TEST SUMMARY

Quest Diagnostics®

Nichols Institute

Cytochrome P450 2C9 and *VKORC1* Mutation Analysis

Test code: 16160X

Clinical Use

- Predict patient-specific warfarin response
- Assist in determining initial dose of warfarin

Clinical Background

Warfarin, a derivative of coumarin, is the most widely prescribed anticoagulant for the prevention and treatment of arterial and venous thromboembolic disease including deep vein thrombosis (DVT), pulmonary embolism (PE), ischemic stroke, myocardial infarction (MI), and atrial fibrillation. Warfarin therapy is complicated by a narrow therapeutic range, ie, individuals with international normalized ratio (INR) values <1.8 are at risk for recurrent thromboembolism, while those with INR values >4 are at risk for increased bleeding.[2] Additionally, a wide variation in response among individuals is seen; eg, some patients may require a dose of 1 mg to achieve anticoagulation, while others require a dose of up to 20 mg.[2]

Warfarin exerts its pharmacodynamic effect by inhibiting the enzyme vitamin K epoxide reductase (VKOR). VKOR assists in production of vitamin-K dependent clotting factors; thus, inhibition of VKOR reduces the level of vitamin K-dependent clotting factors leading to anticoagulation. VKOR is encoded for by the gene *VKORC1*. The -1639G→A mutation in *VKORC1* leads to a reduced level of VKOR and, therefore, a decrease in the level of vitamin-K dependent clotting factors. The mutation is thus associated with an increased sensitivity to warfarin (ie, anticoagulation is achieved at a lower dose).[4,5]

Warfarin is metabolized in the liver by the enzyme cytochrome P450 2C9, encoded by the gene *CYP2C9*. The rate of warfarin metabolism is influenced by intake of vitamin K, ethnicity, illness, age, gender, other medications, and body mass index (BMI).[1] Variants of *CYP2C9* (*CYP2C9*2, CYP2C9*3, CYP2C9*5,* and *CYP2C9*6*) are associated with decreased warfarin metabolism, thus leading to increased sensitivity (ie, anticoagulation is achieved at a lower dose).[6,7]

Together, *VKORC1* and *CYP2C9* polymorphisms account for up to 60% of the variability in an individual's response to warfarin (*VKORC1*, 25%-44%; *CYP2C9*, 10%-15%), and are independently associated with increased sensitivity to warfarin therapy.[1,8] Approximately 42%-46% of Caucasians, 3%-13% of Africans, and 90%-95% of Asians carry at least one copy of the -1639G→A allele.[1,5] Likewise, approximately 33% of Caucasians, 3%-13% of Africans, and 2%-8% of Asians are positive for at least one of the *CYP2C9* poor metabolizer variants.[1,6]

Knowledge of a patient's genotype, along with his/her demographic data, can assist in determining the appropriate warfarin dose; this may lead to a decreased incidence of bleeding complications, especially during the initiation (first 30 days) of therapy.[1,8,9] In an AEI-Brookings study, McWilliam et al predicted that identifying an optimal maintenance dose through genetic testing will prevent 85,000 serious bleeding episodes and 17,000 recurrent strokes per year for an annual savings of 11 million to 2.2 billion dollars.[10] In August 2007, the FDA updated warfarin labeling to indicate that individuals with variations in *VKORC1* and *CYP2C9* may require lower warfarin doses.

A number of genotype-based algorithms have been developed to assist in determining patient-specific doses.[11-12] Use of one such algorithm shows the affect of genotype when warfarin dose is calculated for a 65-year-old person whose height is 175 cm (Table).[11]

Individuals Suitable for Testing

- Individuals slated for, or receiving, warfarin therapy

Specimen Requirements

5 mL room temperature whole blood (lavender- or yellow-top tube preferred); 3.0 mL minimum

Alternatively, submit 2 mL room temperature saliva

Table. Illustration of Warfarin Dose Reduction Based on Genotype[a]

CYP2C9 Genotype	*VKORC1* Genotype		
	GG	AG	AA
*CYP2C9*1/*1*	●●●●●	●●●●	●●●
*CYP2C9*1/*2*	●●●●	●●●	●●◦
*CYP2C9*1/*3*	●●●◦	●●◦	●●
*CYP2C9*2/*2*	●●●	●●◦	●●
*CYP2C9*2/*3*	●●◦	●●	●◦
*CYP2C9*3/*3*	●●◦	●●	●

[a] *CYP2C9*1/*1*, GG is the non-mutated genotype and the 5 circles represent a "normal" warfarin dose. A decreased number of circles represent the relative decrease of warfarin dose for that genotype. This table is for illustration purposes and was created using the dosing algorithm of Sconce et al, which is based on age, height, and genotype.[10] The actual warfarin dose is affected by many factors including weight, tobacco use, concomitant medical conditions, medications, etc.

Method

- PCR amplification of *VKORC1* promoter and exons 3, 5, and 7 of *CYP2C9*
- Single nucleotide primer extension
- Fluorescent detection of extension products
- Aliases: 2C9, *CYP2C9*, vitamin K epoxide reductase complex 1 (*VKORC1*), warfarin (Coumadin®) sensitivity
- CPT codes*: 83891; 83900; 83901 (x4); 83896 (x5); 83909; 83912

Reference Range

VKORC1: negative for -1639G→A
CYP2C9: negative for poor metabolizer variants

Interpretive Information

The presence of -1639G→A in the *VKORC1* gene and *CYP2C9*2, CYP2C9*3, CYP2C9*5,* and *CYP2C9*6* in *CYP2C9* are independently associated with increased sensitivity to warfarin; individuals with one or more of these mutations may require a lower warfarin dose to achieve anticoagulation. Warfarin sensitivity increases with the number of mutations present. For example, an individual homozygous for -1639G→A will be more sensitive to warfarin and require a lower dose than an individual with only one copy of -1639G→A. Likewise, an individual homozygous for -1639G→A who also carries one of the poor metabolizer variants of *CYP2C9* will have an additional increase in sensitivity to warfarin and require an even lower dose (Table).

The appropriate warfarin dosage can be calculated by going to www.nicholsinstitute.com/coagulation and entering the patient's demographic and genotypic data.

While DNA-based testing is highly accurate, results should be interpreted in light of clinical and familial data. Genetic variations other than those tested for in this assay may affect warfarin sensitivity as do many other factors including age, weight, tobacco use, concomitant medical conditions and medications, etc. Assistance with the interpretation of results is available by calling 1-866-GENEINFO.

References

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*The CPT codes provided are based on AMA guidelines and are for informational purposes only. CPT coding is the sole responsibility of the billing party. Please direct any questions regarding coding to the payor being billed.

This test was developed and its performance characteristics were determined by Quest Diagnostics Nichols Institute, San Juan Capistrano. Performance characteristics refer to the analytical performance of the test.

This test is performed pursuant to a license agreement with Orchid Biosciences, Inc.

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