

Cytochrome P-450 2D6 Genotyping

CYP2D6 (cytochrome P450 2D6) acts on one-fourth of all prescription drugs, including the selective serotonin reuptake inhibitors (SSRI), tricyclic antidepressants (TCA), beta-blockers, opiates, neuroleptics, antiarrhythmics and a variety of toxic plant substances. Some 7-14% of the population has a slow acting form of this enzyme and 7% a super-fast acting form. Thirty-five percent are carriers of a non-functional CYP2D6 allele, especially elevating the risk of adverse drug reactions when these individuals are taking multiple drugs. Drugs that CYP2D6 metabolizes include Prozac, Zoloft, Paxil, Effexor, Hydrocodone, Amitriptyline, Claritin, Cyclobenzaprine, Haldol, Metoprolol, Rythmol, Tagamet, Tamoxifen, and the over-the-counter diphenylhydramine drugs, Allegra, Dytuss, and Tusstat. CYP2D6 is responsible for activating the prodrugs codeine and other opioids into their active forms. The analgesic activity of the drugs is therefore reduced or absent in CYP2D6 poor metabolizers. Refer to list for substrates, inhibitors and inducers of CYP2D6.

Genelex offers improved detection rates using an extended Cytochrome P-450 2D6 DNA mutation panel. This test identifies 17 small nucleotide variants and two gene rearrangements in PCR-multiplex format, providing increased sensitivity and quality performance. This CYP2D6 Mutation Detection Panel is the most extensive on the market and covers over 93-97% of poor metabolizer phenotypes. Analytical specificity and sensitivity for detection of these mutations are >99%.

Indication for Testing

For individuals with a personal or family history of adverse drug reactions to medications metabolized by CYP2D6. Confirm presence of genotypes that affect the metabolism of drugs such as tamoxifen that are metabolized by CYP2D6.

Specimen Information

Please call Client Services at 800-523-3080 to obtain specimen kits.

- **Buccal Swabs:** 4 sterile Buccal Swabs
- **Blood:** 5-10 cc whole blood lavender-top EDTA or Yellow-top ACD-A tubes
- **Turnaround Time:** 10 days, 5 Day STAT

CPT Codes

CYP2D6 Mutation DNA Analysis (provided for your guidance only)
1 X 83891, 4 X 83892, 1 X 83900, 2 X 83901, 19 X 83914, 1 X 83909

Clinical Significance

Phenotype prevalence is approximately 10 % PM, 7% UM, and 35% IM.
Drugs metabolized by this enzyme - approximately 25%.
Low-capacity, high-affinity enzyme.

Cytochrome P450 2D6 (CYP2D6) is a highly polymorphic liver enzyme of the cytochrome P450 super family involved with the metabolism and elimination of many commonly prescribed drugs. Genetic polymorphism in CYP2D6 is common and can affect therapeutic response to these drugs. The enzyme activity is expressed at highly variable levels.

Detecting genetic variations in drug-metabolizing enzymes is useful for identifying individuals who may experience adverse drug reactions with conventional doses of certain medications. Individuals who

possess CYP2D6 poor metabolizer variants may exhibit different pharmacokinetics (drug levels) than normal individuals. As a result, such individuals may require non-conventional doses of medications that require CYP2D6 activity for biotransformation. Conversely, medications that do not require CYP2D6 biotransformation may be preferentially selected for patients with potentially impaired CYP2D6 metabolic capacity to avoid adverse drug reactions.

CYP2D6 is considered a low-capacity, high-affinity enzyme and CYP2D6 will preferentially metabolize drugs at lower concentrations. As the concentration of a drug increases, the metabolism spills over to CYP3A4 and CYP1A2, which are high-capacity, low-affinity enzymes. Thus if a drug that has several metabolic pathways but relies on CYP2D6 as its major pathway is given to a patient with poor CYP2D6 activity, the other P-450 enzymes that are high capacity, low affinity will clear the drug, but clearance will be slower and less efficient, and drug levels will increase, increasing the risk for adverse drug reactions.

Four phenotypes are identified: poor metabolizers (PM), ultrarapid metabolizers (UM), intermediate metabolizers (IM) and normal metabolizers (NM).

Normal functional activity alleles of the CYP2D6 gene are designated CYP2D6*1 and CYP2D6*2. Homozygous normal allele individuals have a normal metabolizer phenotype (NM). The alteration of alleles from normal fall into six mutant allele categories: one amino acid change or deletion, frameshift, splicing defect, stop codon, insertion and entire gene deletion. Ultrarapid metabolizers (UM) have duplicate or multiple copies of the complete CYP2D6 gene.

Some 7-14% of Caucasians are poor metabolizers (PM) and lack functional CYP2D6. The genetic basis for poor metabolizers is now well defined. The four most common mutant alleles are CYP2D6*3, CYP2D6*4, CYP2D6*5, and CYP2D6*6 and account for 93-97% of the PM phenotypes in the Caucasian population. The most common of these alleles, CYP2D6*4 has a reported frequency of 21.5-28.6% and involves a base substitution from G to A at position 1846, which causes a splicing defect in exon 3. CYP2D6*3 has a reported frequency of 2.7% and involves a deletion of at position 2549, causing a frameshift in exon 5. In the relatively common PM allele CYP2D6*5 (2.6%) the entire CYP2D6 gene is deleted. Individuals who are homozygous for PM alleles do not display CYP2D6 enzyme activity, nor do any those who carry combinations of these alleles. Additional alleles CYP2D6*6-8, *11-16, *19-20 and *38 are also associated with lack of enzyme activity identified with buparolol, dexromethorphan, debrisoquine or sparteine. However, these alleles are rare. Some 35% of Caucasians are intermediate metabolizers (IM) with a combination of one functional CYP2D6 and one mutant CYP2D6 allele.

There are ethnic differences in distribution of PMs, IMs and UMs. PMs are reported to make up 7-14% of populations of European origin (Caucasian) with CYP2D6*4 being the predominant non-functional allele observed. A recent review indicated that Asians, Pacific Islanders, African and African Americans have higher percentages of reduced functional or non-functional CYP2D6 alleles (between 40% and 50%) than do Europeans (26%). Therefore the percentages of PMs in the former groups are most likely higher. Pacific Islanders have a high frequency (41%) of a reduced functional allele CYP2D6*10, indicating slower metabolism. Non-functional PMs and reduced function IMs represent about 50% of African populations (non functional CYP2D6*17 represents 35% of allele variation). African Americans show twice the allele frequency of PMs compared with Africans (14.5% vs 6.3%).

Not anticipating that patients may be PMs is potentially dangerous. Any drug that is primarily metabolized by CYP2D6 and ingested by a PM will have a delayed metabolism. A drug may also be less effective for a PM at CYP2D6 if the drug needs to be activated by CYP2D6. PMs usually require lower doses to achieve desired effects. The PM will accumulate the parent drug and incur the risk of enhanced side effects. The drug may be secondarily metabolized by another P450 enzyme that is higher in capacity

but that has a lower affinity for the drug or substrate. Often the alternative is CYP3A4. This shifting to a less efficient enzyme leads PMs to have higher drug levels of the parent compound. Evidence suggests that PMs at CYP2D6 have a poorer tolerance of side effects and higher drug levels, even though the CYP3A4 route is available.

Individualized drug therapy based on knowledge of genetic polymorphisms can be used to improve drug therapy and decrease the incidence of adverse drug effects.

Laboratory Test Interpretation

Genelex offers improved detection rates using an extended Cytochrome P-450 Tag-It™ 2D6 DNA mutation panel. This test identifies 17 small nucleotide variants and two gene rearrangements in PCR-multiplex format, providing increased sensitivity (93-97% of poor metabolite phenotypes) and quality performance. The 17 variants tested for in this assay represent the most prevalent and phenotypically relevant variations within the CYP2D6 gene.

Cytochrome P-450 2D6 Mutations Detected		
CYP2D6 allele	Variant	Effect on Enzyme Metabolism
*1	None (wild type)	Normal
	Gene Duplication	Increased activity
*2	-1584C>G, 1661G>C ^a , 2850C>T ^a , 4180G>C ^a	Normal
*3	2549A>del	Inactive
*4	1846G>A; 100C>T ^b	Inactive
*5	Gene Deletion	Inactive
*6	1707T>del	Inactive
*7	2935A>C	Inactive
*8	1758G>T	Inactive
*9	2613-2615 delAGA	Partially active
*10	100C>T ^a	Partially active
*11	883G>C	Inactive
*12	124G>A	Inactive
*14	1758G>A	Inactive
*15	138InsT	Inactive
*17	1023C>T, 2850C>T ^a	Partially active
*41	2988G>A	Partially active

For additional information see the CYP2D6 allele nomenclature database at

<http://www.cypalleles.ki.se/cyp2a6.htm>

^b For *4, no *4I designation exists

^a 1661G>C, 2850C>T, and 4180G>C are found in a wide range of alleles

Testing places individuals in one of four categories:

- **Normal metabolizers (NM)** represent the norm for metabolic capacity. In general normal metabolizers can be administered drugs which are substrates of the CYP2D6 enzyme following standard dosing practices. Genotypes consistent with the normal metabolizer phenotype include two active CYP2D6 alleles or one active and one partially active CYP2D6 allele. Increased caution may be appropriate for individuals having one partially active allele.
- **Intermediate metabolizers (IM)** may require lower than average drug dose for optimal therapeutic response to medications with the exception of prodrugs. For the majority of drugs consider decreased dosage. For prodrugs, like tamoxifen, that require activation by CYP2D6, an alternative treatment or increased dose should be considered. Genotypes consistent with the intermediate metabolizer phenotype are those with one active and one inactive CYP2D6 allele, one inactive and one partially active CYP2D6 allele, or two partially active CYP2D6 alleles.
- **Poor metabolizers (PM)** are at increased risk of drug-induced side effects due to diminished drug elimination or for prodrugs, like tamoxifen, lack of therapeutic effect resulting from failure to generate the active form of the drug. Alternative treatment should be considered. Genotypes consistent with the poor metabolizer phenotype are those with no active CYP2D6 alleles.
- **Ultra metabolizers (UM)** exhibit higher than average rates of metabolism. Ultra metabolizers are at increased risk of therapeutic failure due to increased drug elimination and thus may require an increased dose of drugs that are inactivated by CYP2D6. For prodrugs, ultra metabolizers may also be at increased risk of drug-induced side effects due to increased exposure to active drug metabolites, in which case they may require lower than average doses. Genotypes consistent with ultra metabolizer phenotype include three or more active CYP2D6 alleles due to duplication of an active allele.

Co-administration of other drugs. Genotype results should be interpreted in context of the individual clinical situation. In all cases monitor for co-administration of CYP2D6 inhibitors which may convert patients to poor metabolizer status. Potential adverse outcomes included overdose toxicity or treatment failure particularly for prodrugs. For more information see GeneMedRx drug-drug and drug-gene interaction software and Cytochrome P450 Metabolism Inhibitor/Inducer Tables. Access GeneMedRx via the patient access code provided at www.GeneMedRx.com/DNAlogin.

Dosage Recommendations

Therapeutic drug monitoring is recommended in patients with metabolic variations. Keep in mind that subjects with metabolic deficiency will have decreased drug clearance and require additional time to achieve steady-state. In contrast, subjects with increased metabolic activity (UMs) have increased drug clearance and will achieve steady-state sooner than extensive metabolizers.

A complicating factor in correlating CYP2D6 genotype with phenotype is that many drugs may reduce or increase CYP2D6 catalytic activity. Consequently, an individual may require a dosing decrease greater than predicted based upon genotype alone. It is important to interpret the results of testing in the context of other co-administered drugs.

Patients with an extensive or intermediate metabolizer genotype may have CYP2D6 enzyme activity inhibited by a variety of medications or their metabolites, including many TCAs, SSRIs, many histamine H1 receptor antagonists, amiodarone, celecoxib, cimetidine, cocaine, methadone, quinidine, and ritonavir, as well as several other drugs. Treatment with drugs that are inhibitors of CYP2D6, or produce inhibitors through metabolism, may generate a poor metabolizer phenotype in an individual who has an extensive or intermediate metabolizer genotype.

CYP2D6 activity also is dependent upon hepatic and renal function status, as well as age. CYP2D6 activity does not appear to change with age; however, CYP2D6 activity may appear to be altered because of age-associated changes in hepatic blood flow or a decrease in renal elimination of metabolites. It is important to interpret the results of testing and dose adjustments in the context of renal and hepatic function and age.

- **Poor Metabolizers**

Avoid medications that are altered to their active form through CYP2D6, such as tamoxifen and opioids. (For instance, 10% of a codeine dose is transformed to morphine through demethylation in the liver.)

Reduce dosage 6-10 fold for medications that are administered in their active form and metabolized through CYP2D6 as are many antidepressants. (Desipramine, for example, is absorbed from the gastrointestinal tract following oral administration and is extensively bound to tissue and plasma proteins in the order of 90-95%. It is metabolized by hydroxylation and by further demethylation in the liver.)

If you are uncertain, contact the drug manufacturer or look up the pharmacology data. Therapeutic drug monitoring is recommended for PMs to confirm that steady-state drug concentrations are within the therapeutic target interval.

- **Ultrametabolizers**

Increase dosage 2-5 fold depending on the number of duplications noted in the report. Success has also been achieved by concurrently administering another substrate or an inhibitor of CYP2D6.

- **Intermediate Metabolizers**

Start IMs at lowest efficacious dose and avoid multiple drug therapy that inhibits or activates through the same pathway.

Changes in metabolic capacity for an individual does not change the pharmacologic action of the medication. Therefore standard therapeutic drug concentration target intervals can be used to optimize dosage titration. The advantage of knowing the subject's genotype is in predicting the general dosage range for initiation and recognizing changes in time to achieve steady-state for interpretation of blood concentration monitoring.

For specific dosages see charts and tables adapted from Julia Kirchheiner, et al Molecular Psychiatry Feature Review, 9 442-473 (2004), "Pharmacogenetics of antidepressants and antipsychotics: the contribution of allelic variations to the phenotype of drug response," a meta analysis of published research from 1970-2003 on the relevance of pharmacogenetic effects of CYP 2D6 and CYP 2C19 on 36 antidepressants and 38 antipsychotics.

Test Methodology and Limitations

DNA extraction / Polymerase Chain Reaction (PCR) / Bead Hybridization.

This assay detects all common and most rare CYP2D6 variants with known clinical significance. Laboratory specimens were analyzed using the xTAG™ Mutation Detection system for P450-2D6 (Luminex Molecular Diagnostics) which detects 17 nucleotide variants and two gene rearrangements in a multiplex polymerase chain reaction and allele-specific primer extension format. The performance of the xTAG™ Mutation Detection system for P450-2D6 for use with the Luminex 100 xMAP IS System was validated by Genelex Corporation. Rare CYP2D6 variants may not yet have been observed at Genelex

(<1% of the population). As with all laboratory testing there is a possibility of error. Genelex Corporation is certified by the Clinical Laboratory Improvement Amendments (CLIA No. 50D0980559) and as Washington State Medical Test Site No. MTS-3919 is qualified to perform high complexity clinical testing. Genetic counseling is recommended.

DNA testing will not detect all the known mutations that result in decreased or inactive CYP2D6. Absence of a detectable gene mutation or polymorphism does not rule out the possibility that a patient has an intermediate or poor metabolizer phenotype. This test does not detect polymorphisms other than those listed. Other polymorphisms in the primer binding regions can affect the testing, and ultimately, the genotyping assessments made. Rare diagnostic errors may occur due to primer site mutations. Mutations in other genes associated with drug metabolism will not be detected. Drug metabolism may be affected by non-genetic factors. DNA testing does not replace the need for clinical and therapeutic drug monitoring.

Drug Metabolism Guide

This list is not all inclusive and is for your guidance only.

Substrates Metabolized through Cytochrome P-450 2D6

Substrates refers to drugs that are either activated or deactivated by the pathway.

italics = brand name {brackets} = minor or less potent bold = potent (p) = pro-drug

alprenolol	dextromethorphan in cough syrup	metoclopramide	Rythmol
amitriptyline	diphenhydramine	metoprolol	{sertraline}
amphetamines	dolasetron(p)	mexiletine	tamoxifen(p)
aripiprazole	doxepin	mirtazapine	thioridazine
atomoxetine	duloxetine	nebivolol	timolol
benztropine	Ecstasy	nortriptyline	tolterodine
<i>Bystolic</i>	encainide	{oxycodone}	tramadol
carvedilol	flecainide	paroxetine	venlafaxine
chlorpheniramine	fluoxetine	perazine	zuclopenthixol
chlorpromazine	flvoxamine	perphenazine	
clomipramine	haloperidol	propafenone	
codeine(p)	hydrocodone (p)	propranolol	
desipramine	imipramine	<i>Reglan</i>	
<i>Detrol</i>	MDMA	risperidone	

Inhibitors of Cytochrome P-450 2D6

Inhibitors refers to drugs that reduce the ability of the pathway to process drugs. Co-administration will decrease the rate of metabolism of drugs through the metabolic pathway listed, increasing the possibility of toxicity.

amiodarone	clomipramine	halofantrine	pimozide
amitriptyline	cocaine	haloperidol	propafenone
bupropion	desipramine	hydroxyzine	quinidine/quinine
celecoxib	diphenhydramine	imipramine	ritonavir
chlorpheniramine	doxepin	levomepromazine	Sensipar
chlorpheniramine	duloxetine	methadone	sertraline
chlorpromazine	escitalopram	metoclopramide	terbinafine
cimetidine	fluoxetine	moclobemide	thioridazine
cinacalcet	fluvoxamine	paroxetine	ticlopidine
citalopram	goldenseal	perphenazine	

Inducers of Cytochrome P-450 2D6

Inducers refers to drugs that increase the activity of a pathway. Co-administration increases the rate of excretion for drugs metabolized through the pathway indicated, reducing the drug's effectiveness.

none

References

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