be one nucleotide, but for other alleles, there may be several mutations/polymorphisms that are inherited together (haplotype).

Table 2: Major Human CYP2C19 Alleles and Associated Single Nucleotide Polymorphisms (SNPs)[8]

CYP2C19 Genotypes	Single Nucleotide Polymorphisms
*1	None
*2	19154G>A
*3	17948G>A
*4	1A>G
*5	90033C>T
*6	12748G>A
*7	19294T>A
*8	12711T>C
*9	12784G>A
*10	19153C>T
*17	-806C>T

Frequency of CYP2C19 Variation

Genetic variation in the CYP2C19 gene plays a major role in inter-individual variability in drug response.[3] Variant CYP2C19 alleles distribute differently across ethnic groups. The most common alleles in Caucasians and African Americans are *1 and *17, and the most common alleles in Asians are *1 and *2 (Table 3). Although *3 is rare in Caucasians, and has not been found in African Americans, it is found in five to nine percent of Asians. Conversely, *17 is relatively common in Caucasians and African Americans, and more rare in Asians.

Major CYP2C19 Alleles, Enzyme Activity, and Frequency in Population[3, 9]

CYP2C19 Allele	CYP2C19 Enzyme Activity	Frequency in Population		
		Caucasians	African American	Asian
*1	Wild Type	87%	75%	50-62%
*2	None	12-15%	15%	29 to 35%
*3	None	<0.5%	0%	5-9%
*4♦	None	<1%	<1%	<1%
*5♦	None	<1%	<1%	<1%
*6♦	None	<1%	<1%	<1%
*7♦	None	<1%	<1%	<1%
*8♦	None	<1%	<1%	<1%
*9♦	Decreased	<1%	<1%	<1%
*10♦	Decreased	<1%	<1%	<1%
*17	Increased	16-21%	16%	3%

♦The frequencies of alleles *4 to *10 have not been rigorously studied because they are relatively rare. Typically the frequencies are less than 1%.[7]

CYP2C19 Metabolizer Phenotypes

The CYP2C19 genotype of a patient affects the level of enzyme activity ("phenotype"). CYP2C19 phenotype can be classified into four groups:[9]

- **Extensive metabolizers (EMs)** have normal enzymatic activity and carry two wild-type alleles.
- **Intermediate metabolizers (IMs)** have reduced enzymatic activity, and have one wild-type allele plus one decreased activity or null allele, or one increased activity allele plus one decreased activity or null allele.
- **Poor metabolizers (PMs)** have very low or absent enzymatic activity, and carry two decreased activity or null alleles.
- **Ultra-rapid metabolizers (UMs)** have increased enzyme activity, and carry either one wild-type allele and one increased function allele, or two increased function alleles. The increased function allele in CYP2C19 (the *17 allele) results from a mutation in the gene promoter. This mutation increases the gene transcription of the CYP2C19 mRNA, and therefore increases the level of the CYP2C19 protein product. The *17 allele is the only UM allele identified to date for CYP2C19.[10]

The prevalence of CYP2C19 genotypes varies in the general population (Table 4). The UM phenotype can cause therapeutic resistance or inefficacy at standard doses of a drug; higher doses may be required in UMs to obtain efficacy.[10] The PM phenotype is associated with reduced clearance and increased risk of adverse reactions.[12]

Table 4: Prevalence of CYP2C19 Phenotypes in the General Population[10]

2C19 Phenotype	Percent of patients with phenotype
Extensive	35-50
Intermediate	18-45
Poor	2-15
Ultra-rapid	5-30

CYP2C19 variants affect metabolism of many clinically important drugs. For example, the antiplatelet drug clopidogrel is metabolized to its active metabolite by CYP2C19. Because IMs and PMs for CYP2C19 have reduced ability to produce the active metabolite, the efficacy of clopidogrel treatment is decreased and the patient's risk for cardiovascular events increases.[13]

In 2010, the FDA added a black box warning to the label of clopidogrel stating that CYP2C19 PMs exhibit higher cardiovascular event rates, following acute coronary syndrome or percutaneous coronary intervention (PCI), than patients with normal CYP2C19 function. The warning also states that tests are available to determine a patient's CYP2C19 genotype, and that alternative treatments should be considered in CYP2C19 PMs.

The efficacy of proton pump inhibitor therapy is also affected by CYP2C19 genotype. Patients that carry one or more loss of function variants have higher plasma drug levels, increasing the efficacy of *Helicobacter pylori* eradication with therapy that includes proton pump inhibitors. However, patients with the EM or UM phenotype may have an insufficient response and may require a higher dose.[14]

Many antidepressant drugs are partially metabolized through CYP2C19, which affects the efficacy of treatment as well as the risk of side effects. For citalopram, the CYP2C19*2 allele has been associated with lower tolerance of the side effects of the medication, but remission rates are higher in patients who can tolerate the drug.[15] PMs may also have a higher risk of ADRs with sertraline and imipramine, and may require a lower dose. UMs may require a higher dose.[3]

Voriconazole is an antifungal with a narrow therapeutic range, and CYP2C19 genotype has a significant effect on voriconazole plasma levels, efficacy of treatment, and risk of ADRs. Carriers of the CYP2C19*17 allele are at risk of sub-therapeutic voriconazole levels, and treatment failure.[16,17] CYP2C19 PMs may be at higher risk of voriconazole toxicity.[18]

Clinical Application of CYP2C19 Testing

Cardiology: Thrombosis Associated with Clopidogrel Resistance After Coronary Stenting

A 73 year old woman was admitted to the hospital with acute myocardial infarction (MI). Five days earlier she had undergone coronary stenting with a drug-eluting stent. She had been receiving aspirin and clopidogrel for two weeks prior to stenting. The MI was determined to be caused by subacute thrombosis, despite the preventative drug therapy. The patient's genotype was investigated and found to be CYP2C19*2/*3, and she was classified as a poor metabolizer. Cilostazol, as an additional antiplatelet agent, was added to the patient's medications, in order to overcome the low responsiveness to clopidogrel. Four months later, the patient had experienced no further cardiac events.[19]

*CYP2C19 genotyping has the potential to help decision-making regarding antiplatelet therapy. Several clinical trials have shown that CYP2C19 genotype affects the efficacy of clopidogrel treatment. Many of the studies showing an effect of CYP2C19 genotype were done in acute coronary syndrome patients treated with clopidogrel, undergoing percutaneous coronary intervention. In these patients, carrying a *2 or other loss-of-function allele was associated with a increased risk of stent thrombosis, cardiac mortality, or myocardial infarction.[18-25] As a result, the FDA boxed warning on the clopidogrel label states that alternative treatments should be considered in patients who are CYP2C19 PMs.*

Drug Interactions: Serotonin Syndrome from CYP Polymorphisms and Drug Interactions

A 46 year old woman with HIV and hepatitis C was admitted to the hospital for bacterial peritonitis and evaluation of hepatic transplantation. She began a new antiretroviral drug regimen of darunavir, ritonavir, and emtricitabine/tenofovir. The patient had already been taking escitalopram for depression for five years, and had started taking esomeprazole three days prior to the new antiretroviral regimen. She developed nausea, confusion, dilation of the pupils, myoclonus, deep tendon hyper-reflexia, and rigidity. Serotonin syndrome was suspected, and escitalopram was discontinued.

Escitalopram serum level was measured, as well as CYP2D6 and CYP2C19 genotypes, and CYP3A4/3A5, CYP2D6, and CYP2C19 phenotypes. The serum level of escitalopram was elevated, and the half-life was almost doubled. The patient's CYP2C19 genotype was *1/*2, predicting metabolism between EM and IM, and the CYP2D6 genotype was *5/*10, which predicts a PM phenotype. Phenotyping results showed PM status for both CYP2C19 and CYP2D6, and reduced activity for CYP3A4/5. After escilatopram was discontinued, the serotonin syndrome resolved.[26]

Identification of the patient's CYP2C19 and CYP2D6 genotypes, along with knowledge of inhibitors of CYP2C19 and CYP3A4/5 helped resolve a potentially fatal adverse event that resulted from a drug-to-drug interaction.

Ineffective Therapy: Voriconazole Therapeutic Failure in a CYP2C19 Ultrarapid Metabolizer

A 19 year old male with primary immunodeficiency disease (chronic granulomatous disease) was admitted to the hospital with sepsis secondary to a bacterial infection. The patient had pneumonia, acute respiratory distress syndrome, and multi-organ failure. The bacterial infection was treated with intravenous antibiotics: cefotaxime, meropenem, ceftazidime, trimethoprim/sulfamethoxazole, doxycycline, levofloxacin, and ciprofloxacin. Because of the extreme illness of the patient, and his susceptibility to invasive aspergillosis, empiric treatment with voriconazole was added on day ten. Additional medications included: intravenous pantoprazole or oral lansoprazole and, as needed, acetaminophen, fentanyl, noradrenaline, ketamine, rocuronium, midazolam, and furosemide.

The first measurement of voriconazole in plasma, three days after the initiation of therapy, showed a very high level, so the dose was decreased. Methylprednolisone pulses were given as well, since the patient's condition was continuing to worsen. Subsequent to the initially high plasma level of voriconazole, plasma levels became undetectable even after dose escalation.

Genotyping was performed to detect CYP2C19 variants, and the patient's genotype was found to be *1/*17. The patient was then switched to oral itraconazole for fungal infection prophylaxis. The initially high voriconazole plasma level was attributed to the severe systemic inflammatory response, which is known to inhibit CYP enzyme activity. Once the inflammation was reduced, the patient's CYP2C19 ultrarapid metabolizer phenotype was expressed, and therapeutic plasma levels of drug were not achieved. Although the patient received many concomitant medications, there were not believed to be any clinically significant drug interactions contributing to the low voriconazole plasma levels.[27]

In this case study, CYP2C19 testing allowed the clinician to select an antifungal therapy that would be efficacious for this patient. Since the patient was a CYP2C19 UM, voriconazole plasma concentrations were too low to provide effective fungal infection prophylaxis. Therefore, itraconazole was used instead.

Conclusion

The three case scenarios illustrate the importance of understanding the genetic basis of CYP2C19 drug metabolism for a physician to be able to detect the genotype, and interpret the genotype as an aid in determining therapeutic strategy to predict the variability in drug response.

The drug-metabolizing phenotype of an individual can be determined using genotyping assays. Genotyping results are not affected by drugs, diet or environmental factors. Genotyping assays by molecular methods are fast, reliable and accurate. The interpretation of the genotype result to the phenotype is based mainly on literature, and on the physician's judgment.

There are several FDA-cleared CYP2C19 genotyping assays currently available in the market. The Luminex xTAG® CYP2C19 Kit v3 assay offers a cost-effective genotyping solution with coverage of major, clinically relevant CYP2C19 alleles demonstrating accurate performance across a large number of samples.[7] Preemptive genotyping for 2C19 may help identify patients at risk and may help improve treatment outcomes.

Identification of patient CYP2C19 genotypes may help physicians tailor drug treatment to patients through the selection of appropriate therapies. These measures may improve a physician's ability to impact patient outcome by ensuring maximum drug efficacy with minimal adverse drug reactions.[20]

xTAG CYP2C19 Kit v3 (US-IVD) Intended Use:

The xTAG CYP2C19 Kit v3 is an *in vitro* diagnostic test used to simultaneously detect and identify a panel of nucleotide variants found within the highly polymorphic CYP450 2C19 gene, located on chromosome 10q24, from genomic DNA extracted from EDTA or citrate anticoagulated whole blood samples. The xTAG CYP2C19 Kit v3 is a qualitative genotyping assay which can be used as an aid to clinicians in determining therapeutic strategy for the therapeutics that are metabolized by the CYP2C19 gene product, specifically *2, *3, and *17. The kit is not indicated for stand-alone diagnostic purposes. This test is not intended to be used to predict drug response or non-response.

The xTAG CYP2C19 Kit v3 is indicated for use with the Luminex® 100/200™ instrument or MAGPIX® with xPONENT® software systems.

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