Cytochrome P450 2D6 is thought to be active in the breakdown of 20-25% of all medications prescribed. The potential benefit of CYP2D6 genotype testing is that patients with genotypes that are associated with a higher risk of ineffective therapy or of an adverse event may be identified, and alternative drug may be administered to these patients.

- Clinical Pharmacogenetics Implementation Consortium (CPIC), 2012

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 SNPs that may help predict drug response.

There are several genes responsible for differences in drug metabolism and response. Among the most common are the cytochrome P450 (CYP) genes. 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 breakdown and clearance of clinically prescribed drugs.

Cytochrome P450 2D6 (CYP2D6) Enzyme

Drugs may be metabolized by more than one pathway involving several enzymes of the cytochrome P450 class. Cytochrome P450 enzyme 2D6 (CYP2D6) alone is thought to be active in the enzymatic breakdown of 20-25% of all medicines prescribed[1] including antidepressants, antipsychotics, opioids, beta-blockers, antiarrhythmics, and tamoxifen.

Cytochrome P450 2D6 Nomenclature[3]

CYP = cytochrome P450 2 = genetic family
D = genetic sub-family 6 = specific gene *1 = allele

Note: This nomenclature is genetically based: it has NO functional implication

Many psychotherapeutic drugs are substrates for, or inhibitors of CYP2D6, so this enzyme is of particular interest for patients on such drugs. Drugs metabolized by CYP2D6 are called 'substrates'. Drugs that inhibit CYP2D6 activity reduce availability of CYP2D6 enzyme and are called CYP2D6 inhibitors (Table 1). Inhibitors are likely to increase plasma concentrations of interacting drug.

Table 1: Substrate and Inhibitors of CYP2D6[2]

CYP2D6 Substrates		
Beta Blockers	S-metoprolol Propafenone	Timolol
Antidepressants	Amitriptyline Duloxetine Clomipramine Paroxetine	Desipramine Venlafaxine Imipramine
Antipsychotics	Haloperidol Risperidone	Thioridazone Aripiprazole
Anti-arrythmics	Flecainide Mexiletine	Propafenone
Opioids	Codeine Tramadol	Oxycodone
Others	Dextromethorphan Ondansetron	Tamoxifen

CYP2D6 Inhibitors		
Bupropion Amiodarone Clomipramine Fluoxetine Cimetidine	Chlorpheniramine Paroxetine Doxepin Mibefradil Quinidine	Haloperidol Ritonavir Duloxetine Methadone

Genetics of CYP2D6

Every individual has two CYP2D6 alleles (except for individuals with gene duplications), one inherited from each parent. The combination of these two alleles ('genotype') determines the overall level of CYP2D6 enzyme activity, or phenotype, particular to that combination.

All of the identified polymorphisms associated with CYP2D6 are autosomal recessive. For example, only individuals who are homozygous (such as *3/*3) or compound heterozygous (such as *3/*4) for these polymorphisms are poor metabolizers. Individuals who are heterozygous, with 1 normal gene and 1 polymorphic gene (*1/*3), will have metabolism intermediate between the extensive (normal) and poor metabolizers.[17]

The CYP2D6 gene is a hotbed for germ line mutations, i.e. mutations in the CYP2D6 gene are heritable. Up to 105 different variations in the gene sequence of the CYP2D6 have been described for CYP2D6.[3] The CYP2D6*1 allele is considered the wild-type or "normal" allele, with "normal" enzyme activity. Other CYP2D6 alleles have various mutations compared to CYP2D6*1, that result 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'). Major alleles found in the human cytochrome P450 2D6 enzymes and SNPs associated with those alleles[3] are listed in Table 2.

Table 2: Major human cytochrome P450 2D6 alleles and SNPs[5]

CYP2D6 Genotypes	Single Nucleotide Polymorphisms
*1	None
*2	-1584C>G, 1661G>C, 2850C>T, 4180G>C
*3	2549A>del
*4	100C>T, 1661G>C, 1846G>A, 4180G>C, 2850C>T
*5	deletion
*6	1707T>del, 4180G>C
*9	2613delAGA
*10	100C>T, 1661G>C, 4180G>C
*17	1023C>T, 1661G>C, 2850C>T, 4180G>C
*29	1659G>A, 1661G>C, 2850C>T, 3183G>A, 4180G>C
*41	1661G>C, 2850C>T, 2988G>A, 4180G>C

Pharmacogenetics 101
(Reference: http://www.genome.gov)

- The genetic variation between CYP2D6 alleles may be single nucleotide polymorphisms (SNPs), or it may be structural variations such as deletions or insertions.
- **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.
- **Haplotype** - Combinations of several SNPs together on the same chromosome or in the same gene are called haplotype.
- **Alleles** - Alternative forms of a gene that arise by mutations in the DNA.
- **Genotype** - A genotype is 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, called the phenotype.

CYP2D6 Metabolizer phenotypes

Genetic variation in the CYP2D6 gene plays a major role in inter-individual variability in drug response.[16] This is because the CYP2D6 genotype of a patient to a large extent determines the level of enzyme activity ('phenotype'). CYP2D6 phenotype can be classified into four groups:

- **Extensive metabolizers (EMs)** have normal enzymatic activity, and carry either two wild-type alleles, or one wild-type allele and one decreased activity or null allele.
- **Intermediate metabolizers (IMs)** have decreased enzymatic activity, and carry either two decreased activity alleles, or one decreased activity allele and one null allele.
- **Poor metabolizers (PMs)** have absent enzymatic activity, and carry two null alleles.
- **Ultra-rapid metabolizers (UMs)** have increased enzyme activity, and have gene duplications or multiplications of the CYP2D6 gene (more than two copies of the gene)[6]

The PM phenotype is associated with reduced clearance that may result in inadequate therapeutic effect or an increased risk of adverse reaction.[8] For example, CYP2D6 is an important enzyme in the metabolic pathway of tamoxifen, a medication used for the treatment of metastatic breast cancer, as well as for the adjuvant therapy of breast cancer in post-menopausal women. CYP2D6 is responsible for the conversion of tamoxifen to its active metabolite, endoxifen. Some studies have demonstrated that in poor metabolziers and intermediate metabolizers of CYP2D6, therapeutic effect of tamoxifen is sub-optimal due to lower levels of endoxifen.[10]

On the other hand, due to increased enzyme activity in the UM phenotype, potential for higher toxicity, risk of ADRs or in some cases, therapeutic resistance or inefficacy can result.[7] For example, the opioids such as codeine, tramadol and oxycodone are metabolized by CYP2D6 into active forms. In poor metabolizers, due to reduced or absent metabolite formation, reduced analgesic effect is observed.[11]

In ultra-rapid metabolizer phenotype fatal and serious toxicity have been reported after codeine administration, particularly in pediatric patients and neonates of breastfeeding mothers.[12] In 2013, the Food and Drug Administration (FDA) added a boxed warning to the label of codeine informing prescriber the risk of using codeine to manage pain in children after a tonsillectomy and/or adenoidectomy.

Dose recommendations for certain antidepressant drugs based on CYP2D6 genotype have also been published.[9]

Phenotypic variability from altered CYP2D6 gene is well characterized in the Caucasian population, while data is limited for African and Asian populations (Table 3). Up to 10% of Caucasians, 20% of Africans and 21% of Saudi Arabians (not listed in table) have poor metabolizer phonotypes.[13] CYP2D6*4 is the most frequent poor metabolizer phenotype seen in 18-20% of the Caucasians and 6-9% of the African American population.[5]

Table 3: Variability in CYP2D6 Phenotype[4]

2D6 Phenotype	Caucasians	Africans	Asians
Extensive	60-80%	-	-
Intermediate	10-15%	~30%	~50%
Poor	5-10%	highly variable	Rare
Ultra-rapid	1-10%	-	-

Clinical Applications of CYP2D6 Testing

Opioids: Death of a Breastfeeding Infant Due to Opioid Toxicity

A full-term healthy male infant presented with intermittent difficulty with breastfeeding and lethargy starting at day 7 after birth. At a well-child checkup on day 11, he had regained his birth weight. However, on day 12 he had grey skin and decreased milk intake, and on day 13 he was found dead. A post-mortem analysis found that he had no anatomical abnormalities, but he had an extremely high blood concentration of morphine, at 70 ng/mL. Typical serum concentration for a breastfeeding infant is zero to 2.2 ng/mL.

The baby's mother had been taking codeine and paracetamol (known in some countries as acetaminophen) for episiotomy pain (30 mg codeine and 500 mg paracetamol initially every 12 hours, then after day 2, half of that amount), for 14 days after the birth. Her stored breast milk was tested for morphine (the active metabolite of codeine) and the concentration was very high: it was 87 ng/mL, and typical concentrations in breast milk are 1.9 to 20.5 ng/mL, at a maternal codeine dose of 60 mg every 6 hours.

CYP2D6 genotyping showed that the mother had a gene duplication of CYP2D6. This genotype results in the UM phenotype, and for pro-drugs like codeine, leads to increased plasma concentration of the active metabolite, morphine. This is consistent with the baby's symptoms of opioid toxicity, and with the high levels of morphine found in the stored breast milk and in the baby's blood.[14]

Antipsychotics: Serious ADR to Risperidone Due to CYP2D6 Variants

A 41 year old Japanese woman, diagnosed with schizophrenia at age 24, had relapsed with schizophrenic symptoms while taking quetiapine. The patient was admitted to the hospital and quetiapine dose was increased. However, the patient did not stabilize, so risperidone was added to her treatment.

Two days after beginning risperidone, the patient experienced excessive sweating, hypertonia and altered consciousness. The patient had an elevated creatine phosphokinase level, indicating injury to muscle tissue, heart or brain.

These signs led to a suspicion of neuroleptic malignant syndrome, a severe and sometimes lethal adverse effect associated with antipsychotics. The patient was treated in the ICU and antipsychotic medication was discontinued. She was suspected of having sensitivity to risperidone based on this episode and a previous episode of atypical neuroleptic syndrome while taking a lower dose of risperidone. Since risperidone is primarily metabolized by CYP2D6, the patient was tested for CYP2D6 variants. PMs for CYP2D6 have an increased risk of ADRs on antipsychotics since reduced metabolism of drug leads to a higher plasma concentration.

Genetic testing of this patient revealed two CYP2D6 variants that would result in a PM phenotype. After recovery from the ADR, the patient's schizophrenia was successfully treated with olanzapine, which is not principally metabolized by CYP2D6.[15]

Cardiology: Drug Toxicity and Delirium Due to Drug Interaction

A 69 year old Caucasian female was admitted to the hospital for confusion and paranoia that had been worsening over several days. She had been undergoing treatment in a rehabilitation center for complications from a laminectomy for lumbar stenosis. The patient was taking multiple medications: carvedilol 12.5 mg twice a day, warfarin 2 mg/day, flecainide 100 mg twice a day, folic acid 1 mg/day, levothyroxine 100 µg/day, pantoprazole 40 mg/day and paroxetine 40 mg/day. The most recent addition to the patient's medications was the anti-arrhythmic drug, flecainide, which had been initiated 2 weeks prior to atrial fibrillation. The flecainide concentration upon hospital admission was very high, outside of the expected range. Other blood work was within normal ranges.

After hospital admission flecainide dosage was reduced. The hypothesis for this patient was that the flecainide plasma concentration

was high because of a drug interaction with paroxetine, and the high plasma concentration was causing an adverse drug reaction to flecainide. After 3 days of discontinuing paroxetine, the confusion and paranoia resolved.

Paroxetine had been started 5 years prior for a major depressive episode and is known to inhibit CYP2D6 activity. Flecainide is metabolized by CYP2D6 and therefore the plasma concentration of flecainide could be affected by drugs that inhibit CYP2D6 activity.[16] Genotyping to understand the metabolizer status of patient may have helped determine the right dosage of flecainide for the patient.

Conclusion

The three case scenarios illustrate the importance of understanding the genetic basis of CYP2D6 drug metabolism. Detecting the genotype can help predict the variability in drug responses.

Laboratory techniques to detect drug response variability exist currently. Phenotyping and /or genotyping are primary methods used. Phenotyping is carried out by measuring CYP2D6 enzyme activity directly using a probe drug whose metabolism is known to be solely dependent on CYP2D6. The excretion of parent compound and/or metabolite in urine allows calculating the metabolic ratio, which is a measure of an individual's CYP2D6 enzyme activity. Phenotyping is the only way to identify post-translational variation of CYP2D6 activity. However, using a probe drug to measure individual phenotypes has limitations.

The drug-metabolizing phenotype of an individual can also be predicted using assays that determine genotype from a patient sample. 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 two FDA-cleared CYP2D6 genotyping assays currently available in the market. The Luminex xTAG® CYP2D6 Kit v3 assay offers a cost-effective genotyping solution with comprehensive coverage of major, clinically relevant CYP2D6 alleles demonstrating accurate performance across a large number of samples.[5]

Identification of patient CYP2D6 genotypes can 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.[16]

Intended Use

xTAG® CYP2D6 Kit v3 is a device used to simultaneously detect and identify a panel of nucleotide variants found within the highly polymorphic CYP2D6 gene located on chromosome 22 from genomic DNA extracted from EDTA and citrate anticoagulated whole blood samples. This kit can also identify gene rearrangements associated with the deletion (*5) and duplication genotypes. xTAG CYP2D6 Kit v3 is a qualitative genotyping assay which can be used as an aid to clinicians in determining therapeutic strategy for therapeutics that are metabolized by the CYP2D6 gene product. This kit is not indicated for stand-alone diagnostic purposes. This test is not intended to be used to predict drug response or non-response.

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