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Warfarin Pharmacogenetics: does more accurate dosing benefit patients?

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Abstract

After a decade of clinical investigation, pharmacogenetic guided initial dosing of warfarin is at a crossroads. Genotypes for two single nucleotide polymorphisms (SNP) in cytochrome P 450 2C9 gene, affecting warfarin metabolism, and one SNP in Vitamin K reductase complex 1 gene, affecting warfarin sensitivity, account for approximately 30% of therapeutic warfarin dosing variability in Caucasians and Asians. Incorporating this genetic information with patient's age, body size, and other clinical information improves the accuracy of initial warfarin dosing. Currently, there is insufficient evidence to support the clinical benefits and cost effectiveness of routine warfarin pharmacogenetics. Results from ongoing international randomized clinical trials should provide clarity about warfarin pharmacogenetics place in personalized medicine.

Keywords

warfarin; oral anticoagulant therapy; vitamin k antagonist; pharmacogenetics

INTRODUCTION

Warfarin, and other vitamin K antagonists derived from coumarin, was the only type of oral anticoagulant therapy for over 50 years. As reviewed in Dr. Witt's contribution to this issue, warfarin therapy is effective for treatment and prevention of arterial and venous thromboembolic events. However, warfarin has a narrow therapeutic range, defined by INR targets, and patients' maintenance doses are widely variable and unpredictable¹. Initial trial and error dosing results in greater INR volatility and bleeding complications compared to the chronic phase of therapy²⁻³. Multiple clinical investigators have shown that by combining pharmacogenetic (PGx) and clinical information about a patient, initial dosing can more accurately match the eventual therapeutic dose⁴. Application of currently available pharmacogenetic information distinguishes patients who require lower than average maintenance warfarin doses from those who do not, but does not identify patients who require very large doses to induce anticoagulation, i.e. warfarin resistance. Presently, there is no convincing evidence of the clinical utility and cost effectiveness of routine pharmacogenetic testing before starting warfarin. Several ongoing large randomized clinical trials of PGx dosing will hopefully provide sufficient evidence to either support or refute

adoption of warfarin pharmacogenetics within the context of personalized medicine. In addition, the outcomes of these studies are likely to affect the rate at which expensive, new direct oral thrombin and factor Xa inhibitors replace warfarin.

WARFARIN PHARMACOGENETICS

Over the past 20 years, there have been many advances in our understanding of the biochemical and molecular basis of warfarin pharmacokinetics and pharmacodynamics (Figure)⁵⁻⁷. Pharmaceutical warfarin contains equal amounts of R and S enantiomers: asymmetric chemical structures which are not superposable. S-warfarin is much more pharmacologically active, and undergoes hydrolysis by cytochrome P 450 (CYP)2C9 whereas R-warfarin metabolism is more complex and involves CYP 1A1, 1A2 and 3A4 (Figure). While there are at least 30 known CYP 2C9 variants, two nonsynonymous, (nucleotide substitution changes amino acid in peptide product), single nucleotide polymorphisms (SNP), *2 (420C>T) and *3 (1075A>C) have definite effects on the therapeutic warfarin dose, as they reduce S-warfarin metabolism by 30-40% and 80-90% respectively⁸ compared to individuals homozygous for the reference or major CYP 2C9 allele (*1). The allele frequencies for CYP 2C9 *2 and *3 SNP are highest in people of European ancestry⁹ (Table 1). Several rare CYP 2C9 SNPs (*5, *6, *8, *11) that are associated with decreased warfarin metabolism are relatively more prevalent among people of African ancestry¹⁰.

Warfarin antagonizes vitamin K reductase complex (VKORC1) conversion of oxidized vitamin K epoxide to functional reduced vitamin K (Figure). Following discovery of the VKORC1 gene in 2004¹¹⁻¹², Rieder and colleagues identified a group of SNPs in high linkage disequilibrium in the VKORC1 gene that are associated with increased sensitivity to warfarin¹³. One of the SNPs which can be used to predict inheritance of a warfarin sensitive VKORC1 allele or haplotype is -1639G>A which is in the promoter region of the VKORC1 gene, and appears to be a functional SNP since it correlates with decreased VKORC1 gene transcription and protein expression¹³⁻¹⁴. Asians and Caucasians have higher prevalence's of VKORC1 sensitive haplotypes (identified by genotyping for -1639G>A, or another "tag"SNP in high linkage disequilibrium: 1173C>T) compared to people of African ancestry (Table 1).

Additional genetic variants associated with warfarin dosing have been identified through candidate gene resequencing¹⁵, tag SNP analysis¹⁶ and whole genome associated sequencing analyses¹⁷⁻¹⁹. To date, only the 1297C>T SNP in CYP 4F2 gene, that encodes a valine to methionine substitution at position 244 (V244M), has consistently shown a correlation with warfarin dose²⁰⁻²¹. Using a liver microsome expression system, investigators showed the 4F2 V244M variant is a slow metabolizer of vitamin K²², which is consistent with its clinical association with higher warfarin doses. The allelic frequency of CYP4F2 1297C>T is highest in Asians and lowest in Africans and African Americans (Table 1). Thus, it is likely the common genetic variants with a large impact on warfarin dosing have been discovered, and they are limited to CYP 2C9, CYP 4F2, and VKORC1. Identification of additional genetic variants with marginal affect on warfarin dose will

require different discovery strategies including whole genome sequencing of targeted populations.

DOSING ALGORITHMS USING PHARMACOGENOMIC INFORMATION

Over the past decade, many investigators have assembled clinical, demographic, and PGx databases from stably anticoagulated patients to identify independent variables associated with therapeutic dose and create dosing algorithms based on regression equations for these variables⁴. In Caucasians and Asians, the most important variable is VKCOR1 haplotype, represented by –1639G>A or 1173C>T), followed by age, body surface area, CYP 2C9 *3, and CYP2C9 *2 SNPs. Other minor, but significant in some models, variables include gender, warfarin indication, INR target, African ancestry, smoking, and co morbidities (stroke, diabetes). Certain medications with known affects on CYP 2C9 activity are noteworthy,(e.g. inhibit--amiodarone, azoles, some statins, induce-rifampin), and are included in some, but not all, models due to either low prevalence or incomplete medication histories in databases. Overall, PGx algorithms, that include clinical attributes, can account for about 50% of the variability warfarin dosing in Caucasians and Asians cohorts, in whom SNPs associated with VKORC1 sensitivity and/or CYP 2C9 slow metabolism are common (Table 1). However, in people of African ancestry, these SNPs are less common and therefore most PGx algorithms (which include clinical variables as well) account for less (approximately 30%) of warfarin dosing variability¹⁰. Perera and colleagues recently reported a PGx algorithm accounting for 40% of dosing variation in a small African American cohort, which includes CYP 2C9 SNPs *5, *6, *8, and *11, and one novel SNP in CYP2CP and another inVKORC1²³.

There has been interest in developing algorithms for children as some require chronic oral anticoagulation with vitamin K antagonists, most commonly for congenital cardiac conditions. Two groups have developed pediatric PGx warfarin dosing algorithms²⁴⁻²⁵, based on 120 and 83 children respectively, in a populations that were 90% Caucasian. The most important variable was height, followed by VKORC1 –1639 genotype. The both models accounted for about 70% of warfarin dosing variation, and await prospective validation.

A group of investigators formed the IWPC (international warfarin pharmacogenetics consortium) and pooled genetic and clinical data on a racially and ethnically diverse sample of about 4000 stably anticoagulated patients to derive PGx dosing algorithms²⁶. They validated the algorithm with data from an additional 1000 patients, which accounted for 43% of warfarin dosing variation. The details and performance of the IWPC algorithm are very similar to one derived and validated by Gage and colleagues²⁷. Clinicians can input clinical data, with or without PGx information, into either of these algorithms using a non-profit open access decision support web application:WarfarinDosing.org to obtain therapeutic dose estimates. The website is maintained by Dr. Brian Gage at Washington University in St. Louis.

There is no utility to genotyping patients whose therapeutic warfarin dose is known due to past or current anticoagulation treatment and INR monitoring. The ideal time to apply PGx

algorithms is prior to the first dose in a warfarin naïve patient. However, some experts do not adjust day 1 or 2 warfarin doses for slow metabolizing CYP 2C9 *2 or *3 SNPs in order to shorten the time to first therapeutic INR²⁸, and WarfarinDosing.org provides a “loading dose option” calculation for the first two days in patients with *2 or *3 SNPs. Therefore, if genotype results may not be available until after a patient has taken warfarin for 1-2 days, initial empiric dosing followed by a PGx based adjustment for subsequent doses is reasonable.

The INR response subsequent to starting warfarin provides important information about a patient’s unique pharmacology. Therefore, Gage and colleagues developed additional PGx algorithms to apply after 4-5 days of treatment. Incorporating INR response and warfarin dose with PGx data into a dose refinement algorithm, improves the accuracy of PGx dosing²⁹⁻³⁰, and WarfarinDosing.org has dose refinement calculation capabilities.

Recognizing the potential for PGx dosing to reduce warfarin associated adverse events, the United States, the Food and Drug Administration (FDA), revised the package insert for warfarin in 2007 to include general information about pharmacogenetics, and to inform prescribers that patients with genetic predisposition toward slow metabolism of, or increased sensitivity to, warfarin require lower therapeutic doses. In 2010, The FDA revised the package insert again, adding a table with suggested warfarin dose ranges for different combinations of CYP2C9 *2,*3, and VKORC1 –1639 SNPs (Table 2). When applied to a retrospective cohort of genotyped, stably anticoagulated patients, FDA genotype table derived doses were more accurate than an empiric dose of 5 mg/day³¹. However, a validated PGx algorithm performed better than the FDA genotype table, predicting the dose within 20% of actual therapeutic dose in 52% and 43% of subjects, respectively, indicating that there is additional predictive information provided by patients’ age, body size, race, smoking status, INR target, and indication for warfarin.

Prospective applications of warfarin dosing algorithms include case-control studies, several small randomized control studies (RCT), and one effectiveness trial. In orthopedic patients, PGx-based dosing was more accurate than a clinical-based algorithm applied to a control group 32-33. Due to the relatively low frequencies of major bleeding and thromboembolic events, the primary endpoints of most RCT on warfarin dosing have been laboratory targets including INR time within therapeutic range, INR > 4.0, and time to first therapeutic INR. Until recently, completed RCT were underpowered, used different dosing algorithms and strategies for PGx dosing and control arms, and produced inconsistent results for different INR dependent endpoints^{28, 34-37}. Given insufficient evidence to support the clinical utility of PGx-based algorithmic dosing of warfarin, in August, 2009, the Center for Medicare and Medicaid Services (CMS) announced it would not reimburse genetic testing claims for this indication. In addition, the American College of Chest Physicians³⁸ does not endorse PGx guided warfarin dosing. In order to address the clinical utility evidence gap, large warfarin PGx RCTs are underway³⁹⁻⁴¹ (Table 3), and the results of the CoumaGen-II trial by Anderson and colleagues were recently published⁴². This study addressed two issues: first a randomized, double blinded trial compared an initial warfarin PGx dose algorithm (similar to IWPC), with subsequent dose adjustments based on an INR nomogram, to a more complicated strategy that combined an initial PGx dose (ignoring CYP2C9 genotype until

the day 3 dose to permit “loading doses” of warfarin on days 1 and 2 for slow metabolizers) and a revision PGx algorithm to adjust doses after the 4 or 5 days of warfarin therapy, with subsequent dose adjustment by INR nomogram. There was no difference between groups for multiple INR endpoints, including percent time within therapeutic range and the percentage of out of range INRs, suggesting that PGx information is only needed for the initial dosing of warfarin when combined with a standard nomogram for subsequent dose adjustments. However, in the COAG trial, a revision algorithm is used for both clinical and PGx algorithms after the 4th or 5th warfarin dose, and in the GIFT trial, different revision algorithms, for both clinical and PGx dosing, are used whenever an INR is obtained between days 2-10 after starting warfarin. The results from all three trials should be helpful to decide if PGx dose revision algorithms are clinically useful or not.

The second part of the completed CoumaGen-II trial was a non-random, effectiveness analysis comparing the INR outcomes for the combined randomized controlled trial PGx patients (n=504) to a concurrent cohort of patients (n=1866) starting warfarin. Although the potential for bias and unaccounted confounding variables exists, the INR outcomes at 1 and 3 months were statistically better for the PGx group. For example, percent times INR in therapeutic range at 1 month were: PGx 68.9%, routine care 58.4% (p<0.001). These are encouraging findings, and suggest application of PGx in general practice could improve clinical outcomes.

In the United States, there are three commercial sources of FDA cleared reagents and instruments to perform CYP2C9 *2, *3 and VKORC1 –1639 or 1173 SNP analysis: Infiniti (Auto genomics, Vista CA), eSENSOR, (GenMarkDx, Carlsbad CA), and Verigene (Nanosphere, Northbrook, IL). In addition, investigators have validated laboratory developed test (LDT) methods using methods such as pyrosequencing and real time PCR methods, for example. Method comparison studies indicate that genotyping has accuracy rates of 95-100% for both commercial and LDT methods ⁴³⁻⁴⁵. Reagent costs for genotyping one patient can range from <\$25 for some LDT methods to about \$200 for commercial platforms, and clinical laboratory charges can range from \$400-\$800 ⁴⁶.

CONCLUSIONS

The current status of warfarin PGx can be viewed as either a glass which is half full, or one that is half-empty. Validated PGx based algorithms can account for about 50% of warfarin dose variability in Caucasian adults, and possibly higher in Caucasian children. The recently completed CoumaGen-II study supports better laboratory and possibly clinical outcomes when comparing PGx dosing to standard nomogram dosing. However, the “standard of care” management group was not randomly selected raising concerns for confounding and bias. The results from several ongoing large prospective, randomized studies will provide substantial evidence for analysis by the major stakeholders, and hopefully will support a consensus on the clinical utility of warfarin PGx as well as accurate cost-benefit analysis to guide reimbursement decisions.

It will be challenging to account for the remaining ~50% of warfarin dose variation. GWAS and candidate gene investigations have not identified additional polymorphisms with the

potential impact of CYP2C9 *2/*3 or VKORC1 –1639 SNPs, suggesting the remaining unexplained dosing variation may be due to a large number of weak genetic variants and non-genetic variables which are difficult to quantify or control such as vitamin K intake.

However, given the current state of inconsistent policies, between regulatory advocacies from the FDA, professional organization proscription, and denials of reimbursement by companies, physicians and patients considering PGx testing before starting warfarin should weigh the potential advantages or more accurate dosing against the likely unreimbursed financial costs. If the decision is to proceed with genotyping, use of an online algorithm resource like WarfarinDosing.org is preferable to a nomogram approach to initial dosing. Finally, if feasible, clinicians should refer patients to one of the ongoing randomized control trials of PGx.

Abbreviations used in manuscript

PGx	pharmacogenetics
SNP	single nucleotide polymorphism Tag
SNP	tagged single nucleotide polymorphism
CYP	cytochrome P 450
VKORC1	vitamin K reductase complex 1
COAG	Clarification of Optimal Anticoagulation Through Genetics
EU-PACT	European Pharmacogenetics of Anticoagulant Therapy
GIFT	Genetics Informatics Trial
AF	atrial fibrillation
VTE	venous thromboembolic event
RCT	randomized clinical trial
DB	double blinded
SB	single blinded
OL	open label
TTR	time within therapeutic range

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Figure.

Warfarin pharmacodynamics and pharmacokinetics: Lower portion left to right: In hepatocytes, post-translational gamma carboxylation of selected glutamic acid residues converts hypofunctional factors X, IX, VII, and II to functional coagulation factors, while the cofactor, reduced vitamin K, is converted to an oxidized epoxide. Upper portion: vitamin K epoxide is recycled to reduced vitamin K by vitamin K reductase complex 1 (VKORC1). Warfarin indirectly produces an anticoagulated state by inhibiting vitamin K recycling and reducing gamma carboxylation. The S-enantiomer of warfarin is more potent than the R-enantiomer, and undergoes hydrolysis by cytochrome P 450 2C9.

Table 1

Variant allele frequencies for VKORC1, CYP2C9, and CYP4F2 polymorphisms in different populations

SNP (reference)	European American	African American	Asian
VKORC1-1639G>A (9)	0.37	0.22	0.91
CYP2C9*2 (9)	0.14	0.02	0
CYP2C9*3 (9)	0.06	0.01	0.04
CYP4F2C>T (21)	0.30	0.07	0.24

Table 2

FDA recommended daily warfarin dose ranges (mg/day) based on CYP2C9 and VKORC1 genotypes

<u>VKORC1:–1639G>A</u>	<u>CYP2C9*1/*1</u>	<u>CYP2C9*1/*2</u>	<u>CYP2C9*1/*3</u>	<u>CYP2C9*2/*2</u>	<u>CYP2C9*2/*3</u>	<u>CYP2C9*1/*3</u>
GG	5-7	5-7	3-4	3-4	3-4	0.5-2
GA	5-7	3-4	3-4	3-4	0.5-2	0.5-2
AA	3-4	3-4	0.5-2	0.5-2	0.5-2	0.5-2

Reproduced from warfarin (Coumadin) product label 9/15/2010.

Table 3

Ongoing prospective randomized warfarin initiation pharmacogenetic trials

<u>Name (ref)</u>	<u>Enrollment goal</u>	<u>Duration</u>	<u>Indication</u>	<u>Design/Dosing</u>	<u>Primary endpoint</u>
COAG (39)	1238	2009-2012	AF, VTE, THR or TKR	DB/PGx vs clinical algorithms Initial + revision	INR TTR during 1 st month
EU-PACT(40)	970	2010-2013	AF, VTE	SB/ PGx vs clinical algorithms	INR TTR during 3 months
GIFT (41)	1600	2011-2014	THR or TKR age>65	PGx vs clinical algorithms Initial + revision	Composite: VTE, major bleeding, INR 4, death during 4-6 wks
Singapore PGx [Clinicaltrials.gov NCT00700895]	320 198	2006-2013 2008-2013	starting warfarin	OL/PGx algorithm vs routine practice	#INR dose titrations after 1,2,and 3 months
WARFPGX [Clinicaltrials.gov NCT00904293]			AF, VTE, valve replacement	DB/PGx vs clinical Algorithm	INR TTR during 3 months

COAG: Clarification of Optimal Anticoagulation Through Genetics; EU-PACT: European Pharmacogenetics of Anticoagulant Therapy; GIFT: Genetics Informatics Trial; WARFPGX: Genotype-Guided Warfarin Therapy Trial; Pox: pharmacogenetics; AF: atrial fibrillation; VTE: venous thromboembolic event; DB: double blinded; SB: single blinded; OL: open label; TTR: time within therapeutic range.