

Cytochrome P-450 CYP2C9 and VKORC1 Genotyping

Cytochrome P450 2C9 (CYP2C9) acts on 15% of drugs in current clinical use. About 35% of Caucasians have a slow acting form of this enzyme. CYP2C9 is an important drug-metabolizing enzyme that catalyzes the biotransformation of many other clinically useful drugs including angiotensin II blockers, nonsteroidal anti-inflammatory drugs, the alkylating anticancer prodrugs, sulfonyleureas, some antidepressants, tamoxifen and many others. Of special interest are those drugs with narrow therapeutic index, such as S-warfarin, tolbutamide and phenytoin, where impairment in CYP2C9 metabolic activity might cause difficulties in dose adjustment as well as toxicity. Indications for testing include lack of therapeutic effect or difficulties with side effects to any of the drugs metabolized by CYP2C9.

CYP2C9 and VKORC1 variation greatly affect the half life of warfarin (Coumadin) and time to a stable dose. VKORC1 is the site of action of warfarin. The level of the enzyme is under genetic control according to the DNA sequence present in the control region of the gene. Inherited differences in VKORC1 increase or decrease the amount of warfarin needed to inhibit the formation of the clotting factors. When the amount of warfarin exceeds what is needed, the risk of bleeding is increased. Indications for testing include lack of therapeutic effect or difficulties with side effects to warfarin.

This test identifies the eight most common variants of CYP2C9 (see table below) and a VKORC1 polymorphism (-1639G>A) which influences warfarin maintenance dose. 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 warfarin and/or other medications metabolized by CYP2C9. Confirm presence of genotypes that affect the metabolism of any drugs that are metabolized by CYP2C9. Confirm presence of CYP2C9 and VKORC1 (-1639G>A) genotypes that affect metabolism of warfarin (Coumadin).

Specimen Types

Please call Client Services at 800-523-6487 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

CPT Codes

CYP2C9 Mutation DNA Analysis (provided for your guidance only)
1 X 83891, 3 X 83892, 1 X 83900, 2 X 83901, 11 X 83914, 1 X 83909

Clinical Significance

CYP2C9 phenotype prevalence is 2-4% Poor Metabolizer, >35% Intermediate Metabolizer for CYP2C9. Drugs metabolized by this enzyme approximately 15%.

Allele frequencies: (Note: genotype frequencies are higher than allele frequencies as each person has two alleles):

CYP2C9*2: Caucasians 8-19%, African 3-4%, Asians ~ 1%.

CYP2C9*3: Caucasians 3-17%, African 2%, Asians 1-7%

CYP2C9*4: Caucasians <1%, Asians 2%.

CYP2C9*5: African 2%.

CYP2C9*6: African 2%.

CYP2C9*8: African 5-9%, Asians and Hispanics ~ 1%.

CYP2C9*11: Caucasians ~1%, African 3%.

CYP2C9*13: Asians 2%

CYP2C9 and VKORC1 mutations affect warfarin clearance:

- CYP2C9*2 (430C>T) reduces metabolism of s-warfarin by approximately 30%; extends half-life of drug, requiring longer to achieve steady state; average daily Warfarin requirement is approximately 17% lower in patients with one copy of CYP2C9*2 (CYP2C9 *1/*2); allele frequency in Caucasians is approximately 11%.
- CYP2C9*3 (1075A>C) reduces metabolism of Warfarin by approximately 80%; extends half-life of drug, requiring longer to achieve steady state; average daily Warfarin requirement is approximately 37% lower in patients with one copy of CYP2C9*3 (IM CYP2C9 *1/*3); allele frequency in Caucasians is approximately 7%.
- CYP2C9*8 has a metabolism impact similar to that of CYP2C9*3. Inclusion of CYP2C9*8 alone reclassifies the predicted metabolic phenotypes of almost 10% of African Americans, and when combined with CYP2C9*5, *6 and *11, more than 15%.
- VKORC1 (-1636G>A). Average daily Warfarin requirement is approximately 20% lower in patients with one copy of the VKORC1 (-1636G>A) as compared with patients with no VKORC1 mutations (heterozygous G/A); allele frequency in Caucasians is approximately 40%.
- Combinations of mutations from one or more genes will reduce the average daily warfarin requirement further. CYP2C9 accounts for up to 18% of the variability in Warfarin dosing. VKORC1 (-1636G>A) accounts for about 30% and combining genotypes with clinical factors may account for up to 79% of variability in warfarin dosing.

CYP2C9 is a highly polymorphic liver enzyme of the cytochrome P450 super family involved with the metabolism and elimination of many commonly prescribed drugs. Genetic polymorphisms in CYP2C9 are common and can affect therapeutic response to drugs. The enzyme activity is expressed at highly variable levels. Three phenotypes are identified: poor metabolizers (PM), intermediate metabolizers (IM) and normal metabolizers (NM).

This assay detects the most common CYP2C9 and VKORC1 variants with known clinical significance. The two CYP2C9 allelic variants detected in this CYP2C9 genotyping test provide greater than 98% coverage of the variant alleles found for this gene. The wildtype allele of the CYP2C9 gene is designated CYP2C9*1. Homozygous wild-type individuals have a normal metabolizer phenotype (NM). The most common poor metabolizer phenotypes have been identified as CYP2C9*2 and CYP2C9*3. CYP2C9*2 (C430T) and CYP2C9*3 (A1075C) each differ from the normal CYP2C9*1 by a single nucleotide substitution, which leads to impaired enzyme activity. Lee et al (2002) determined that these two poor

metabolizer types CYP2C9*2 and CYP2C9*3 were found in up to 35% of Caucasians (42% Croatians). Among different white populations CYP2C9*2 and CYP2C9*3 are of significance with allelic frequencies of 8-19% and 4-16% respectively. In Africans and Asians both variants are much less frequent (0.5-4%). Homozygosity for the CYP2C9*3 or CYP2C9*2 genotype is relatively rare (~1-2 %) in Caucasians.

Detecting genetic variations in drug-metabolizing enzymes is useful for identifying individuals who may experience adverse drug reactions (ADRs) with conventional doses of certain medications. Individuals who possess CYP2C9 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 CYP2C9 for biotransformation. Conversely, medications that do not require CYP2C9 biotransformation may be preferentially selected for patients with potentially impaired CYP2C9 metabolic capacity to avoid ADRs.

Clinical studies have demonstrated that patients with at least one copy of the CYP2C9*2 allele require a mean daily warfarin dose 17% less than the homozygous wild-type patients. In addition patients with at least one copy of CYP2C9*3 allele had a mean daily warfarin dose 37% less than the homozygous wild-types. In a separate study the risk of over anticoagulation (INR>3) during the first 2 weeks of therapy was approximately double for patients classified as CYP2C9*2 (*1/*2 or *2/*2) or CYP2C9*3 (*1/*3, *2/*3, or *3/*3) compared to NM phenotypes.

Single nucleotide polymorphisms in the VKORC1 gene have been associated with lower dose requirements for warfarin. One study demonstrated that 30% of the variance in warfarin dose could be attributed to VKORC1 polymorphisms.

Laboratory Test Interpretation

Genelex offers improved detection rates using a Cytochrome P-450 CYP2C9 and VKORC1 DNA mutation panel. This test identifies eight of the most common variants of CYP2C9 (see table below) and a VKORC1 polymorphism (-1639G>A) which influences warfarin maintenance dose.

Cytochrome P-450 2C9 Mutations Detected		
CYP2C9 allele	Nucleotide change	Effect on Enzyme Metabolism
*1	None (wild type)	Normal
*2	430C>T	Decreased
*3	1075A>C	Decreased
*4	1076T>C	Decreased
*5	1080C>G	Decreased
*6	818delA	None
*8	449G>A	Decreased
*11	1003C>T	Decreased
*13	269T>C	Decreased

For additional information see the CYP2C9 allele nomenclature database at <http://www.cypalleles.ki.se/cyp2c9.htm>

VKORC1 Mutations Detected		
VKOR allele	Nucleotide change	Effect on Enzyme Metabolism
-1639	-1639G>A	Decreased Transcription

CYP2C9 interpretation:

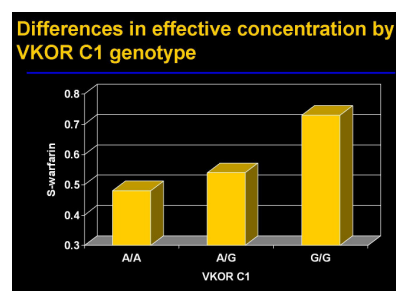
CYP2C9 testing places individuals in one of three categories:

- **Normal metabolizers (NM)** represent the norm for metabolic capacity. In general normal metabolizers can be administered drugs which are substrates of the CYP2C9 enzyme following standard dosing practices. Genotypes consistent with the normal metabolizer phenotype include two active CYP2C9 alleles.
- **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, that require activation by CYP2C9, an alternative treatment or increased dose should be considered. Genotypes consistent with the intermediate metabolizer phenotype are those with one active and one inactive CYP2C9 allele.
- **Poor metabolizers (PM)** are at increased risk of drug-induced side effects due to diminished drug elimination or for prodrugs 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 CYP2C9 alleles.

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 CYP2C9 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.

VKORC1 interpretation:

The -1639 G>A polymorphism of the vitamin K epoxide reductase complex subunit 1 (VKORC1) gene is associated with warfarin sensitivity and decreased maintenance dose requirements of the medication. Presence of the variant A nucleotide confers high sensitivity to warfarin and is associated with a lower warfarin daily dose requirement than the common wild-type, low sensitivity G nucleotide. The VKORC1 (-1639G>A) test identifies how sensitive an individual is to warfarin: low sensitivity (GG), high sensitivity (AA), or intermediate sensitivity (GA).



Warfarin Dosage Recommendations

Current warfarin maintenance dose ranges are subject to extensive inter-individual variability.

Food and Drug Administration¹. New labeling information for warfarin (Coumadin); Approved 1/22/2010 (see Table 1). Ranges are derived from multiple published clinical studies. Other clinical factors (e.g., age, race, body weight, sex, concomitant medications, and comorbidities) are generally accounted for along with genotype in the ranges expressed in the Table. VKORC1 -1639G>A variant was used in this table. Other co-inherited VKORC1 variants may also be important determinants of warfarin dose. Patients with CYP2C9 *1/*3, *2/*2, *2/*3, *3/*3 may require more prolonged time (>2 to 4 weeks) to achieve maximum INR effect for a given dosage regimen¹. These ranges are only recommendations and should be considered along with patient specific clinical factors such as age, race, body weight, sex, concomitant medications, vitamin K intake and co-morbidities.

Table 1: Range of Expected Therapeutic Warfarin Doses Based on CYP2C9 and VKORC1 Genotypes #

VKORC1 genotype	CYP2C9 genotype					
	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3
GG	5-7 mg	5-7 mg	3-4 mg	3-4 mg	3-4 mg	0.5-2 mg
AG	5-7 mg	3-4 mg	3-4 mg	3-4 mg	0.5-2 mg	0.5-2 mg
AA	3-4 mg	3-4 mg	0.5-2 mg	0.5-2 mg	0.5-2 mg	0.5-2 mg

Coumadin Product Insert, Approved 1/22/2010

The time it takes warfarin serum concentrations to reach steady state varies according to CYP2C9 genotype as outlined in the table below. Dose should be adjusted according to phenotype on day 5 to avoid an increase beyond desired levels for IM and PM metabolizers.

CYP2C9 genotype	Time to stable warfarin dose
*1/*1 normal metabolizer	4 - 5 days
*1/*2 intermediate metabolizer	8 - 10 days
*1/*3, *2/*2, *3/*3 intermediate or poor metabolizer	>2 - 4 weeks

WarfarinDosing.org . The Warfarin Dose Refinement Collaboration and International Warfarin Pharmacogenetics Consortium are international collaborations of biostatisticians, geneticists, pharmacists, and physicians who share anonymous data to improve warfarin dosing. Free access is available at www.warfarindosing.org. This algorithm allows input of genetic factors as well as patient factors including INR, age, ethnicity, sex, height, weight, smoking, and liver disease.

Sconce Algorithm . This algorithm allows input of genetic factors as well as patient factors height and age. Access at www.GeneMedRx.com.

It is important to note that the maintenance doses listed are estimates and should be viewed as an example of how this information should be taken into consideration by the physicians a part of the overall patient management strategy.

A complicating factor in correlating CYP2C9 genotype with phenotype is that many drugs may reduce or increase CYP2C9 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.

CYP2C9 activity also is dependent upon hepatic and renal function status, as well as age. Patients also may develop toxicity if hepatic or renal function is decreased. Consider the results of testing and dose adjustments in the context of renal and hepatic function and age.

Therapeutic drug monitoring in Poor Metabolizer and Intermediate Metabolizer patients is highly recommended. Again standard measures of efficacy (INR for Warfarin or therapeutic target interval for Phenytoin, for example) can be applied to ensure optimal therapy.

Test Methodology and Limitations

DNA extraction / Polymerase Chain Reaction (PCR) / Allele-specific hybridization.

Laboratory specimens were analyzed using PCR based technologies that detect the eight most common CYP2C9 variants and the VKORC1 (-1639G>A) variant that influences warfarin maintenance dose. The performance of this assay was validated by Genelex Corporation. 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 CYP2C9. 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 2C9

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

<i>{Avandia}</i>	fluvastatin	{naproxen}	{tamoxifen}
<i>Amaryl</i>	glimepiride	nateglinide	tenoxicam
<i>Atacand</i>	glipizide	Orinase	tetrahydro-cannabinol (marijuana)
bosentan	Glucotrol	phenobarbital	Tracleer
candesartan	glyburide	phenytoin	tolbutamide
celecoxib	ibuprofen	piroxicam	Tomide
chlorpropamide	indomethacin	<i>{rosiglitazone}</i>	toremide
Cozaar (p)	irbesartan	{sertraline}	valproic acid
<i>DiaBeta</i>	<i>Lescol</i>	Starlix	zafirlukast
diclofenac	lornoxycam	sulfa drugs	
fluoxetine	losartan	suprofen	
flurbiprofen	meloxicam	S-warfarin	

Inhibitors of Cytochrome P-450 2C9

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.

Accolate	delavirdine	fluvoxamine	teniposide
Arimidex	efavirenz	isoniazid	valproic acid
Tricor	fenofibrate	phenylbutazone	voriconazole
Vfend	fluconazole	sertraline	zafirlukast

Inducers of Cytochrome P-450 2C9

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.

aprepitant-long term	bosentan	lopinavir/ritonavir	St John's Wort-long term
barbituates	carbamazepine	rifampin-chronic	

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