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### Pharmacogenetics and Determining Warfarin Dosage

Pharmacogenetics is the study of how genetics affect a patient's response to a drug. The field contributes to the fairly new concept of personalized medicine. The same drug in the same dosage can elicit a variety of responses. Some patients will respond well, some will experience adverse drug reactions, and others may see no effect at all.

Research is ongoing to determine how pharmacogenetic information can be used in clinical practice. According to A.C. Wu and A.L. Fuhlbrigge in an article about the economic feasibility of pharmacogenetic tests, "Pharmacogenetic tests have the potential to (i) predict intended response, the goal outcome of the medication; (ii) predict unintended response to the medication, such as adverse events; (iii) titrate medication dose; and (iv) inform the development of novel therapeutics." A number of clinical trials have recently focused on using pharmacogenetics in determining proper dosing for patients using the drug warfarin.

Warfarin is an anticoagulant, a very popularly prescribed medication. This drug is used in the prevention of thromboembolic events due to conditions such as atrial fibrillation, venous and arterial thrombosis, and prosthetic heart valves.

Thromboembolism occurs when a blood vessel is blocked by a blood clot that has broken away from its original site. Warfarin is an anticoagulant, which works by preventing the formation of blood clots in the blood vessels. The drug decreases the

ability of the blood to form clots by preventing the synthesis of several clotting factors (II, VII, IX, and X) and several anticoagulant proteins (C and S) (Kuruville).

Currently in the United States, 2 million people are being treated with warfarin. However, there are dangers associated with taking warfarin. It has a very small therapeutic region, measured by INR (international normalized ratio). In order for the drug to be safe and effective, patients should have an INR between 2.0 and 3.0. If the INR is lower than 2.0, there is an increased risk of a thromboembolic event, such as a stroke. If the INR is above 3.0, there is an increased risk of bleeding. Doctors must be cautious when prescribing warfarin to patients, as there is often much adjustment of the dose required before the patient reaches a stable dosage. Warfarin dosing is also complicated by drug-drug and drug-food interactions.

Adding to the difficulty of prescribing warfarin is the great variability in how patients respond to the drug. It is difficult to predict how each patient will react, as the level of response varies among patients even when using the same dose. This variability is associated with two locations in particular, in the genes CYP2C9 (cytochrome p450) and VKORC1 (vitamin K epoxide reductase complex 1). CYP2C9's function is to metabolize the S-enantiomer of warfarin. The VKORC1 gene is involved in the vitamin K cycle, reducing vitamin K to vitamin K<sub>H2</sub>. Vitamin K<sub>H2</sub> is an important co-factor in the activation of clotting factors. Warfarin's anticoagulant function comes from its ability to inhibit the VKORC1 protein. Of course, a patient's genetic makeup is not the only factor that must be considered when determining drug dosage. Doctors must still consider the patient's age, weight, diet, and other potential drug interactions.

There is significant evidence that these two genes (CYP2C9 and VKORC1) are important in determining proper warfarin dosage. In fact, the FDA now requires the drug label for warfarin to state that, “The patient’s CYP2C9 and VKORC1 genotype information, when available, can assist in a selection of the starting dose” (Pirmohamed).

For example, patients with the \*2 and \*3 alleles of CYP2C9 show greater risk of bleeding, and their warfarin dosage must be adjusted accordingly. Looking at the VKORC1 gene, having the minor A allele reduces the expression of the gene to half that of patients with the normal G allele. Therefore, lower warfarin doses are needed in patients exhibiting the A genotype. The frequency of this A allele differs greatly between ethnic groups. According to Figure 1, (from “Warfarin Pharmacogenetics” by Julie Johnson), it occurs in 86% of Asians, 37% of Europeans, and only 10% of African Americans. Therefore, the dose of warfarin suggested for patients of each of these ethnicities would be drastically different.

**Figure 1**  
Minor allele frequencies by ethnicity.

Variant	European	African Americans	Asian
<i>VKORC1-1639A</i>	0.37	0.10	0.86
<i>CYP2C9*2</i>	0.13	0.02	ND
<i>CYP2C9*3</i>	0.06	0.01	0.02
<b><i>CYP2C9*5</i></b>	<b>ND</b>	<b>0.01</b>	<b>ND</b>
<b><i>CYP2C9*6</i></b>	<b>ND</b>	<b>0.01</b>	<b>ND</b>
<b><i>CYP2C9*8</i></b>	<b>ND</b>	<b>0.06</b>	<b>ND</b>
<b><i>CYP2C9*11</i></b>	<b>ND</b>	<b>0.02</b>	<b>ND</b>
<b><i>CYP4F2 433Met</i></b>	<b>0.29</b>	<b>0.07</b>	<b>0.29</b>
<b><i>rs12777823</i></b>	<b>0.14</b>	<b>0.25</b>	<b>0.32</b>

ND = not detected or rare. Variants shown in bold text were not included in either the COAG or EU-PACT study.

Although it is widely accepted that a patient's genotype can affect their response to warfarin, pharmacogenetically determined dosing is not yet used regularly by doctors. Several clinical trials have been conducted in the last few years to examine the difference between pharmacogenetically-guided dosing and standard clinical dosing. These trials have had mixed results, with some showing that pharmacogenetically-guided dosing is superior and others showing no significant difference between the two methods of determining warfarin dose. I will now examine the methods and results of two clinical trials, one with each of these outcomes.

The first clinical trial, led by M. Pirmohamed, was published in the New England Journal of Medicine in 2013. Conducted by a group from EU-PACT (European Pharmacogenetics of Anticoagulant Therapy), the trial aims to measure whether genotype-guided dosing is actually more effective than regular dosing. In this study, warfarin was used to treat patients with atrial fibrillation or venous thromboembolism. It was a randomized, controlled trial with an experiment group, in which the dosage was determined by a patient's genotype, and a control group, in which the standard clinical dosage was given.

For the first five days of treatment, patients in the genotype-guided group received doses assigned based on pharmacogenetic algorithms. After this, they received the standard clinical dosage. The pharmacogenetic algorithm was based on the effect of different alleles of the gene CYP2C9 on warfarin metabolism. Patients in the control group received the standard clinical dose on all days. The patients in the

trial continued to receive treatment for 3 months, with INR measured at various points throughout the trial.

The study concluded that genotype-guided dosing, based on pharmacogenetics, was better for patients, as they spent more time in the therapeutic range, had less difficulty with excessive anticoagulation, and reached the therapeutic range faster. Patients in the genotype-based dosing group also required fewer adjustments to the dose of warfarin before reaching a stable dose.

The main measure of comparison in this clinical trial was percentage of time spent in the therapeutic range (defined by having an INR between 2.0 and 3.0). The results showed the genotype-guided group at 67.4% and the control group at 60.3% of time in the therapeutic range. This is a large and statistically significant difference. Genotype-guided dosing was better according to other measures as well. 82% of patients in the genotype-guided group reached a stable dose within three months of beginning treatment, while only 70.4% of control group patients reached a stable dose in this same amount of time. Also, looking at the many measurements taken of INR throughout the trial, genotype-guided patients were less likely to have an INR of greater than 4.0, which is shown to increase the risk of bleeding.

Researchers found that the difference in INR between the genotype-guided group and the control group was the largest during the initial stages of treatment, suggesting that genotype-determined dosing is more effective at the start of a warfarin regimen. This trial was performed using patients in the United Kingdom and Sweden;

therefore, most patients were of European descent. Researchers acknowledged that the results might be different in patients of other ethnicities.

A second clinical trial investigating the effects of pharmacogenetically determined dosing was published in the same issue of the New England Journal of Medicine last December. This clinical trial, led by S. Kimmel and also known as the COAG (Clarification of Optimal Anticoagulation through Genetics) trial, was conducted very similarly to that of the EU-PACT. However, a different result was obtained. After the first four weeks of treatment, it was found that the average time spent in the therapeutic region (also measured by an INR between 2.0 and 3.0) was not significantly different for the genotype-guided group at 45.2% versus the clinically guided (control) group at 45.4%. This study also didn't find any difference in the time above or below the therapeutic range between the groups. Neither group appeared to have more thromboembolic events than the other.

This trial found slightly fewer bleeding events, measured over a 6-month period after beginning treatment, in the genotype-guided group than in the standard dosage group. 4% of patients in the control group experienced major bleeding events, compared to only 1% in the genotype-guided group. Although the difference is not statistically significant ( $P = 0.021$ ), this is something to be investigated in future trials. If pharmacogenetically based dosing allows patients to spend more time in the therapeutic range and to reach this point earlier, they will most likely be in less danger of severe bleeding events while taking the medication.

The one difference found was that the effectiveness of the dosage varied depending on the patient's ethnicity. For example, African American patients in the genotype-guided group spent less time in the therapeutic region than those in the control group. However, the difference was still not statistically significant, with a P-value of 0.01. Non-black patients had a slightly higher percentage of time in the therapeutic range in the genotype-guided group than in the control group. This data demonstrates how a patient's response to warfarin can vary based on race. It may indicate in this case that African American patients respond less well to warfarin in general, or that pharmacogenetic-guided dosing is less effective. Or this difference could be attributed to the frequency of the CYP2C9 alleles in different racial groups. As Russ Altman wrote in his response to Kimmel's article in the New England Journal of Medicine, "the single-nucleotide polymorphisms used in the study's pharmacogenetic dosing algorithm are known to occur at significantly lower frequencies in persons of African descent than in persons of European descent... Studies testing the usefulness of pharmacogenetics in a specific population should test variants with high frequency and measurable effect in that population."

Several genome-wide association studies have shown yet another SNP to be important in determining warfarin response in African American patients (Cavallari and Nutescu). A certain allele at this location, near the gene CYP2C18, contributes to a lower required dose of warfarin in patients of African descent. Over 40% of African Americans carry this allele, making this an important factor in clinical trials involving warfarin dosage.

There are a number of possible reasons why the results of the COAG study were not consistent with the EU-PACT clinical trial discussed above. It is interesting to note that for the COAG trial, the percentage of time spent in the therapeutic range is lower in both groups than in the other trial. The COAG study saw values around 45% while the EU-PACT trial saw values around 60%. This could be attributed to several factors, including the length of the study or the racial breakdown of the patients. The COAG trial ran for only a month, compared to 3 months in the EU-PACT trial. The increased number of INR measurements in the EU-PACT trial may have led to a greater average percentage of time spent in the therapeutic region. Also, the COAG trial included patients of varying ethnicity, while the EU-PACT trial was conducted mainly in white participants. In the COAG trial, about 30% of the participants were African American and 6% were Hispanic. If, as suggested above, pharmacogenetically guided dosing is less effective in black patients, this would have reduced the overall average time spent in the therapeutic range.

Another major difference between these two trials was the dosing method for the control group (Cavallari and Nutescu). The EU-PACT trial used fixed dosing, which is the method typical in clinical practice today. Patients received 10 mg on the first day of treatment, 5 mg on the next two days, followed by doses determined based on each individual patient's INR measurements. The COAG trial used a clinical dosing algorithm, which is based on a patient's age and body size, among other factors. Studies have shown that the clinical dosing algorithm (used in the COAG trial) is superior to the fixed dosing method (used in the EU-PACT trial). So this may explain why there was no



discernible difference between the control and genotype-guided groups. However, since the clinical dosing algorithm is rarely actually applied in clinical practice, the EU-PACT trial gives a better sense of how doctors assign the dosage for patients.

Given the conflicting data, researchers and clinicians are as of yet unsure whether pharmacogenetics should be applied to warfarin dosing. As L.H. Cavallari and E.A. Nutescu conclude in their review of several clinical trials, "If a patient has genotype data readily available, it is difficult to argue against the use of these data to assist with warfarin dosing, especially if they reveal the VKORC1-1639AA genotype associated with increased warfarin sensitivity or a CYP2C9 genotype associated with poor warfarin metabolism." In other words, if a patient's genome has already been sequenced, it is simple to determine if they have a genotype that might require a nontraditional dose of warfarin. However, the question now is whether all patients beginning a warfarin regimen should first be sequenced to determine if they have one of these genotypes. The clinical trial data is as of yet unclear on whether the effects of genotype-guided dosing are great enough to warrant genome sequencing of every warfarin patient.

Many other trials are currently being run or planned to more conclusively determine the effectiveness of genotype-guided warfarin dosing. One trial, which is currently ongoing, is also investigating the effect of pharmacogenetic vs. clinical dosing of warfarin. This trial, conducted by E.J. Do and colleagues, includes about 1600 patients who have recently undergone a hip or knee replacement and are therefore at risk for both thrombosis and bleeding. This trial, expected to be complete in 2015, will look further into the difference in the number of thrombotic and major bleeding events

between the experiment and control groups. This will provide further data on which to base conclusions about whether genotype-guided dosing affects the severity or number of adverse reactions (such as thrombosis or bleeding) due to warfarin.

Genotype-guided warfarin dosing has even already been implemented as general practice in some hospitals. For example, starting in August 2012, the University of Illinois Hospital & Health Sciences System introduced genotype-guided warfarin dosing for patients beginning to take warfarin (Nutescu). All patients starting warfarin during hospitalization are genotyped. A clinical pharmacogenetics consulting service was used to interpret the genotype data and recommend proper doses. The hospital concluded that, "Providing routine genotype-guided warfarin dosing supported by a pharmacogenetics consult service is feasible from a procedural standpoint."

In conclusion, there is much research still to be done in this area. It is well established that warfarin dosing will vary depending on the patient. And it is agreed that a large portion of this variation in a patient's reaction to warfarin can be attributed to genetics. Several genes, including VKORC1 and CYP2C9, have been connected to warfarin response, and certain genotypes require that the warfarin dose be increased or reduced in order to maximize safety and effect on the patient. Clinical trials and genome-wide association studies have discovered that a patient's response to warfarin also depends on their ethnicity, and that the genotypes associated with warfarin dosing vary greatly across racial groups. Further clinical trials need to be conducted to examine the effect of pharmacogenetically guided dosing in minorities. While it appears that pharmacogenetics are helpful in determining proper dosing in some cases, more clinical

trials need to be conducted before genotype-guided warfarin dosing becomes standard practice in the clinic.

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