

Warfarin Sensitivity (*CYP2C9* & *VKORC1*) 3 Mutations

TO IDENTIFY PATIENTS WITH INHERITED VARIANTS THAT AFFECT METABOLISM AND/OR EFFICACY OF WARFARIN

Clinical Background

- Warfarin (Coumadin®) is a widely used anticoagulant and one of the most commonly prescribed drugs in the United States.
- Individual response to warfarin varies considerably due to factors such as age, gender, body mass, diet, concomitant medications, and heredity.
- Overdosing and underdosing can result in life-threatening events (e.g., bleeding or thrombosis).
- It is estimated that 1 percent of patients die due to bleeding complications associated with warfarin and up to 15 percent of patients experience minor bleeding complications.
- Dose adjustments are often necessary and are based on measuring the prothrombin time in blood and calculating an international normalized ratio (INR).
- Identifying inherited variants involved with warfarin metabolism or efficacy can be used to optimize selection of warfarin doses and reduce the time needed to achieve a therapeutic INR.
- Pharmacogenetic testing to address variable pharmacokinetics and pharmacodynamics with warfarin has been studied and dosing algorithms based on these genes have been devised.

Epidemiology

- Allele frequencies of *CYP2C9* and *VKORC1* variants differ among ethnic groups.

Gene	Variant (nucleotide)	Allele designation	Protein change	Protein effect	Allele frequency ^a
<i>VKORC1</i>	c.-1639G>A		Protein level	Decreased expression	C: 0.42 A: 0.89 AA: 0.08
<i>CYP2C9</i>	c.430C>T	*2	R144C	Decreased activity	C: 0.08-0.13 A: 0.02-0.06 AA: <0.01
<i>CYP2C9</i>	c.1075A>C	*3	I345L	Abolished activity	C: 0.06-0.10 A: <0.01 AA: 0.01-0.04

^a C- Caucasian; A- Asian; AA- African American

Genetics

- The primary enzyme involved in metabolism and subsequent inactivation of s-warfarin is the cytochrome P450 isozyme 2C9 (*CYP2C9*).
- The *CYP2C9* gene has two common variants: *CYP2C9**2 and *CYP2C9**3. These variants result in a protein with decreased function and nearly abolished function, respectively.
- Vitamin K epoxide reductase (*VKOR*) is the site of action for warfarin. The associated gene, *VKORC1*, has a common promoter variant (c.-1639G>A) that reduces the expression of the gene, and therefore lowers the amount of *VKOR* and leads to warfarin sensitivity. This variant is in strong linkage disequilibrium with the intron 1 *VKORC1* variant c.173+1000C>T
- The combination of two *CYP2C9* variants (*2 and *3) with the *VKORC1* promoter mutation is estimated to account for 40–63 percent of the variability in therapeutic warfarin dose.^{1,2}

Mechanisms of Action

- Individuals with *CYP2C9**2 and *CYP2C9**3 exhibit impaired metabolism of s-warfarin, leading to a longer half-life and rate of clearance.
- Standard 5 mg/day dosing of warfarin in patients with *CYP2C9* and/or *VKORC1* variants can lead to excessive warfarin exposure, resulting in an exaggerated anticoagulant response and a risk of serious or life-threatening bleeding complications.^{3,4}
- Patients with impaired *CYP2C9* function are likely to require more time to achieve steady state and a stable INR due to the longer half-life of the drug. Thus, dosing adjustments and INR determinations should be made less frequently when *CYP2C9* variants are known to allow steady-state concentrations to be achieved.
- Variations in *VKORC1* have been associated with both warfarin sensitivity and warfarin resistance. The common promoter mutation (c.-1639G>A) may explain much of the pharmacological variability in warfarin sensitivity.
- Warfarin's primary mechanism of action is to inhibit vitamin K epoxide reductase (*VKOR*), which recycles vitamin K and activates clotting factors II, VII, IX, and X. Thus, warfarin exerts anticoagulant effects by reducing the concentration of activated clotting factors.

- Example of predicted maintenance warfarin doses based on genotype:

CYP2C9 variants	warfarin (mg/d)					
	VKORC1 GG		VKORC1 AG		VKORC1 AA	
	calculated initial dose	% reduced from 5.6	calculated initial dose	% reduced from 5.6	calculated initial dose	% reduced from 5.6
None	5.6		4.5	20%	3.5	38%
CYP2C9*2	4.5	20%	3.5	38%	2.7	52%
CYP2C9*3	4.0	29%	3.1	45%	2.3	59%
CYP2C9*2*2	3.5	38%	2.7	52%	2.0	64%
CYP2C9*2*3	3.1	45%	2.3	59%	1.6	71%
CYP2C9*3*3	2.6	54%	1.9	66%	1.3	77%

Indications for Ordering

- Patients being considered for warfarin therapy.
- Patients with a personal or family history of difficulty in dosing warfarin or other drug substrates of *CYP2C9*.

Interpretation

- Genotype should be interpreted with clinical information; consultation with a clinical pharmacist is recommended.
- Negative: No mutations were detected. This genotype does not predict an increased risk for warfarin sensitivity.
- Positive: The detection of a *CYP2C9* or *VKORC1* mutation(s) predicts a reduced rate of warfarin metabolism, prolonged drug half-life, and/or the potential for drug sensitivity. Consideration of effects on warfarin clearance relative to dose adjustments and interpretation of INR results is recommended. Lower maintenance doses are predicted when *CYP2C9* and/or *VKORC1* mutations are detected.

Methodology

- Polymerase chain reaction (PCR) followed by unlabeled probe and melting-curve analysis to detect *CYP2C9* *2 (c.430C>T), *CYP2C9* *3 (c.1075A>C), and *VKORC1* c.-1639G>A.

- Greater than 90 percent of deleterious *CYP2C9* mutations are detected in Caucasians; clinical sensitivity is unknown in other ethnicities.
- Analytical sensitivity and specificity for the mutations detected are 99 percent.

Limitations

- *CYP2C9* mutations other than *2 and *3 and *VKORC1* mutations other than c.-1639G>A will not be detected.
- Mutations in other genes and non-genetic factors that may affect drug metabolism (e.g., drug-drug interactions) are not detected.
- Rare diagnostic errors can occur due to primer-site mutations.
- The detection of genetic variants does not replace the need for therapeutic drug monitoring or other appropriate clinical monitoring.

References

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4. Takahashi H, et al. Different contributions of polymorphisms in *VKORC1* and *CYP2C9* to intra- and inter-population differences in maintenance dose of warfarin in Japanese, Caucasians and African-Americans. *Pharmacogenet Genomics* 2006;16:101–10.
5. International Warfarin Pharmacogenetics Consortium. Estimation of the warfarin dose with clinical and pharmacogenetic data. *N Engl J Med* 2009;360(8):753–64.
6. Takeuchi F, et al. A genome-wide association study confirms *VKORC1*, *CYP2C9*, and *CYP4F2* as principal genetic determinants of warfarin dose. *PLoS Genet* 2009;5(3):e1000433.

Test Information

0051370

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For specific collection, transport, and testing information, refer to the ARUP Web site at www.aruplab.com.

For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.