Genetic Test for Warfarin Therapy

Background Information

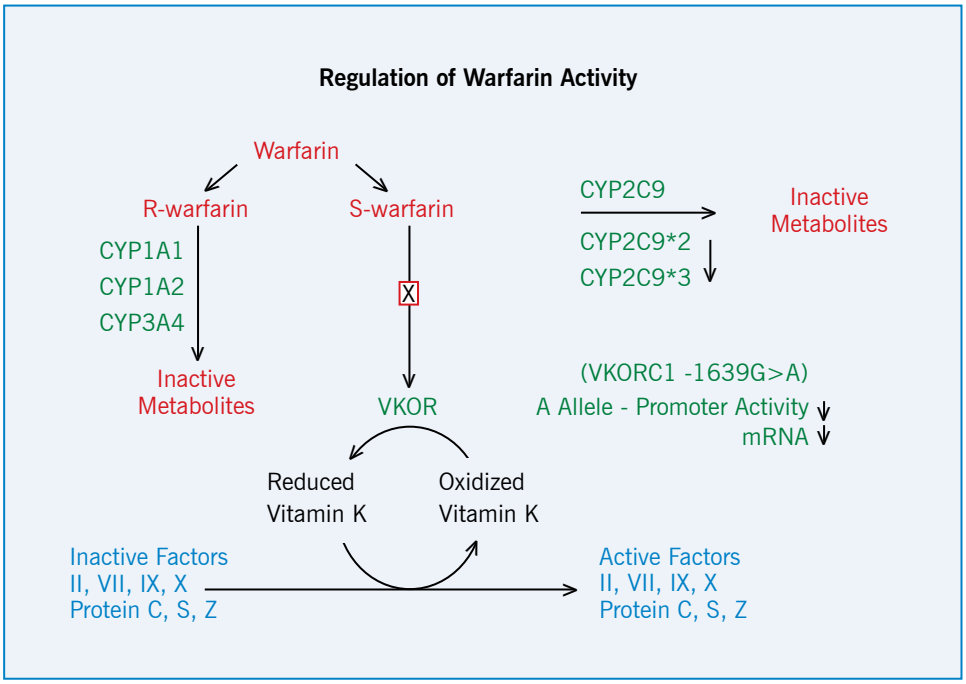
Warfarin (Coumadin) is a commonly prescribed oral anticoagulant to prevent or treat clotting disorders, including those associated with venous thrombosis, pulmonary embolism, atrial fibrillation, cardiac valve replacements, stroke and acute myocardial infarction. Warfarin has a narrow therapeutic range, which can influence the optimal dose for individual patients, ranging from < 2 mg/day to > 10 mg/day. While underdosing can result in the formation of blood clots, overdosing can increase the risk for bleeding.

The current approach to determining an appropriate warfarin dose for an individual considers age, gender, weight, height, current medications and diet and monitors the patient's prothrombin time (PT) and international normalized ratio (INR). It is a lengthy process with an increased risk for ADE.

Alternatively, molecular diagnostic tests based on the patient's genetic information can be used to determine an appropriate dosing strategy to achieve a stable maintenance dose. Using this technique, recommended dosing and risk level can be determined quickly, providing safer, more effective warfarin therapy (www.warfarindosing.org).

Clinical Indication

Warfarin is a vitamin K antagonist composed of S- and R-isomers. The more potent S-warfarin is metabolized by an enzyme cytochrome P450 2C9 (CYP2C9), encoded by the *CYP2C9* gene. Warfarin exerts its anticoagulant effect by inhibiting its target enzyme, Vitamin K epoxide reductase (VKOR), encoded by the Vitamin K epoxide reductase complex subunit 1 (*VKORC1*) gene. VKOR is required to recycle oxidized vitamin K to reduced vitamin K, a co-factor necessary for the production of activated clotting factors II, VII, IX and X.



Genetic variations in the *CYP2C9* and *VKORC1* genes can affect warfarin's efficacy and the dose required to achieve stable INR. Specifically, two variants in the *CYP2C9* gene (*CYP2C9*2* and *CYP2C9*3*) result in an enzyme with reduced activity, leading to increased active warfarin levels. A variant in the *VKORC1* gene (*VKORC1*- 1639/3673 G>A) can lead to reduced gene expression, resulting in decreased level of VKOR. Together, these three variants can account for 40 to 60% of the variability in warfarin dosage. These allelic variations of individual patients can be factored in while determining the warfarin dose to reduce the risk of ADE.

This genetic test is used to identify patients at risk for ADE due to impaired warfarin metabolism and sensitivity by:

- Detecting two genetic variants in the warfarin metabolizing gene *CYP2C9* (*CYP2C9*2* and *CYP2C9*3*)
- Detecting one genetic variant in the warfarin target gene *VKORC1* (-1639G>A).

Interpretation

Interpretation of the assay is based on the presence of specific genetic variants of *CYP2C9* and *VKORC1*:

Genetic Variant	Phenotype
CYP2C9 *1/*1	Extensive (normal) metabolizer
CYP2C9 *1/*2	Intermediate metabolizer
CYP2C9 *1/*3	Slow metabolizer
CYP2C9 *2/*2	Slow metabolizer
CYP2C9 *2/*3	Slow metabolizer
CYP2C9 *3/*3	Very slow (poor) metabolizer
VKORC1 -1639 GG	Low sensitivity to warfarin
VKORC1 -1639 AG	Medium sensitivity to warfarin
VKORC1 -1639 AA	High sensitivity to warfarin

1. Individuals with *CYP2C9* variant alleles designated *2 and *3 have reduced enzyme activity compared to *1 (wild-type) individuals, leading to increased levels of the active S-warfarin, typically require reduced maintenance doses of warfarin and take longer to achieve steady state.
2. *VKORC1* variants are alleles designated G/G, A/G, and G/G that alter the activity of VKOR enzymatic activity, leading to differences in warfarin dose required and time to achieve steady state. Individuals with the A/A genotype have decreased VKOR activity leading to increased inhibition to warfarin and are at increased risk of bleeding complications from anticoagulant overdose. They typically require reduced doses of warfarin to achieve therapeutic INR sooner compared with A/G individuals.
3. Prevalence of gene variations in *CYP2C9* and *VKORC1* differs with ethnicity.

Ordering Recommendations

- This test should be ordered prior to starting warfarin, or within the first week to help guide dosing.
- This test is not recommended for patients on stable warfarin therapy.

Limitations of the Assay

Analysis for specific genetic variants detected in this test does not rule out the possibility of the presence of other variant alleles that may influence drug effect and metabolism. *CYP2C9* variant alleles are important in the metabolism of drugs other than warfarin, such as phenytoin, glyburide, glimepiride, tolbutamide, sulfamethoxazole and certain nonsteroidal anti-inflammatory agents (NSAIDs). A *2 or *3 result for *CYP2C9* is associated with a poor metabolizer phenotype for all drugs metabolized by CYP2C9.

Methodology

An array-based diagnostic test kit employing Infiniti analyzer (AutoGenomics Inc., CA) is used for genotyping *CYP2C9-VKORC1* variants. The assay involves a multiplex PCR amplification of genomic DNA followed by allele-specific (AS) primer extension using fluorescently labeled dCTP and hybridization onto a microarray coated with capturing oligonucleotides (zipcodes), which are specific for complementary oligonucleotides (anti-zipcodes) linked to the allele specific primer-extended products.

A built-in confocal microscope is used to capture fluorescent signal from the pre-determined hybridization spots corresponding to specific products and genotypes deciphered from the signal ratio.

References

1. American Medical Association, Arizona CERT and Critical Path Institute. Personalized Health Care Report 2008: Warfarin and Genetic Testing.
2. Flockhart DA, O'Kane D, Williams MS, Watson MS, Flockhart DA, Gage B, Gandolfi R, King R, Lyon E, Nussbaum R, O'Kane D, Schulman K, Veenstra D, Williams MS, Watson MS. ACMG Working Group on Pharmacogenetic Testing of CYP2C9, VKORC1 Alleles for Warfarin Use. Pharmacogenetic testing of CYP2C9 and VKORC1 alleles for warfarin. *Genet Med.* 2008;10:139-150.
3. Gage BF, Eby C, Johnson JA, Deych E, Rieder MJ, Ridker PM, Milligan PE, Grice G, Lenzini P, Rettie AE, Aquilante CL, Grosso L, Marsh S, Langaee T, Farnett LE, Voora D, Veenstra DL, Glynn RJ, Barrett A, McLeod HL. Use of pharmacogenetic and clinical factors to predict the therapeutic dose of warfarin. *Clin Pharmacol Ther.* 2008;84:326-331.
4. McClain MR, Palomaki GE, Piper M, Haddow JE. A rapid-ACCE review of CYP2C9 and VKORC1 alleles testing to inform warfarin dosing in adults at elevated risk for thrombotic events to avoid serious bleeding. *Genet Med.* 2008;10:89-98.

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Test Overview

Test Name	Warfarin Sensitivity Genotyping Genetic test for warfarin therapy
Reference Range	An interpretive report will be provided.
Specimen Requirements	Testing Volume/Size: 5 mL; Type: Whole blood; Tube/Container: EDTA (Lavender); Coll. Temperature: Refrigerated
Ordering Mnemonic	WARSEN
Billing Code	84613
CPT Codes	83891, 83900, 83901, 83914 x6, 83912, 83912-26

Technical Information Contacts:

Christine Dziekan, MT(ASCP)
216.444.8444
dziekac@ccf.org

Wendy Nedlik, MT(ASCP)
216.444.8410
nedlikw@ccf.org

Scientific Information Contacts:

Kandice Kottke-Marchant, MD, PhD
216.444.2484
marchak@ccf.org

Joyce Heesun Rogers, MD, PhD
216.445.2719
rogersj5@ccf.org

Felicitas L. Lacbawan, MD, FCAP, FACMG
216.445.0781
lacbawf@ccf.org