Warfarin Pharmacogenetics: New Life for an Old Drug

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Warfarin was first introduced in the 1950s and quickly became the most commonly used oral anticoagulant for the prevention of thromboembolism in patients with deep vein thrombosis, atrial fibrillation, or prosthetic heart valve replacement. Warfarin is highly effective in treating these diseases; however, several factors prevent it from even wider use, especially in Asian populations. It is difficult for patients on warfarin to reach desired anticoagulation due to its narrow therapeutic index and highly variable dose response. The major adverse event is bleeding which is associated with overdose of warfarin. Clinical and genetic factors such as polymorphisms in CYP2C9 and VKORC1 associated with an individual's warfarin maintenance have been identified. More than 20 dose prediction algorithms incorporating both genetic and clinical factors have been developed, and some of them have been tested clinically. However, most of the algorithms were tested in small populations. Several major clinical trials are now underway. This review aims to provide an overview of the field of warfarin which includes information about the drug, genetics of warfarin dose requirements, dosing algorithms developed and the challenges of clinical implementation of warfarin pharmacogenetics.

Key Words: CYP2C9 • Pharmacogenetics • VKORC1 • Warfarin

ABOUT THE DRUG

Warfarin, first introduced in the 1950s, has now become the most commonly prescribed oral anticoagulant for the prevention of thromboembolism in patients with deep vein thrombosis, atrial fibrillation, or prosthetic heart valve replacement.[1-5] Total prescriptions have reached 0.5-1.5% of the world's total population.[6] Warfarin exists as a racemic mixture of (R) and (S) – enantiomers with the (S) form being more potent than the (R) isomer.[7] Warfarin works by blocking the vitamin K regeneration cycle to achieve its anticoagulation effect. The blood coagulation pathway requires gamma-carboxylation of vitamin K-dependent clotting factors such as factor II, VII, IX and X. During the coagulation process, the gamma carboxylase used vitamin K and oxygen to add a carbon dioxide molecule to the side chain of glutamic acid of the clotting factors, which resulted in the oxidation of vitamin K to vitamin K 2,3-epoxide. Vitamin K 2,3-epoxide is then regenerated to the reduced vitamin K for another cycle of catalysis.[8,9]

Although warfarin is very effective in treating the diseases described above, it is plagued by a narrow therapeutic window, and large dose variations for achieving the desired anticoagulation. Differences in warfarin dose requirements among different individuals can range 10-20 fold.[10] There are also significant dose differences between different ethnic groups; Asian populations usually require lower doses relative to Caucasian or African populations.[11,12]

Thus, the anticoagulation status of a patient has to be monitored due to the broad range of warfarin dosing requirements. The International Normalized Ratio (INR)

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has been developed to facilitate proper dosing, which is a measure of the prothrombin time (PT) and calculated by dividing a patient's PT with mean normal prothrombin time (MNPT) which is the geometric mean of PT of the healthy adult population. A normal individual usually has an INR of 1 and the clotting time doubles if one has INR of 2. The desired INR range for warfarin treatment depends the patient's indication; the most widely accepted range is 2.0-3.0.[13]

To maintain INR in the target range can be difficult, and thus deviations from the target INR are very common which resulted in over- and under- anticoagulation. Thrombosis is often associated with under-anticoagulation, and over-anticoagulation can lead to bleeding. Bleeding is the most common adverse reaction during warfarin treatment.[14-16] Warfarin associated adverse events accounted for one-third of drug related hospitalizations[17] and has become one of the top 10 drugs for drug-related hospitalization in the United States. Elevated INR is frequently associated with an increased incidence of adverse bleeding. Several studies have demonstrated that incidence of bleeding is increased when the INR exceeds 4, and the risk rises significantly with values > 5.[18] In the event of high INR or bleeding, the anticoagulation effect of warfarin is the reverse of using vitamin K, which is the antagonist of warfarin. The fear of bleeding led to a significant underuse of warfarin, especially in Asian countries. For example, only 24.7% of the atrial fibrillation (AF) patients received appropriate treatment in Taiwan.[19]

GENETIC DETERMINANTS INFLUENCING WARFARIN RESPONSE

Candidate gene approach was first used to identify genetic factors affecting warfarin dose more than 20 years ago. It was soon identified that polymorphisms in the warfarin metabolizing enzyme cytochrome P450 2C9 (CYP2C9), which metabolizes the potent (S)-warfarin[20] CYP2C9 were associated with reduced warfarin dose requirement.[21] The most common allele is CYP2C9*1 which is considered as the wild type allele in all populations. The CYP2C9*2 (rs1799853) allele which has an $Arg^{144}Cys$ substitution and CYP2C9*3 (rs1057920) has an $Ile^{359}Thr$ substitution are the two most prevalent variants in European ancestry.[22-24] The minor allele of these two variants produces a metabolically impaired enzyme with activities reduced by 30% (CYP2C9*2) and 80% (CYP2C9*3).[21] Other CYP2C9 variants with reduced metabolic capacity (CYP2C9*5, *6, *8 and *11) are also identified in African Americans.[25-27] The frequency of CYP2C9 polymorphisms show ethnic differences. CYP2C9*2 is virtually absent in some Asian populations (Table 1A). Since these variants produced reduced function of CYP2C9, individuals who carry these variants require lower doses of warfarin, especially for those with 2 copies of the *3 allele. The risk of bleeding also increases significantly for these individuals and they require a longer time to achieve stable target INR.[28,29]

CYP1A1[30] and CYP3A5[31] are metabolizing enzymes for the (R)-warfarin. Since (R)-warfarin play a minor role on anticoagulation, polymorphisms identify in these genes are clinically insignificant. Genes in the vitamin K regeneration cycle, clotting factors and genes which interact with warfarin were also investigated. These genes include CYP2C18, CYP2C19, PROC, ABCB1, APOE, EPHX1, CALU, GGCX, ORM1, ORM2, factor II, factor V, factor VII, Factor IX, and NR112.[27,31-47] However, the associations of these genes to warfarin dose variation were norminal and were not replicated in all of the populations tested.

Major advances came in 2004 when vitamin K epoxide reductase subunit 1 (VKORC1), the second major genetic determinant, was identified.[48,49] VKORC1 is responsible for the regeneration of vitamin K-epoxide to vitamin K.[39] Several single nucleotide polymorphisms (SNPs) in VKORC1 were identified to be associated with warfarin dose requirement and these SNPs were in strong linkage disequilibrium (LD).[50] The most commonly used VKORC1 variant (VKORC1 -1639 G>A, rs9923231) lies in the promoter region of VKORC1. This SNP alters a transcription binding site which resulted in decreased promoter activity[51] and thus, individuals that carry the G allele require higher warfarin doses than those with the A allele. This association was soon confirmed in populations across the globe.[12,52-56] The VKORC1 SNP also show population difference (Table 1B). Several rare nonsynonymous mutations in VKORC1 were also identified which conferred warfarin resistance.[57]

Gene chip technologies (DMET, genome-wide association study) were then used to identify additional genes that contribute to variations in warfarin dosing.[58-61]

Table 1. Genotype frequencies for VKORC1 and CYP2C9

(A) CYP2C9 genotype (*1,*2,*3) frequencies

	Chinese	Caucasian	African
*1/*1	93%	64%	90%
*1/*2	0%	20%	6%
*1/*3	7%	10%	4%
*2/*2	0%	2%	0%
*2/*3	0%	3%	0%
*3/*3	0%	1%	0%

(B) VKORC1-1639G>A frequencies

	Chinese	Caucasian	African
AA	80%	15%	2%
AG	17%	50%	18%
GG	3%	35%	80%

All these studies confirmed the associations of CYP2C9 and VKORC1 with warfarin dosing variation. In addition, they identified CYP4F2 as the additional genetic factor. CYP4F2 is involved in the oxidation of vitamin K1 and the non-synonymous polymorphism resulting in decreased enzymatic activity.[62] One meta-analysis which involved more than 9000 participants from thirty studies[63] confirmed the association of the CFP4F2 variant (rs2108622). However, the effect size of this association is much lower than those contributed by VKORC1 and CYP2C9, and therefore its clinical use needs to be validated.

DIETARY AND CLINICAL FACTORS AND CONCOMITANT DRUGS AFFECTING WARFARIN RESPONSE

As described above, vitamin K is required for the coagulation cascade. Thus, the amount of vitamin K intake positively correlates with warfarin dose.[64] Dark green vegetables such as broccoli and spinach that are rich in vitamin K could potentially interfere with warfarin dose requirement. In order to minimize variations in warfarin response, the National Institutes of Health (NIH) has recommended consistent vitamin K intake for patients on warfarin treatment.[65] Additionally, excessive alcohol consumption has been shown to influence warfarin metabolism and can elevate INR.[66]

In addition to dietary intake, concomitant use of drugs can also affect warfarin response. Amiodarone is perhaps the most common concomitant drugs for warfarin treatment. Warfarin dose should be reduced when amiodarone is co-administered as amiodarone decreases the clearance of both (R)- and (S)-warfarin.[67] Antibiotics can also influence warfarin response via different mechanisms: metronidazoles increases warfarin response by reducing the metabolism of warfarin, and broad-spectrum antibiotics enhance the effect of warfarin by altering the balance of intestinal flora.[68] Some drugs do not act directly on warfarin but can also affect warfarin response. For example, non-steroidal anti-inflammatory drugs (NSAIDs) increase the incidence of bleeding due to gastrointestinal erosion caused by NSAID, even for patients with the desired range of INR.[68] Anti-platelets drugs, such as aspirin and clopidogrel, increase the risk of hemorrhage.[69] Finally, drugs that interfere with the vitamin K cycle (such as acetaminophen whose metabolite inhibits the vitamin K cycle) can also affect warfarin response.[70]

It has been well-established that age, height, and weight positively correlate with warfarin dose. Patients with renal impairments also require less warfarin than patients with normal renal function.[71]

DOSING ALGORITHMS FOR BETTER PREDICTION OF WARFARIN DOSE

Early on, enormous efforts were made to try and to reduce the warfarin-induced adverse events; the development of INR is the prime example of this undertaking. To date, INR is still the “gold standard” in warfarin therapy. In addition, dosing algorithms or computerized programs using clinical variables were also developed, aimed to guide physicians prescribe warfarin.[72-75] However, these were not widely adopted because the programs were not effective in real clinical settings and the clinical prediction algorithms can only explain low dosing variability (~20%). The development of dosing algorithms incorporating both clinical and genetics variables were then the main focus in improving warfarin safety after the identification of the genetic variants associated with warfarin response. The aims were to develop dosing algorithms which could explain a greater variation in dosing and more accurately predict the

maintenance dose. The pharmacogenetic dosing algorithms were initially developed using just CYP2C9 genotypes.[75,76] After the identification of VKORC1, VKORC1 genotypes were incorporated into subsequently developed algorithms. CYP4F2 and other genetic factors were also included in recently developed algorithms.[77,78] More than 20 dosing algorithms have been developed for the world's major populations.[12,79-81] Regression analysis used to generate these algorithms showed that demographic factors (age, gender, weight, height) and the use of concomitant drugs usually accounted for approximately 25% of the variability in dosage. CYP2C9 and VKORC1 genotypes account for an additional 35-50% of the variability. However, most of these algorithms were developed from a single ethnic group using a relatively small sample size. To solve this problem, the International Warfarin Pharmacogenetics Consortium (IWPC) was formed with clinical and genetic data collected from over 5000 patients covering three major ethnicities (Asian, African and Caucasian). Information from 4042 individuals was first used to develop two algorithms, one with clinical factors only and the other with both genetic and clinical variables. They were then tested and replicated on an additional 1009 patients.[12] The IWPC study showed that the algorithm with both genetic and clinical factors outperformed the non-genetic clinical algorithm and fixed dose. The study also demonstrated that patients requiring less than 21 mg/week or greater than 49 mg/week of warfarin had the greatest benefit for pharmacogenetics dosing. These patients accounted for more than 40% of the total patients. These algorithms, including the IWPC algorithm, were not suitable for use in pediatric patients as they were developed using mostly adult populations. New algorithms using pediatric patients have been developed.[82,83] In addition, the IWPC researchers also demonstrated that incorporating the VKORC1 -1639 G>A SNP is sufficient for dosing algorithms, adding additional VKORC1 SNPs or the uses of haplotypes does not further improve warfarin dose prediction.[52]

All the algorithms developed so far can at most explain about 80% of the variations in warfarin response.[84] New genetic and clinical variants yet to be identified could explain some of the missing variations. However, it is more likely that the remaining variations cannot be fully explained. For example, food preference can differ greatly even within the same country/ethnic group, and it is extremely difficult to capture variations caused by dietary consumption.

WARFARIN PHARMACOGENETICS: IS IT READY?

The United States Food and Drug Administration (FDA) updated the drug label for warfarin to include the genetic information, given the enormous evidence and the regulatory mandate for drug safety. However, this initial update did not provide any specific dosing recommendation using genetics and the genetic tests were not widely available at the time which resulted in a lot of controversy. A genotype-stratified dosing table was included in the 2010 label revision.

The Clinical Pharmacogenetics Implementation Consortium (CPIC), a partnership of the National Institutes of Health PharmGKB (www.pharmgkb.org) and the Pharmacogenomics Research Network (PGRN; www.pgrn.org) has developed a guideline for phmarcogenetic use of warfarin. This guideline provides interpretation and recommendations for physician use if CYP2C9 and VKORC1 genotype data is available.[6] If genetic information is available, a pharmacogenetic dosing algorithm should be used. The FDA genotype dosing table should still be used if the use of algorithms is not possible. One study demonstrated that the pharmacogenetics dosing algorithms were the most accurate in predicting warfarin dose, followed by the genetic dosing table and empirical dosing.[85] Websites have been established to assist the use of pharmacogenetic dosing algorithms, since the calculations can be complex. Warfarindosing.org (http://www.warfarindosing.org) is a free website which includes the IWPC, the Gage algorithms and information regarding the genetic use of warfarin. The PharmGKB database also generated a spreadsheet for the IWPC algorithm to help estimate warfarin dose (http://www.pharmgkb.org/drug/PA451906).

Even though there is mounting evidence of the genetic association with warfarin dosing, the benefit of clinical use of warfarin pharmacogenetics is still being debated. Several studies, including randomized trials, have been performed to address these issues.[80,86-94] Some of the studies demonstrated that using genetic information in warfarin treatment did lead to a real

therapeutic advantage. The Medco-Mayo Warfarin Effectiveness study is perhaps the most notable of these investigation.[89] It showed that if the treating physicians were given VKORC1 and CYP2C9 genotypes, patients were less likely to be hospitalized for a bleeding episode (28% less). However, this study came under heavy criticism as the genotype data was not provided before treatment initiation, instead, genotype data were provided, on average, 32 days after the start of treatment. Also, the major problem for these studies is that the sample sizes were too small to have adequate power to detect the genetic effects.

The first clinical pharmacogenetic study of warfarin with sufficient sample size and adequate power was the CoumaGen-II study,[95] with 504 cases in the pharmacogenetic-guided dosing arm, and 1911 controls in standard dosing arm. This study demonstrated that patients with pharmacogenetics-guided dosing had a higher percentage of patients that stayed in the therapeutic INR range, fewer INR ≥ 4 and ≤ 1.5, and less serious adverse events. Several large randomize trials are also currently ongoing to test the benefits of pharmacogenetics-guided dosing.[6]

CONCLUSIONS

There are several roadblocks which prevent pharmacogenetics of warfarin from being more widely used. Efforts were made on all fronts to remove these roadblocks. The ongoing large clinical trials would provide the evidence required for the clinical use of the genetic information. The CPIC guideline provides genetic data interpretation, physician education and recommendations for courses of action. Four FDA approved tests and several none-FDA tests are also available at http://www.pharmgkb.org/drug/PA451906#tabview=tab0&subtab=34). These tests are designed to provide fast, reliable and economical genotyping. It can often take more than one week before the results are made available due to the fact that these platforms are still not widely available outside of the major medical center setting. Thus, before pharmacogenetics-guided dosing is widely used, it is vital to develop and put in place the infrastructure required.

Due to the large number of patients requiring anticoagulation treatment, several new anticoagulants have emerged on the horizon which aim to replace warfarin. The US FDA has recently approved Dabigatran (a direct thrombin inhibitor)[96] and rivoxaban (factor Xa inhibitor).[97] These new anticoagulants are in some cases superior to warfarin. One recent study showed that intracranial bleeding was lower for dabigatran and the incidence of myocardial infarction was also reduced for patients on dabigatran.[98] However, these new drugs can still cause adverse bleeding, and anticoagulation status monitoring similar to INR is not available. This can cause some difficulties as there is no way to identify who is at risk for bleeding with these drugs. The cost associated with these new anticoagulants is also much higher than warfarin. Furthermore, there is no antidote to reverse the effect of these new drugs, so patients are at greater risk when adverse bleeding occurs. For these reasons, warfarin will not be replaced anytime soon.

There is an enormous amount of data on the safety, clinical use and genetics of warfarin from over 60 years of use. Thus, at least for the near future, warfarin still will be the world's most prescribed oral anticoagulant. The fate of the pharmacogenetics of warfarin relies in part on the ongoing clinical trials. If these large scale clinical trials clearly show the benefit of pharmacogenetics-guiding dosing, with the rapid decrease in the cost of genotyping, there will be new life for warfarin.

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