2. P450 Enzymes and Drug Metabolism

P450

1 gram total / liver

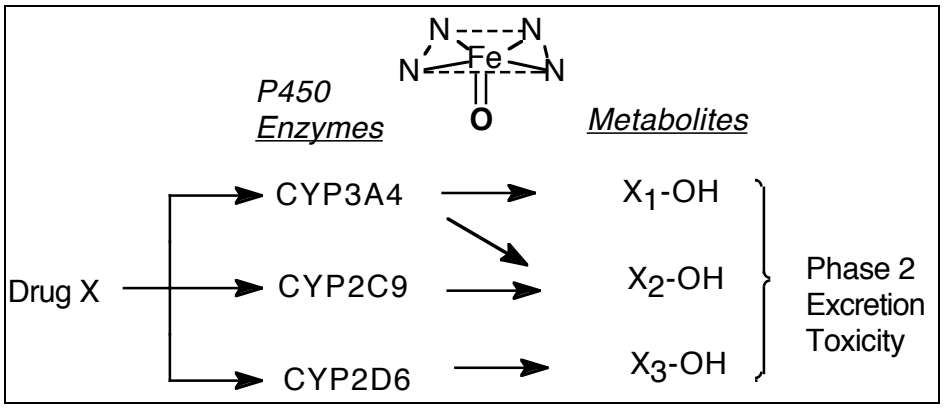
$$NADPH + O_2 + R \longrightarrow R\text{-}OH + H_2O + NADP^+$$

1) Huddled on the endoplasmic reticulum of the liver as well as other tissues such as the enterocytes resides the family of cytochrome P450 enzymes. The P450 enzymes provide the major line of defense against the accumulation of lipophilic compounds in the body. The defense strategy honed by evolution appears to be relatively simple.

   a) Disperse by metabolism into multiple metabolites each invading xenobiotic (drug). Thus the concentration of any metabolite will be lower than the parent drug. The metabolites are polar which facilitates either excretion in the urine or further conjugation reactions and excretion in of the conjugates in urine or bile.

   b) Metabolic dispersion of the drug dose usually involves metabolism of a single compound to multiple metabolites by multiple enzymes.

   c) All other things being equal, conversion of a drug to multiple metabolites by multiple enzymes is a desirable property for drugs and is a major goal for the development of new drugs.



2) Drug metabolism is a major determinant of drug clearance from the body and drug half-life in the blood. We rely on drug metabolism when we dose and would like to treat every individual the same way. Some complications of drug therapy that are due to variability in metabolism:

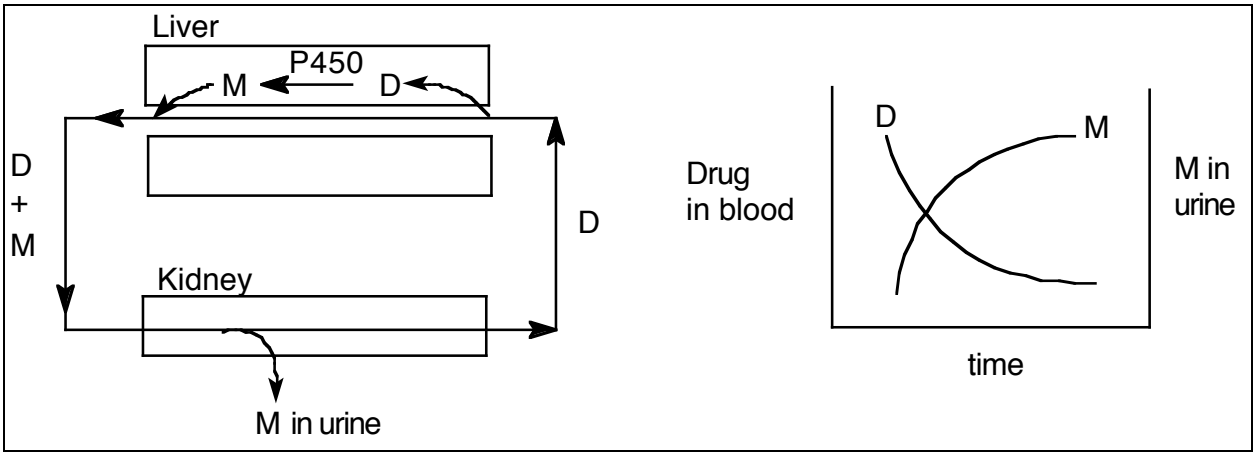
   a) Phenotypic variability (variable levels of enzyme expression in the population at large)

   b) Genotypic variability (polymorphic forms of P450 enzymes)

   c) Enzyme induction (many drugs can cause significant increases in the amounts of P450 enzymes causing drug-drug interactions where the object drug levels fall to possibly subtherapeutic concentrations)

   d) Enzyme inhibition (many drugs can inhibit P450 enzymes causing levels of object drug to rise to potentially toxic concentrations.)

e) Properties of metabolites (metabolites of drugs may have pharmacological or toxic effects)



3) Desirable properties of a given drug from the perspective of metabolism.

a) Don't cause DDIs The drug should have minimal inhibitory and inductive effects on drug metabolism enzyme activities at therapeutic concentrations.

b) Metabolic clearance is constant in the population: The ideal drug should be metabolised significantly by multiple enzymes to decrease (1) interindividual variability in metabolic clearance and (2) susceptibility to drug-drug interactions caused by other drugs or ingested compound (grapefruit juice)

c) Metabolites are benign: Cleared to multiple metabolites to decrease the potential for toxicity due to the effect of circulating metabolites.

d) Dosing should be simple in the population: Drug pharmacokinetic profile (half-life; peak blood levels) should be compatible with desired pharmacological effect (e.g. short acting-long acting).

4) The major human P450 enzymes that are responsible for the metabolism of drugs have names.
a) The P450 nomenclature classifies P450's as CYP_ _ _ (example CYP2D6) based on primary amino acid sequences of the enzymes which reflects relatedness.

i) The first number after the CYP stands for the family (1,2,3,4 etc). Enzymes in the same family are > 40% homologous in their amino acid sequences.
ii) The first letter stands for the subfamily. Enzymes with the same first number and first letter are > 55% homologous.

b) Most P450 dependent drug metabolism is carried out by the CYP1, CYP2, and CYP3 families of enzymes.

c) The different P450 enzymes are sometimes called isoforms or isoenzymes.

d) One thing to be aware of in the P450 nomenclature is that P450's of the same designation (say CYP1A2) in different species (rat, rabbit, man) do not have identical amino acid sequences nor do they exhibit identical substrate and metabolite profiles. This species difference in the structures and activity of the P450 enzymes somewhat limits the usefulness of metabolic studies in animals for predicting human metabolism.

5) Variability in drug metabolism is significant and therapeutically relevant (dosing to effect).

   a) A person's ability to process a particular drug and clear it from the body is largely controlled by the amounts of the relevant P450 enzymes contained in that individual's liver.

   b) What this means is that the clearance of drug from the body and half-life of a given drug in the body is highly dependent on the amount of the P450 enzymes that process that drug that exists in that persons liver at that time.

Active P450 Levels Relative to the Population at Large	Clearance (Cl)	Half-life ($t_{1/2}$)	Effect on Average Blood Levels or AUC	Dose Adjustment Required?
Reduced Enzyme Activity or Amount	↓	↑	↑	↓
Increased Enzyme Activity or Amount	↑	↓	↓	↑

6) <u>Four major factors that can affect active P450 levels or activities of the hepatic CYPs in the population.</u>

   a) Baseline interindividual variability in the constitutive levels of P450 enzymes.

   b) Genetic polymorphisms (inherited) where an enzyme is (a) expressed in an altered, less active, form (different amino acid sequence), (b) not expressed at all or multiple gene copies exist on the genome. **CYP2D6**, CYP2C19, CYP2C9 as well as others less important.

   c) The presence of drugs or dietary constituents that induce the levels of P450 enzymes.

   d) The presence of drugs or dietary constituents that inhibit the activity of P450 enzymes.

7) Factor 1 inter-individual variability in content

   a) CYP 3A4, 2C9, 2C19 and 2D6 are the major drug metabolizing enzymes in terms of numbers

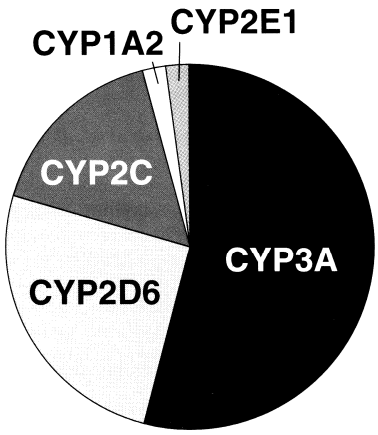


Figure 1–4. The proportion of drugs metabolized by the major cytochrome P450 enzymes.

of drugs metabolized in the liver. CYP2E1 processes and clears the inhalation anesthetics.

b) Reported interindividual variability in activity and content of each P450 enzyme in liver microsomes is significant. Thus we expect variablity in the dose required to achieve a given plasma level in the population. Fortunately we rarely have to vary dose level to match metabolic capacity from individual to individual. Most people cluster around the mean.

Enzyme	Variability in Activity in Liver	Percent of Drugs Metabolized
CYP1A2	40-fold	4%
CYP3A4	30-fold	55%
CYP2D6	30-fold	25%
CYP2E1	7-fold	4%
CYP2C9	5-fold	20%
CYP2C19	50-fold	4%
All P450's total	5-10-fold	>90%

c) Drugs with Narrow Therapeutic Indexes are more difficult to dose effectively due to interindividual variability in metabolism as well other factors that change enzyme activity during therapy (polytherapy; inhibition, induction)

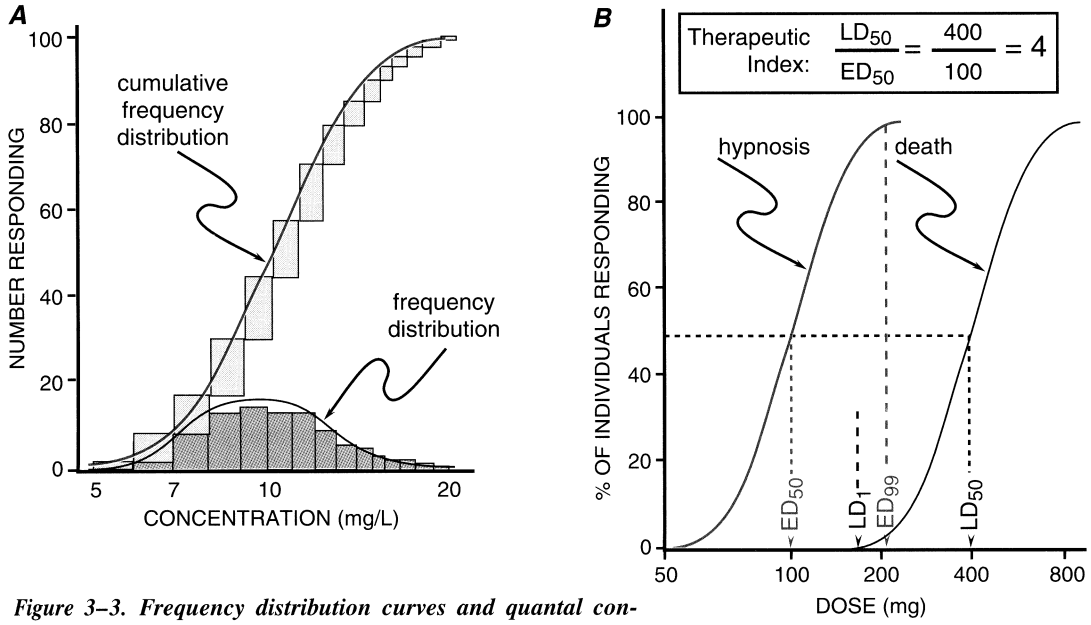


Figure 3–3. Frequency distribution curves and quantal concentration–effect and dose–effect curves.

i) When a drug has a narrow therapeutic index the patient is at relatively higher risk of underdose or overdose. It is often necessary to titrate the dose of the drug in order to match the drug metabolizing capacity in that particular patient. Dosing algorithms can be used to systematically arrive at the best dose while minimizing the risk to the patient. Warfarin, theophylline, phenytoin and the tricyclic antidepressants are examples of these kinds of drugs.

ii) Conversely, when the toxic threshold of a drug is much higher than therapeutic concentrations (wide therapeutic index; desirable) dosing to desired effect is less problematical since a wide range of drug concentrations can achieve the desired pharmacological effect without toxicity.

8) **Factor 2: Genetic polymorphisms create sub-populations of patients that may require individual therapy strategies**: Most P450 genetic polymorphisms can be categorized as follows:

   a) When a variant form of the wild type enzyme P450 with normal, reduced or no activity is expressed due to inherited differences in gene sequences (common)

   b) When the altered DNA sequence does not produce enzyme (sometimes).

   c) When an upstream site of mutation in the DNA exists that changes how much native enzyme is expressed (rare) or duplicate copies of the active gene exist (also rare).

   d) The important polymorphisms are for CYP2D6, CYP2C9 and CYP2C19. Many of these are too rare (low frequency) to worry about.

Table 1. Chromosomal Location of CYP2 Genes and Predicted Enzyme Activities of Related Alleles[9]

CYP	Chrom[a]	Increased	Normal	Decreased	Null
2C9	10	*8?	*1A	*2, *3, *5, *11, *12, *13, *14, *16, *18, *26, *28, *30, *34	*6, *15, *25
2C19	10	*17	*1A	*8, *9, *10, *27	*2, *3, *4, *5, *6
2D6	22	*1xN, *2xN, *35 x 2, *53	*1A, *2A, *27, *33, *35, *39, *48	*9, *10, *17, *18, *29, *36, *41, *47, *49, *50, *51, *54, *55, *57, *59, *69, *72	*3, *4, *5, *6, *8, *11, *12, *13, *14, *15, *16, *19, *20, *21, *31, *38 ... more

Abbreviation: Chrom, chromosome.

9) The most important P450 polymorphism in drug metabolism is the **CYP2D6 polymorphism**. Note all of the known polymorphisms shown above and classified for type of effect. The important null mutant is CYP2D6*4. One reason for that this enzyme polymorphism is so important is that many cardioactive and neuroactive drugs are significantly metabolized by this enzyme (narrow Therapeutic Index).

   a) Thus the pharmacokinetics of CYP2D6 metabolized drugs are partially under genetic control so we need to classify the genotypes in terms of phenotypic response. This nomenclature (EM, IM, PM) classifies via phenotype and is conserved for polymorphisms for the other P450 enzymes.

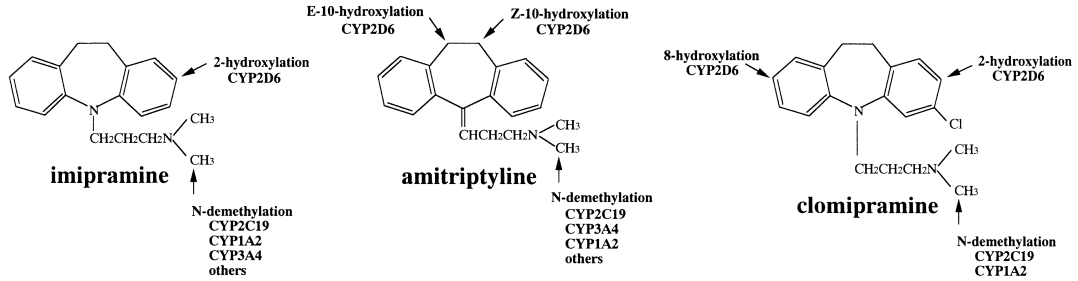
      i) **EM** Extensive Metabolizers (Homzygous for the wild type): Normal, two copies of the wild type gene (normal drug metabolism).

      ii) **IM** Intermediate metabolizers (Heterozygous for wild type: One copy of the wild type and one copy of the mutant gene are present and usually expressed. Impaired metabolism is observed in population studies but is normally not a problem for drug metabolism so practically speaking this group can be lumped with Extensive Metabolizers (normal group)

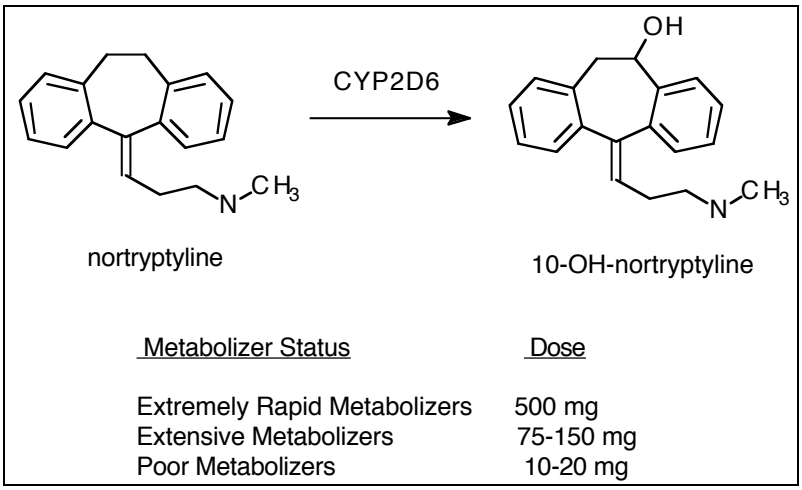
      iii) **PM** Poor Metabolizers (PM's) (Homozygous for a defective gene); About 7-10% of the Caucasian population and 2% of the Asian population do not express any functional enzyme as a result of two copies of "defective" genes. These individuals are called Poor Metabolizers (PM). As far as we can tell the poor metabolizer status has no effect on an individual's health except when it comes to drug therapy.

iv) Extremely-rapid Metabolizers (Multiple copies of wild type) Other individuals have multiple copies of the gene for the active enzyme (up to 13). These individuals have high levels of active enzyme and are called ultra-rapid metabolizers. They are relatively rare.

b) The pharmacokinetics of the tricylic antidepressants are dependent on the activity of CYP2D6. CYP2D6 tends to metabolize at locations about 8 angstroms from an amine functional group.



Here we see the effect of CYP2D6 metabolizer status on the dose requirements of nortryptyline.



Metabolizer Status	Dose
Extremely Rapid Metabolizers	500 mg
Extensive Metabolizers	75-150 mg
Poor Metabolizers	10-20 mg

c) CYP2D6 Poor Metabolizers do not receive analgesic benefit from codeine:

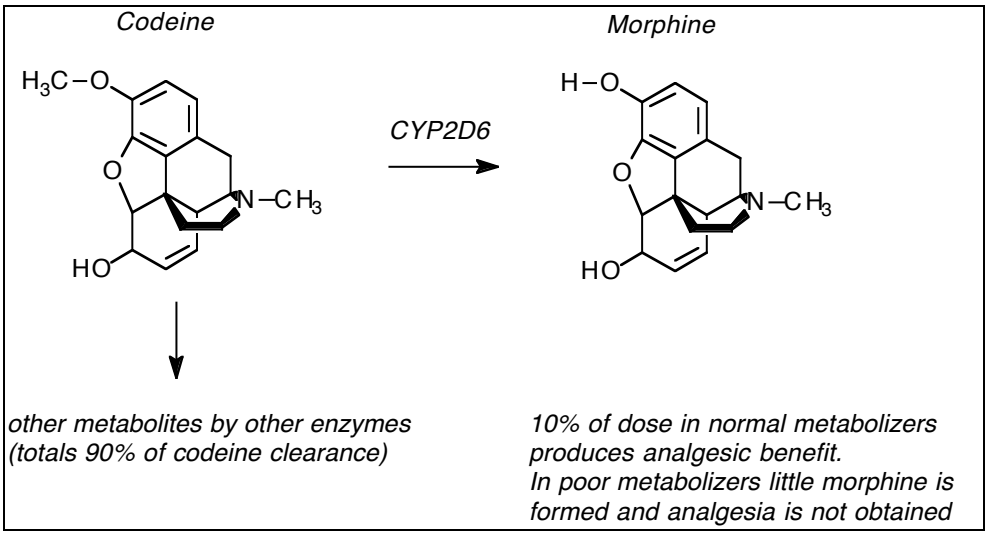
i) An interesting example is codeine which is a drug we have already looked at with respect to metabolism (O-dealkylation). Codeine itself is a cough suppressant.

ii) Codeine is also used for analgesia however it's pain-reducing effects are largely due to a metabolite morphine. Codeine itself does not produce clinically significant analgesia.

iii) CYP2D6 converts approximately 10% of a dose of codeine to morphine via an O-demethylation reaction. Thus CYP2D6 poor metabolizers receive little analgesic benefit from codeine since they cannot convert it to morphine. Cough suppression (a different receptor) is still observed.

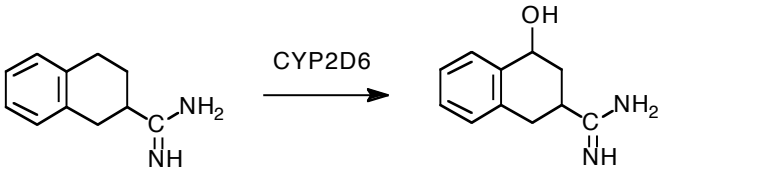
iv) Since only 10% of the codeine dose is cleared by CYP2D6 toxic levels of codeine are not observed in poor metabolizers.

d) Testing for the CYP2D6 polymorphisms: These "defects" are genetic so the following is true.



Once a poor metabolizer, always a poor metabolizer. Since the metabolic clearance of many drugs is controlled by CYP2D6, it would seem that poor metabolizer status would be a good thing to know and include in a patient's medical records.

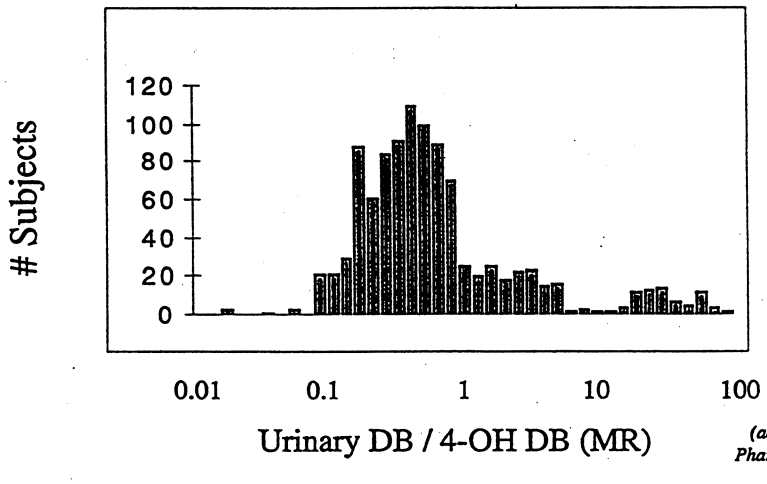
i) Testing for Phenotype using single doses of test drugs and looking for the ratio of parent drug to CYP2D6 metabolite in urine.



Debrisoquine (DB) *4-OH-Debrisoquine (4-OH-DB)*

$$\text{Metabolic Ratio (MR)} = \frac{[\text{DB}]}{[\text{4-OH-DB}]}$$ in a six hour urine after a test dose

MR is inversely related to CYP2D6 dependent clearance so high MR means low clearance (PM'S)



(adapted from Dahl et Pharmacogenetics 3:61.

ii) Testing for Genotype: DNA analysis (bucal swab or blood sample and PCR test) provides genotype information.

CYTOCHROME P450 DNA TYPING REPORT, GENE CYP2D6

Patient Name:_____ Patient ID:_____ LPH ID:_____

Patient Date of Birth:_____ Date of specimen receipt into laboratory:_____

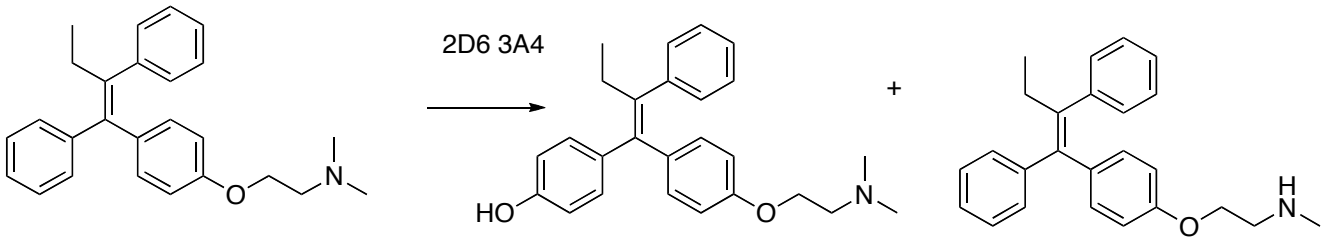
Name of Physician/Authorized person requesting test:_____

ALLELES	CARRIER STATUS	METABOLIZER STATUS
☐ WT		
☐ *5	☐ Gene Duplication	☐ Ultra-rapid
☐ *17		
☐ *6	☐ Normal	☐ Functional
☐ *4		
☐ *3	☐ Carrier	☐ Deficient
☐ *9		
☐ *10	☐ Double	☐ Null
☐ Duplication		
☐ Other: ____		

DNA Drug Sensitivity Test (DST) Cytochrome P450 CYP2D6 alleles tested:

Active alleles: CYP2D6 *1 or *2
Partially active alleles: CYP2D6 *9 or *10 or *17 or *41
Inactive alleles: CYP2D6 *3 or *4 or *5 (deletion) or *6 or *7 or *8 or *11 or *12 or *14 or *15
Gene Duplication: CYP2D6 *1 or *2 or *4 or *10 or *41
Analytical specificity and sensitivity for detection of these mutations are 99%. Other known variants not listed are not detected (< 5% of the population for Caucasians).

iii) Personalized Medicine: A great deal of effort is being expended to develop methods to reduce the error rates in drug therapy. The anticancer agent tamoxifen binds to the estrogen receptor. Metabolites are equal or more active than parent. CYP2D6 makes the metabolites. There is much interest in determining whether genotype can be used to guide therapy. Run a PubMed on "tamoxifen CYP2D6 genotype" and stand back. Note: Dave Veenstra (Dept Pharmacy works on this problem)

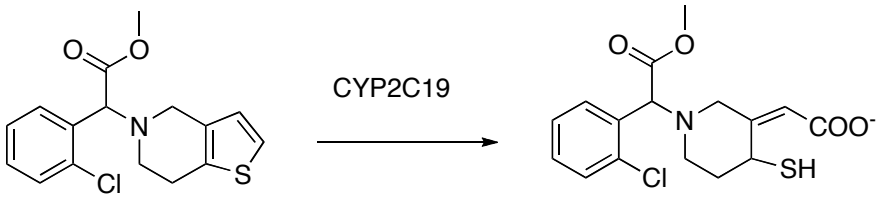


Tamoxifen

acitive metabolites bind much more tightly
to the estrogen receptor than tamoxifen
amoxiphene (hydroxymetabolite) under development

10) **CYP2C19 polymorphism**. Major polymorphism is CYP2C19*2 which is inactive. 2-5% of caucasians and 15-20% of asians are poor metabolisers) .

a) A somewhat controversial black box warning that has been recently put on Plavix which must be metabolized to an active metabolite to have an anticoagulant effect**. CYP2C19 poor metabolizers are at risk of clots after surgery.**



Plavix
Inactive

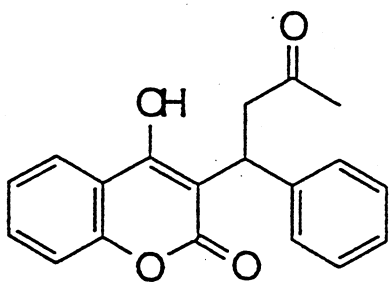
Plavix Metabolite
Active

b) Note that omeprazole (Prilosec) is potent inhibitor of CYP2C19 and causes a significant DDI when coadministered with Plavix.

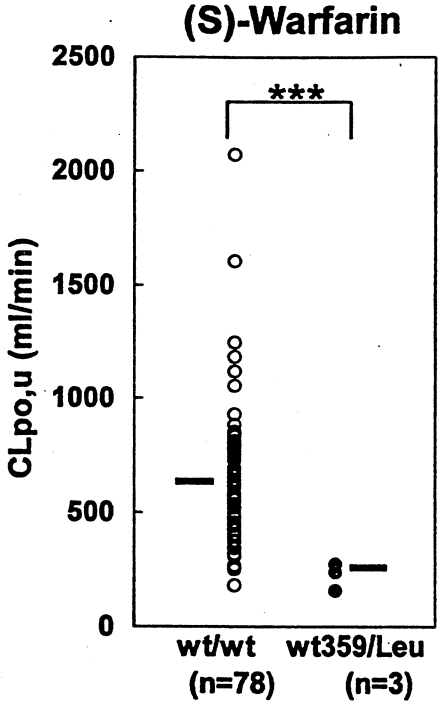
11) CYP2C9 polymorphism The major polymorphism for CYP2C9 is a single amino acid change due to single nucleotide change (SNP) in the gene. Single nucleotide changes are called SNP's. The altered enzyme (CYP2C9*3) is still metabolically active however it is a much poorer catalyst. The metabolism of (S)-warfarin to the 6 and 7 hydroxymetabolites by CYP2C9 is responsible for 85% of the metabolic clearance. The major low therapeutic index drug cleared by CYP2C9 is warfarin. Dose reductions of as much as 10-fold may be required for CYP2C9 PM's although they are rare.

Enzyme	Vmax (nmol/nmol CYP/min)	Km (μM)	Vmax/Km (efficiency)
CYP2C9*1 (wild type)	210	6	35 (100)
CYP2C9*3	41	30	1.3 (4)
Ratio	5 slower metabolism	5 fold lower affinity	25 fold lower intrinsic clearance

a. Below we see that the clearance of (S)-warfarin is lower in individuals that express one copy of the wild type enzyme (CYP2C9*1) and one copy of the leucine to isoleucine mutant (CYP2C9*3). The dose of warfarin in these IM individuals must be reduced to avoid toxic effects because the clearance of warfarin is reduced.



- CYP2C9 is the exclusive catalyst of the 7- and 6- hydroxylation of S-warfarin; major pathway of elimination and for loss of pharmacological activity.



- measurement of *S*-warfarin AUC or the 7-hydroxy formation clearance used as a pharmacogenetic measure *(Takahashi et al., CPT 63:519-28, 1998).*

Fig. 2. Unbound oral clearance ($CL_{po,u}$) for warfarin enantiomers obtained for 78 patients with *CYP2C9* wt/wt (*open circles*) and 3 patients with *CYP2C9* wt/Leu_{359} (*shaded circles*) and median values for the respective enantiomers (*horizontal bars*). ***$p < 0.001$, NS (not significant) for comparisons between the two genotype groups.

b. Warfarin has a narrow therapeutic index and is always dosed to effect (INR) which is a measure of how long blood takes to clot. Warfarin is dosed chronically. IM's require on average a warfarin dose that is 1/3 to 1/2 of the dose in the wild type (EM) population (2 mg/day vs 6 mg/day). The dose requirement of Phenytoin , another CYP2C9 substrate, also is lower in CYP2C9 IM than EM's.

c. The CYP2C9*1/CYP2C9*3 genotype confers intermediate metabolizer status (IM) and is reasonably rare <3%. Two small studies suggest that preknowledge of genotype does not improve the time required to normalize an individual on warfarin. However a large multi-center trial is currently ongoing to test this hypothesis.